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Tannin Concentrations of Gyrinops Tea with Different Leaf Processing Methods and Addition of Herbal Medicine Ingredients

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Abstract. Gyrinops tea is the new type of agarwood tea from Lombok Island, which has a good prospect to be developed. Addition of herbal medicine ingredients could improve the quality of this product as a healthy good taste herbal tea. One of chemical standard to measure quality of herbal tea product is tannin measurement. The aim of this research is to examine tannin concentration of Gyrinops tea with different leaves processing and addition of herbal medicine ingredient. Factorial Completely Randomize Design was used as experimental design with three replications and two treatment factors. i.e. leaf processing methods (P_1= leaves without oxidation, P_2: leaves with oxidation), and addition of herbal medicine ingredients (H_1: *Zingiber officinale*, H_2: *Cymbopogon citratus*, H_3: *Citrus aurantiifolia*). Tannin measurement was carried by titrimetric method using $KMnO_4$ and Indigo carmine reagent. Tannin concentration data were analyzed using analysis of variance (ANOVA) and DMRT at 5% level of significance. The results indicated that both treatment factors showed significant effects on tannin concentrations. However, there was no interaction effect between those two factors. Oxidation of *Gyrinops* leaves increased tannin concentration of the *Gyrinops* tea. Addition of *Cymbopogon citratus* resulted in the highest tannin concentration compared with other ingredients. Thus oxidation of *Gyrinops* leaves combined with addition of *Cymbopogon citratus* could produce herbal tea with high quality of tannin concentration.

INTRODUCTION

Gyrinops versteegii is an endemic agarwood species on Lombok Island. This species could be found on its natural habitat at Senaru Forest, North Lombok [1]. This species could also be found on several agarwood plantation including: Pejaring Village East Lombok [2] and Lingsar District, West Lombok [3]. This species is classified as a high valuable commodity due to the high price of it resin called "Gubal".

Diversification product of this commodity other than "gubal" was an important issue recently on agarwood farmer of Lombok Island. It is because "gubal" productions on agarwood species need at least 12 years of investing time [4]. Agarwood farmers of Lombok Island need alternative usage of agarwood commodity to get income during that long period of investing time. One of the good alternative usages is herbal tea from agarwood leaves [3].

Gyriniops tea is a new type of agarwood tea from Lombok Island. Most Indonesian people know agarwood tea as Aqila tea that originated from Sumatra Island [5]. Aqila tea comes from Aquilaria spp, while Gyrinops tea comes from *Gyrinops versteegii*. Although being classified as new type of agarwood tea, this product has good response from Mataram City Society. Based on hedonic test, respondent give good score for Gyrinops tea on parameter of color and flavor [6]. However, respondent give suggestion to adding more ingredient to Gyrinops tea to make it taste better.

Technically, herbal tea is herbal mixture made from leaves, seeds and/or roots of various plants other than tea (*Camelia sinensis*) [7]. That means, Gyrinops tea could be combined with other ingredient without losing its identity as agarwood herbal tea. Herbal ingredient with medicinal effect has become new trend in beverage production recently [8]. These resources could be a potential additional ingredient for Gyrinops tea. Furthermore, people of Lombok Island have got used to drinking beverage that contains herbal medicine ingredient.

Gyrinops tea mixed with herbal medicine ingredient is an innovative beverage product which needs to be standardized. That standardization should be focused on the main component that responsible for quality

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determination of tea. Tannin is an astringent, bitter plant polyphenol that is commonly found in beverages such as tea, wine and beer [9]. This molecule is important for the sensory quality of beverage [10]. This molecule also has potential role on beverage preferential of consumer based on oral perception and psychosocial aspect [11]. Moreover, tannin is considered as the main property that determines the quality of industrial tea beverage [4], [12].

Recent study of Gyrinops tea also uses tannin as an important photochemical property [2]. This means that tannin is also good parameter for herbal tea beverage. Thus, the aim of this research is to examine tannin concentration of Gyrinops tea with different leaf processing methods and addition of herbal medicine ingredients.

