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Phytochemical screening and antioxidant activity of Gyrinops tea from agarwood plantation on Lombok island, Indonesia

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Abstract. Phytochemical screening and antioxidant analysis are essential standardization procedure for the agarwood tea product. Gyrinops tea is an agarwood tea that has been emerged on Lombok Island recently. This research aims to carry qualitative phytochemical analysis and antioxidant activity assay of Gyrinops tea from several agarwood plantations on Lombok Island. *Gyrinops versteegii* leaves as the raw material of Gyrinops tea were taken from several agarwood plantations on Lombok Island, including Lingsar, Rarung, Kekait, Pejaring, and Mataram. There are three steps on *G. versteegii* leaves processing method into Gyrinops tea, including sterilization, drying, and chopping. *G. versteegii* leaves were brewed in distilled water at 70°C for 5 minutes to make Gyrinops tea. Phytochemical screening of Gyrinops tea was conducted with a qualitative assay identification of Flavonoid, Alkaloid, Saponin, and Tannin. The antioxidant assay of Gyrinops tea was investigated based on DPPH radicals scavenging activity. The result shows that Gyrinops tea positively contains tannin in high dosage and Flavonoid in a moderate dose with no alkaloid and saponin. Gyrinops tea samples have various antioxidant activity from moderate to strong based on IC50 measurement. It could be concluded that different sampling location has affected antioxidant activity of Gyrinops tea.

1. Introduction

Agarwood tea is an excellent alternative utilization of agarwood commodities [1]. This product was made from agarwood leaves that could be harvested periodically by agarwood farmers [2] [3]. The agarwood leaves utilization into agarwood tea could give agarwood farmers additional income during the waiting period of agarwood resin harvesting. Moreover, agarwood tea production involves a simple method quickly learned by agarwood farmers [4].

Agarwood species from *Aquilaria* genus are well known as the raw material of agarwood tea products [3]. In Indonesia, Agarwood tea products from *Aquilaria* genus are mostly well developed on Sumatra Island [5], [6]. This agarwood tea product is well known as “Aqila Tea” with good economic feasibility [1]. However, agarwood species well distributed on Lombok Island is *Gyrinops versteegii* instead of *Aquilaria* sp [7]. Thus, the development of agarwood tea on Lombok Island should be focused on *Gyrinops versteegii* as the raw material source [2]



Gyrinops tea is an agarwood tea made from *Gyrinops versteegii* leaves [8]. This product has been well developed by agarwood farmers on Lombok Island, mostly from the West Lombok region [9]. It is also well accepted by Lombok Island society based on hedonic assay [10]. Thus, Gyrinops tea has an excellent prospect to develop on Lombok Island. To further develop this product as a potential herbal tea commodity, it is essential to carry quality standardization.

Phytochemical screening and antioxidant assay are the most expected quality standardization on agarwood tea products [11]. Several studies have proven that agarwood tea from *Aquilaria* members contains a chemical compound with good antioxidant activity [6], [12]. This antioxidant activity is affected by the environmental condition of the *Aquilaria* habitat [13]. However, phytochemical screening and antioxidant assay of agarwood tea from *Gyrinops versteegii* have rarely been conducted. Phytochemical screening and antioxidant assay of Gyrinops tea are essential for developing this product, especially on Lombok Island [14]. *G. versteegii*, the raw material of this product, is well distributed on Lombok Island. Thus, the phytochemical assay and antioxidant activity of Gyrinops tea should represent a variant of this species in a different region of Lombok Island [15]. This research aims to carry qualitative phytochemical analysis and antioxidant activity assay of Gyrinops tea from several agarwood plantations on Lombok Island.

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2. Materials and methods

2.1. Sampling location and Sample preparation

Gyrinops versteegii leaves were taken from 5 regions on Lombok Island, including Lingsar (West Lombok), Rarung (Central Lombok), Pejaring (East Lombok), Kekait (North Lombok), and Mataram (table 1). Leaves processing and Gyrinops tea extraction were conducted at General Forest Research Laboratory of Pendidikan Mandalika University. Phytochemical Screening was conducted at Agriculture Chemistry Laboratory of Muhammadiyah Mataram University. Antioxidant activity measurements were conducted at the Immunobiology Laboratory of Mataram University.

