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# Potency of Fresh and Rotten Leaves of Gaharu (*Wikstroemia tenuiramis* Miq) Sumatera Endemic as Raw Material of Antioxidant Rich Tea

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**Abstract.** Gaharu (agarwood) distributes in nature and endemic species of Sumatera (W. Tenuiramis Miq). Along with the knowledge of development gaharu leaf has been used as raw material of tea drink. The aim of this study is to see if gaharu species *W. tenuiramis* Miq has potential as an alternative tea, both fresh leaves and rotten leaves by looking at a group of chemical compounds contained in gaharu leaf that serve as anti-oxidants, to know the strength of antioxidant activity from gaharu leaf extract and to search the content of chemical compounds and to test the level of consumer preference with hedonic test. The fresh gaharu leaf from a tree that grows naturally in nature taken based on the stage of growth namely fresh leaves and rotten leaves. The leaf that has been made simplicia was extracted with 96% ethanol solvent. The phytochemical screening includes examination of alkaloids class compounds, glycosides, steroids/triterpenoids, flavonoids, tannins and saponins. The antioxidant activity test was conducted by DPPH method to get the value IC<sub>50</sub> (Inhibitory Concentration). The results showed that the ethanol extract of young leaves and old leaves of gaharu containing the class of flavonoid compounds, glycosides, steroids/triterpenoids and tannins. The results testing of antioxidant activity showed that fresh leaves extract and rotten leaves of gaharu have IC<sub>50</sub> value ethanol solvent and hot water respectively, were 27,78 and 28,80; 25,35 and 28,59 µg/ml, < 50 ppm with category antioxidant activity is very strong, feasible to be developed as an alternative tea rich in antioxidant. Hedonic tests are on a 3-4 scale or are on not sure - like hose, feasible to be developed as an alternative tea.

## 1. Introduction

Thymeleacea, Leguminosae, and Euforbiaceae family produce a resin called gaharu that can be obtained from microbial infection. Gaharu grows naturally in nature and there are endemic species in Sumatra (*Wikstroemia tenuiramis* Miq). The development of science is not only the resin that was



utilized, but the leaves can be used as raw materials. The utilization of gaharu leaf tea is based on chemical compound content of flavonoids group that is flavon, flavonols and isoflavones so that the leaves used as a tea that acts as an antioxidant.

Research of [1] explained that the secondary metabolite compounds of flavonoids, terpenoids and phenol compounds acted as free antiradicals (antioxidant). The natural antioxidants are abundant in some parts of the plant, such as in wood, bark, roots, fruits, flowers, seeds, and leaves [2].

Based on that, the study conducted is to see the feasibility of Sumatra endemic gaharu leaf as the raw materials of gaharu tea that is rich in antioxidant. The aim of this study to know the gaharu type *W.tenuiramis* Miq can be utilized as an alternative tea by testing it with consumers through a hedonic test, to know a group of chemical compounds contained in gaharu leaf that serve as anti-oxidants, to known the strength of antioxidant activity from gaharu leaf extract *W.tenuiramis* Miq.

## 2. Method

### 2.1. Time and Location

The study was done from June to November 2017. Sampling was conducted in Siantona Village, Lembah Sorik Marapi Sub District, Mandailing Natal District. For determination of water content, phytochemical screening and antioxidant test was conducted at Pharmacognosy Laboratory and Research Laboratory, Faculty of Pharmacy, University of Sumatera Utara

### 2.2. Research Procedure

#### 2.2.1. Sampling of Plant

Sampling was conducted purposively without compare with the same plant from another area. Sample that used in this research is gaharu leaf (*W. tenuiramis*) from the Siantona village because this type was not much studied before and grows naturally in nature.

#### *Preparation of Raw Material*

At this stage gaharu leaf samples are cleared of dirt that stick with running water, then spread on parchment paper until the water is absorbed. The material was dried in the drying cupboard to dry and brittle. Drying of leaves material were conducted by artificial drying that is using dryer cabinet with temperature 40°C-50°C The aim of this drying process is to get the raw material that is not easily damaged, so it can be saved for a long time. The dried leaves were made into powder using a blender. The dried powder is inserted into a vessel that is protected from sunlight prior to extraction and testing process.

#### 2.2.2. Measurement of Water Content

Azeotropy method was conducted to determinate the water content (Toluene distillation).

#### 2.2.3. Preparation of Ethanol Extract of Gaharu Leaf

Extract preparation was conducted with maceration by 96% ethanol solvent as much as 200 g of dried powder is put into a glass vessel, poured with 1500 ml of 96% ethanol, closed, left for 5 days protected from light and occasionally stirred. After 5 days the mixture is covered (strained). Washable with 96% ethanol sufficiently up to 2000 ml, then moved in a closed vessel and left in a cool place protected from light for 2 days, then poured and filtered. Macerate is concentrated using a tool rotary evaporator at a temperature of 40°C until a concentrated macerate is obtained, then dried using the freeze dryer so that obtained dry extract.

#### 2.2.4. Testing Antioxidant Ability with UV-Visible Spectrophotometer

The ability of the test sample in reducing the oxidation process of free radical of DPPH in methanol solution (so that the color change of DPPH from purple to yellow) with IC<sub>50</sub> values (concentration of the test sample trapping 50% free radical) as parameter determines the antioxidant activity of the test sample.