MATERIALS AND MEHTODS

This research started by processing Gyrinops leaveas including: drying, chopping and oxidizing (for leaves with oxidation process). Gyrinops leaves then were mixed with *Z. officinale, C. citratus* and *C. aurantiifolia*. All these ingredients were measured for their tannin concentration by titrimetric method using $KMnO_4$ and Indigo carimine (Figure 1)

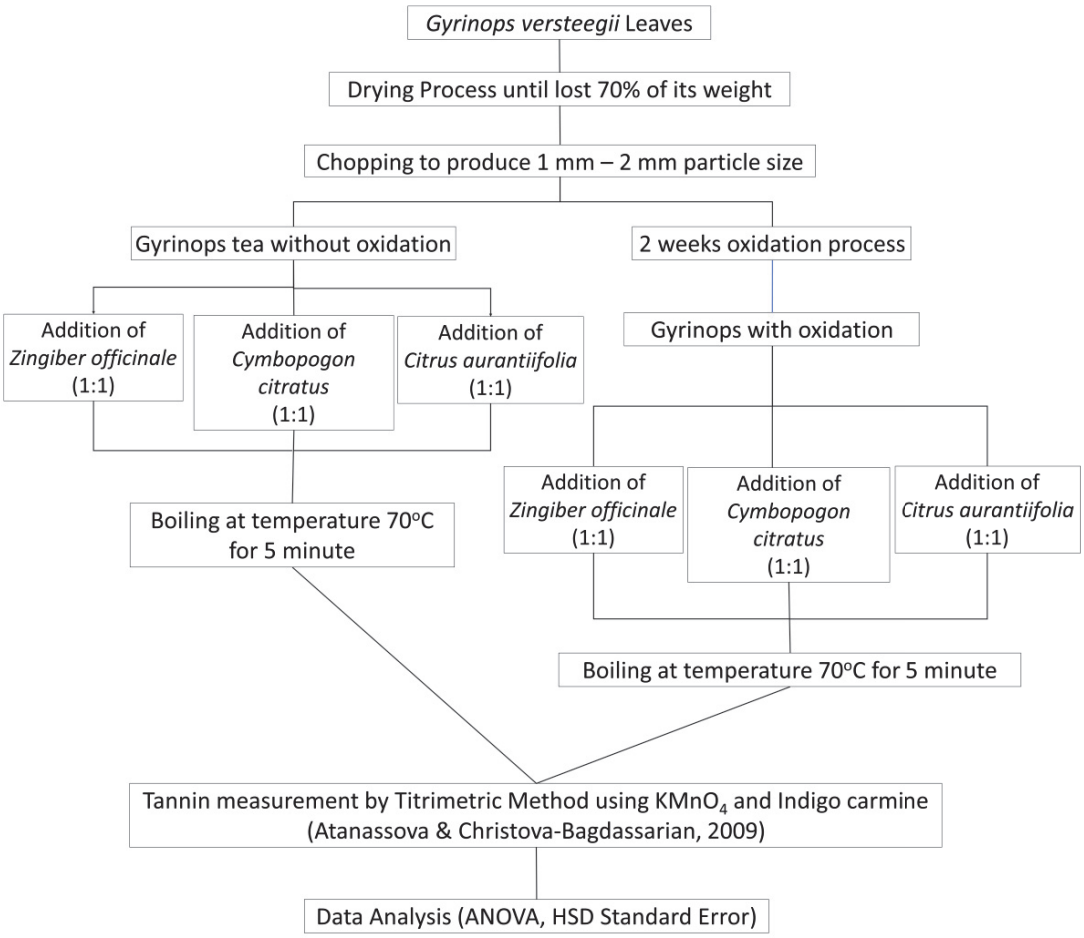

FIGURE 1. Systematic Method of the Research

Experimental Design

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

Processing method of *G. versteegii* leaves

P_1 : Leaves without oxidation process
P_2 : Leaves with oxidation process

Addition of herbal medicine ingredients

H_1 : Addition of *Zingiber officinale*
H_2 : Addition of *Cymbopogon citrates*
H_3 : Addition of *Citrus aurantiifolia*