Table 1. Sampling location of *Gyrinops versteegii* leaves.

Location	Coordinate (Latitude-Longitude)	Elevation (m)
Rarung	8°33'26''S 116°17'38''E	410
Kekait	8°31'26''S 116°07'03'' E	24
Mataram	8°33'58''S 116°07'47'' E	42
Lingsar	8°33'32'' S 116°09'25'' E	72
Pejaring	8°42'28'' S 116°27'11'' E	200

2.2. Sample collection

G. versteegii tree was chosen from agarwood plantation on Lombok Islands (Table 1). Leaves were taken from *G. versteegii* tree after selection based on size, shape, and condition. Leaves with length from 5 cm – 15 cm with no chlorosis and necrosis were chosen as a sample for Gyrinops tea production [9]

2.3. Raw material preparation

G. versteegii leaves were washed with flowing water for three times to clear dirt and dust on them. The leaves then were dried on a drying rack at room temperature for 2 – 3 days until they lost 10% of water content. Dried leaves were chopped using a grinding machine to form a 1 – 2 mm particle size [4]. The particles were stored for further analysis.

2.4. Gyrinops tea extraction

G. versteegii particle leaves were extracted using distilled water to form Gyrinops tea products. Five-gram particles were extracted on 250 ml (concentration 0.02 gr/ml) distilled water and were heated 70oC for 5 minutes. The filtrate was taken by filtration using a qualitative filter paper [14]. The Gyrinops tea samples were stored for further analysis.

2.5. Phytochemical screening

Phytochemical screening is carried by a qualitative chemical assay to examine compounds that have antioxidant activity. Four chemical compounds were examined in this study by qualitative chemical tests, including Tannin, Flavonoid, Alkaloids, and Saponins. These tests using a different method and chemical reagent (Table 2).

Table 2. Phytochemical screening procedure.

Assay	Reagent	Indicator	Reference
Tannin test	FeCl ₃	Changing solution into blackish brown	[16]
Flavonoid test	PbCH ₃ COO	Formation of white precipitates	[17]
Alkaloid test	Wagner's reagent Dragendroff's reagent Mayer's reagent	Formation of precipitant on the solution	[18]
Saponins test	HCl	Formation of stable foam for 5 minutes	[19]

2.6. Antioxidant activity assay

The antioxidant activity of Gyrinops tea was carried by DPPH free radical scavenging method based on [20] with some modification. Serial dilution of Gyrinops tea were carried using absolute methanol to form different concentration including: 150 µg/ml, 125 µg/ml, 100 µg/ml, 75 µg/ml, 50n µg/ml. Pure vitamin C was used as a positive control. Absorbance measurement of each Gyrinops tea concentration was performed by a UV-Vis spectrophotometer at 516 nm wavelength.

Scavenging Activity Percentage were measured using equation [21]

$$\% \text{ Scavenging Activity} = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{sample}}} \right) \times 100\%$$

IC₅₀ measurements were calculated based on the linear regression interpolation of scavenging activity percentage data. The IC₅₀ value is the concentration of Gyrinops tea that could give 50% of scavenging activity inhibition.

3. Results and Discussion

3.1. Phytochemical screening

Phytochemical screening is the preliminary test to examine secondary metabolites that have antioxidant activity. Secondary metabolites on the plant are commonly produced as a defense mechanism of the plant against pressure on its habitats. Two plant organisms but classified into the same species could have different secondary metabolites characteristic if they grow on different habitat conditions [22]. However, in this study, 5 Gyrinops tea samples taken from different regions with different environmental conditions on Lombok Island have shown the same characteristic of secondary metabolite profile (Table 3). All of these Gyrinops tea samples contain tannin and Flavonoid with no alkaloid and saponins. These two compounds are important secondary metabolites that commonly found on agarwood leaves from *Aquilaria* genus [23][24] and *Gyrinops* genus [25].