Collecting of *Gyrinops versteegii* Leaves

Gyrinops versteegii leaves were taken from agarwood plantation at Duman Village, West Lombok District, West Nusa Tenggara Province, Indonesia. The leaves were taken from the branch at the top of *G. versteegii* tree. Selection of leaves was conducted based on several criteria including: leaf size, leaf color and they should be free from pest and disease. The leaves were washed 3 times with distilled water and then treated according to the experimental design [3]

Gyrinops versteegii Leaf Treatment

Gyrinops versteegii leaves were air dried for 3 days until it lost 70% of its water content. Leaves then were chopped using Miyako BL-22 PLY grinding machine. Leaves without oxidation were directly measured its tannin concentration. On the other hand, leaves with oxidation were given oxidation treatment in oxidation chamber for 2 weeks [2].

Preparation of the Additional Ingredients

Zingiber officinale rhizome, *Cymbopogon citratus* leaves and *Citrus aurantiifolia* fruits were collected from Mandalika Market of Mataram City, West Nusa Tenggara Province, Indonesia. *Z. officinale* rhizome and *C. citratus* leaves were air dried for 3 days until they lost 70% of their water content, and then were chopped using Miyako BL-211 PLY grinding machine. Powder of *Z. officinale* and *C. citratus* were mixed with *G. versteegii* leaves with proportion 1:1 (w/w). *Citrus aurantiifolia* fruit were squished to take its lime water. Lime water was mixed with *G. versteegii* leaves with proportion 1:1 (w/v).

Preparation and Standardization of the Reagents

Indigo carmine solution and KMnO4 solution were two main reagents used for tannin measurement. Indigo carmine solution was made by dissolving 6 grams of Indigo carmine into 500 ml distilled water by heating. After the mixture was cooling down, 50 ml of 95% H_2SO_4 was added. The mixture was then diluted until reached 1 L volume by adding distilled water. Indigo carmine solution then was filtered with qualitative filter paper [14]. Solution of $KMnO_4$ was made by diluting 3.3 grams of $KMnO_4$ powder in 1 L distilled water. Solution of KMnO4 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 KMnO4 [15].

Preparation of Gyrinops Tea

One gram mixture of Gyrinops leaves and *Z. officinale* (1:1 w/w), one gram mixture of Gyrinops leaves and *C. citratus* (1:1 w/w), one gram mixture of Gyrinops leaves and *C. aurantiifolia* (1:1 w/v) were added to 50 ml of distilled water and heated at 70°C for 5 minutes. The mixture was cooled and filtered through Whatman No. 1 filter paper. The filtrate was then centrifuged at 4000 rpm for 15 minutes. The supernatants were stored at 4°C for later analysis [2].

Qualitative Estimation of Tannin

Qualitative estimation of tannin was performed based on Kasnabis et al. [12]. This assay was preliminary assay to convince that sample of Gyrinops tea mixed with herbal medicine ingredient contains tannin. Three drops of 5% (w/v) aqueous solution of $FeCl_3$ was added to 1 ml of sample. Formation of greenish precipitate from the sample indicates the presence of tannins. Positive test result was then continued with quantitative estimation of tannin.

Quantitative Estimation of Tannin

Quantitative estimation of tannin was performed by titrating sample with standardized $KMnO_4$. Twenty five ml of sample were mixed with 25 ml indigo carmine solution in 1 L Erlenmeyer flask. Then 750 ml of distilled water was added to the mixture. The mixtures were titrated with standardized KMnO4 until the blue color of the mixture change into green color. Then few drops were added until color of the solution become golden yellow. The blank test was carried out by titration of mixture of 25 ml indigo carmine solution and 750 ml distilled water. All samples were analyzed triplicate. 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 KMnO4 for titration of the sample (ml), V_0 is the volume of 0.1 N KMnO4 for titration of the blank sample (ml), 0.004157 is tannins equivalent in 1 ml of 0.1 N KMnO4, g is mass of the sample taken for analysis (g), 25 is the volume of sample, 50 volume of extraction solvent for sample.

Data Analysis

Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) at 5% level of significant ($\alpha = 0.05$) were used for analyzing the tannin percentage data. The data were also analyzed for their means and standard error then were presented as bar chart with error bars to interpret the patterns of interactions between the two treatment factors. All data analyses were performed using the statistical software CoStat for Windows ver. 6.303.

RESULT AND DISCUSSION

Herbal medicine ingredients have become new trend on beverage industry recently [8]. The use of these ingredients has increased over past three decades with up to 80% people worldwide use it as a primary healthcare [16]. That is why addition of these ingredients to Gyrinops tea was a good innovation to improve quality of this product.

Z. oficinale, *C. citratus* and *C. aurantiifolia* have been chosen as additional herbal ingredients for Gyirinops tea because these ingredients are the common ingredients found in traditional Indonesian beverage that is called “Jamu” [17]. These ingredients are also the most common raw materials for healthy beverage in Lombok Island. Furthermore, these ingredients are well known as a good source of antioxidant and antibacterial agents [18], [19], [20], [21].

Preliminary study about Gyrinops tea suggested that different processing method lead to different taste of this beverage [6]. Also addition of herbal ingredient gives different perspective from respondent about the taste of Gyrinops tea [22]. This qualitative data (different taste of the Gyrinops tea) needs to be analyzed chemically to build a quality standard for Gyrinops tea. One of important chemically assays to inspect quality of tea and herbal tea beverage was tannin measurement [2], [12].

Tannin Measurement of samples shows that leaf processing method had significant effect on tannin concentration of Gyrinops tea based on DMRT result ($\alpha = 0.05$). Tannin percentage of Gyrinops tea from oxidation leaves was higher than tannin percentage of Gyrinops tea from non-oxidation leaves (Table 1). Basically, oxidation process on herbal tea such as Gyrinops tea will have the same result with oxidation of tea (*Camelia sinensis*) based on tannin concentration. Tannin is the main component that responsible for astringent and bitter taste on tea [9]. Oxidation process on tea produces black tea while non-oxidation process on tea produces green tea [23]. Black tea

taste bitterer then green tea. Chemical assay shows that black tea contains higher tannin concentration than green tea [12].

TABLE 1. DMRT Analysis of Tannin Measurement of Gyrinops Tea Samples

Factors	Treatments	Tannin Concentration (%)	Significance
Processing method of *G. versteegii* leaves	P_1 : Leaves without oxidation process	4.78	a
	P_2 : Leaves with oxidation process	3.63	b
	LSD 0.05	0.59	
Addition of herbal medicine ingredient	H_1: Addition of *Zingiber officinale*	5.28	a
	H_2: Addition of *Cymbopogon citratus*	4.48	b
	H_3: Addition of *Citrus aurantiifolia*	2.86	c
	LSD 0.05	0.72	

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

Preliminary research on Gyrinops tea based on hedonic assay shows that Grynops tea with oxidation process taste bitterer and stronger then Gyrinops tea without oxidation [6]. It is suggested that oxidation of Gyrinops tea should contains higher tannin concentration than non-oxidation Gyrinops tea. This research confirms that hypothesis by proving that oxidation of Gyrinops tea resulted in higher tannin concentration than non-oxidation Gyrinops tea. This research also supports former study on Gyrinops tea without addition of any herbal ingredient, which concluded that the longer oxidation process, then the higher tannin concentration of the Gyrinops tea [2].

Addition of herbal medicine ingredients also had significant effect on tannin concentration based on ANOVA and DMRT result. Addition of *C. citratus* dry leaves produced the highest tannin concentration on Gyrinops tea among the others ingredient. On the other hand, addition of *C. aurantiifolia* fruit squash produced the lowest tannin concentration of Gyrinops tea (Table 1). Based on literature study about total tannin concentration on *Z. officinale*, *C. citratus* and *C. aurantiifolia,* it is suggested that the order of tannin concentration of those ingredient from the highest to the lowest is *C. citratus*, *Z. officinale* and *C. aurantiifolia* (Table 2). This data was supported by this research since it produces the same order of tannin concentration among those three ingredients.