Gyrinops tea from Mataram is the only sample with an abundance of tannin concentration. Tannin is an important secondary metabolite that could determine the quality of herbal tea products. Moreover, tannin is a standard compound on agarwood tea product that has mostly been examined. Tannin content on agarwood tea products has a positive correlation with the antioxidant capacity [13]. Thus it could be hypothesized that Gyrinops tea from Mataram region has the highest antioxidant capacity among other regions.

Table 3. Phytochemical screening result of Gyrinops tea sample.

Compounds	Sampling Location				
	Mataram	Langko	Pejaring	Rarung	Kekait
Tannin	++	+	+	+	+
Flavonoid	+	+	+	+	+
Alkaloid	-	-	-	-	-
Saponins	-	-	-	-	-

Gyrinops tea from all regions has shown flavonoid content. Flavonoid is a compound that has been responsible for the antioxidant activity of *G. versteegii* leaves extract [26]. This result could support the hypothesis that Gyrinops tea from all regions should have antioxidant activity.

Saponins compound could not be found on Gyrinops tea from all regions. This chemical characteristic of Gyrinops agarwood tea is different from the chemical characteristic of Aquilaria agarwood tea. Agarwood tea from Aquilaria has been reported to contain saponin based on phytochemical screening [24]. Thus, the taste of Gyrinops agarwood tea should be different from the taste of Aquilaria agarwood tea.

3.2. Antioxidant Assay

An antioxidant assay based on DPPH free radical scavenging method shown different antioxidant activity of Gyrinops tea from different regions. Based on the linear regression slope value, Gyrinops tea from Mataram regions has the highest antioxidant activity among other regions. This result has confirmed previous phytochemical screening results showing that Gyrinops tea from Mataram has the highest tannin concentration. Tannin concentration on agarwood tea has a positive correlation with its antioxidant activity.

3.3. Measurement of IC50

Different antioxidant activity of Gyrinops tea from different regions has affected its different IC50 values (Table 4). Gyrinops tea from the Mataram region has the highest IC50 value since it has the highest antioxidant activity. Gyrinops tea of Mataram also the only sample that has strong antioxidant strength based on its IC50 value.

Table 4. IC50 Measurement Result.

Region	IC50 ($\mu\text{g/ml}$)	Antioxidant Strength Category
Mataram	68.58 \pm 4.69	Strong
Langko	142.78 \pm 4.78	Moderate
Kekait	123.82 \pm 5.23	Moderate
Rarung	233.4 \pm 8.8	Weak
Pejaring	103.94 \pm 3.61	Moderate

Standard Value of Antioxidant Power Category [11]

Very Strong	: < 50 $\mu\text{g/ml}$
Strong	: 50 $\mu\text{g/ml}$ – 100 $\mu\text{g/ml}$
Moderate	: 101 $\mu\text{g/ml}$ – 150 $\mu\text{g/ml}$
Weak	: 151 $\mu\text{g/ml}$ – 200 $\mu\text{g/ml}$

Environmental conditions of plant habitat play an essential role in secondary metabolite production. An unfavorable habitat that leads to plant stress conditions could improve several secondary metabolites for defensive mechanisms [22]. Mataram region is the only *G. versteegii* habitat on Lombok Island that located at urban area. This condition is much worse than the condition of *G. versteegii* natural habitat. Thus, *G. versteegii* from Mataram regions has been forced to produce higher secondary metabolites than the regions with similar conditions with *G. versteegii* natural habitat.

Gyrinops tea has a lower IC50 value compare to *G. versteegii* leaves extract. *G. versteegii* leaves extracted with methanol has very strong antioxidant strength with 22.13 µg/ml IC50 value. On the other hand, Gyrinops tea from this study only has 68.58 µg/ml IC50 value, which is classified into a strong antioxidant strength category. The lower antioxidant strength of Gyrinops tea hypothetically is caused by the low extracting power of water compare to the extracting power of methanol as an organic solvent [27]. However, this study could give the closest phytochemical data for the food industry since it directly using agarwood tea products as a sample. A study about agarwood antioxidant activity was used agarwood leaves extract instead of agarwood tea product.

4. Conclusion

Different sampling location with different environment condition has affected the antioxidant activity of Gyrinops tea. However, based on qualitative screening, no different chemical compounds were detected from Gyrinops tea from those different sampling locations.

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