TABLE 2. Comparison of tannin concentration from *Z. officinale, C. citratus* and *C. aurantiifolia*

Ingredient	Organ	Tannin Concentration (%)	Reference
Z. officinale	Root	0.67	[24]
C. citratus	Leaves	0.87	[25]
C. aurantiifolia	Fruit peel	0.64	[26]

Although there was no significant interaction between leaf processing method and addition of herbal medicine ingredients, the correlation between these two factors could be analyzed by standard error (Figure 2). It could be suggested that additional herbal medicine ingredient has the same effect both on Gyrinops leaves without oxidation and Gyrinops leaves with oxidation. Addition of *C. citratus* always gives highest tannin concentration on Gyrinops leaves both without oxidation and with oxidation. On the other hand, addition of *C. citratus* always gives lowest tannin cocnetration on Gyrinops leaves both without oxidation and with oxidation. Since the additional of herbal medicine ingredient has the same pattern on non-oxidation and oxidation Gyrinops tea, the correlation between those two factors classified into positive correlation. However, no specific interaction has been found between those two factors (Fig. 2).

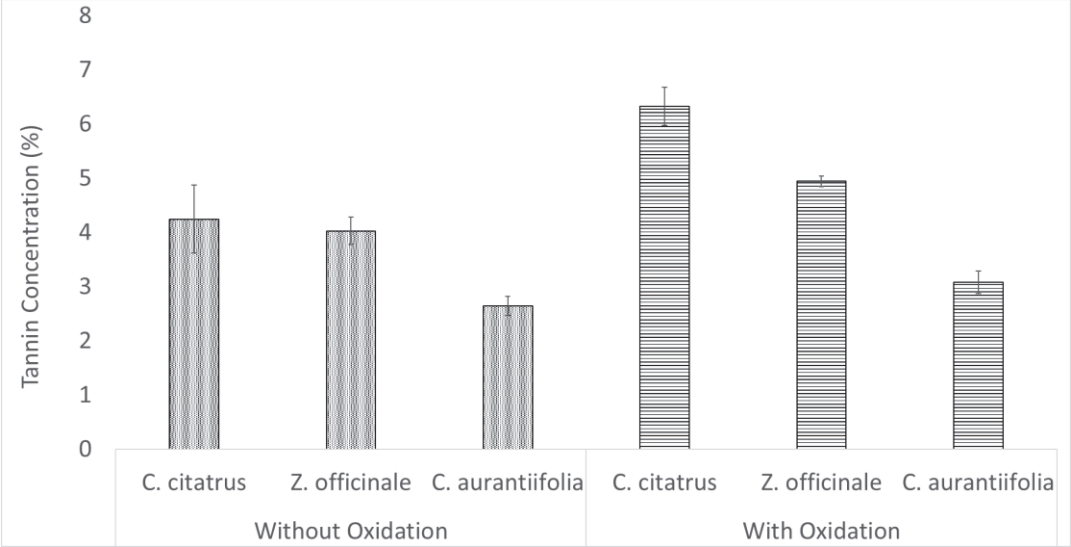

FIGURE 2. Standard Error Analysis of leaves processing factors and additional ingredient factor

Oxidation of Gyrinops tea mixed with dry leaves of *C. citratus* produced the highest tannin concentration among the treatments, suggesting that this ingredient combination could produce high quality beverage based on tannin standard. It also suggests that this ingredient combination produced the strongest color, taste, aroma and texture since it has the highest tannin concentration [22]. Thus, the development of Gyrinops tea product should be based on these two ingredients. However, in beverage industry, consumers sometimes prefer light taste of product. Even consumer with this demand has low proportion on the population, their opinion still need to be considered for product development [8].

CONCLUSION

It could be concluded that oxidation of Gyrinops leaves combined with addition of *Cymbopogon citratus* dry leaves could produce Gyrinops tea with high quality of tannin concentration

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