### 2.2.5. Hedonic Test

The favorite test is also called a hedonic test. In a panelist hedonic test is asked for his personal response to preferences or the opposite of displeasure and suggest a level of fondness or also called a hedonic scale. The test was conducted in sensory (organoleptic) that is determined based on a numerical scale. The test was given to 150 panelists of varying ages (17-50 years), sex and ethnicity for tests on taste, aroma and color. The scale used in Table 1. The limit of rejection is the extent to which gaharu leaf tea is deemed unlike by the consumer to be at a numerical scale  $\leq 3$ .

**Table 1.** Hedonic Scale and Numeric Scale

Hedonic Scale	Numeric Scale
Like very much	5
Like a little	4
Not sure	3
Dislike a little	2
Dislike very much	1

## 3. Results and Discussions

### 3.1. Water Content of Simplicia

Water content needs to be known before the simplicia is tested further. The water content determination was conducted to provide a minimum limit of water content that can still be tolerated in the simplicia. The water content of gaharu leaves of *W. tenuiramis* presented in Table 2. The water content of both types of gaharu leaves are relatively same, because the processing is also same. Water content of simplicia of natural material used to be lower than 10% so that bacteria or fungi do not grow so that the simplicia can be stored for a long time. The water content of the simplicia has fulfilled the standard requirement that is not more than 10% [3].

**Table 2.** Water Content of Gaharu Leaf *W. tenuiramis*

Part of Leaves	Water Content (%)
Fresh leaves	7.69
Rotten leaves	9.70

According to [4] high microbial growth especially fungi is supported by high water content of simplicia that can be trigger the enzymatic reactions so that it can occur decaying chemicals present in simplicia. The water content of fresh leaves is relatively lower than rotten leaves (Table 2). Both of fresh and rotten leaves was free from dirt and the aroma was not deviate because of mucus and fungi.

### 3.2. Determination of Tannin Content

Tannin content are influenced by extract content in tea water because tannins have properties if dissolved in water will form a colloid and have a sour taste and tighten on tea. Value of Tanin Content in Table 3. Tanin is a polyphenol compound that can be complex with proteins forming copolymers. Tanin is present in the leaves, the immature fruit, is an active compound of plants belonging to the flovonoid group that has a sense of sepat on food [5].

The tannin content from Siantona village of *W. tenuiramis* for fresh leaves and rotten leaves are 1.08 and 4.65%. The differences in tannin content in various types of tea are differences in the process of tea management, leaf age, climate differences where samples are taken. In addition, the tannin content in each leaf type (fresh and rotten) is different because the content contained in plants is different.

**Table 3.** Tannin Content of Gaharu Leaf *W. tenuiramis*

Part of Leaves	Water Content (%)
Fresh leaves	1.08
Rotten leaves	4.65

According to [6] a fermentation results the chemical changes in tea caused by enzymes. During the fermentation process, there is oxidation of cells released during milling with oxygen, in the presence of an enzyme that acts as a catalyst.

### 3.3. Value of IC50 (Inhibitory Concentration) test sample

The amount of antioxidant activity is characterized by IC50 value, that is the concentration of sample solution required to inhibit 50% of DPPH free radicals [7]. The value of IC50 obtained based on calculation of the linear regression equation obtained by plotting concentration of test solution and percent DPPH damping as a parameter of antioxidant activity, where the concentration of the test solution (ppm) as abscissa (X axis) and percent value of damping as ordinate (Y axis). The category of strength determination of antioxidant activity can be seen in Table 5.

Supported opinion of [8], the ability of test sample in trap DPPH as free radicals in a methanol solution with a value IC50 (concentrations of the test samples capable of trapping free radicals by 50%) used as a parameter to determine the antioxidant activity of the test sample. Value of IC50 ethanol extract of gaharu leaf species *W. tenuiramis* Miq differentiated based on fresh leaves and rotten leaves are 27,78 and 25,35 µg/ml, while the value of IC50 hot water extract of gaharu leaf species *W. tenuiramis* Miq differentiated based on young leaves and old leaves are 28,80 and 28,59 µg/ml (Table 5). The treatment of fresh leaves and rotten leaves also to show the same value that is in the category very strong.

**Table 4.** The Category of Antioxidant Activity [9]

Category	Concentration (µg/ml)
Very strong	< 50
Strong	50 -100
Medium	101 – 150
Weak	151 – 200

Strong or weak antioxidants are determined by several factors, one of which is the chemical composition it contains the chemical composition of the ingredients is also affected by its habitat [10]. The main compounds that cause strong antioxidants are phenol groups compounds, such as flavonoids. The results of phytochemical test and TLC test of gaharu leaf tea *W. tenuiramis* positive contains flavonoids and tannins, this is the antioxidant cause of gaharu leaf tea is very strong (rich in antioxidants). Both types of gaharu have very strong antioxidant activity, and is in the same value hose that is less than 50, both young leaves and old leaves. All leaves deserve to be used as raw materials for tea rich in antioxidants.

**Table 5.** Antioxidant activity test of Gaharu leaves extract ( $\mu\text{g/ml}$ )

Solvent	<i>W. tenuiramis</i> from Siantona Village	
	Fresh leaves	Rotten leaves
Ethanol	27.78	25.35
Hot water	28.80	28.59

According [11] said that antioxidant can against the free radicals present in the body, which is obtained from the metabolism of the body, air pollution, food contamination and sunlight etc. Various plants commonly consumed in Indonesia contain antioxidant, results of research gaharu leaf tea the antioxidant content is high so that if consumed to be one source of antioxidant body.

### 3.4. Value of Community Preferences (Hedonic Test) of Gaharu Leaf Tea

This hedonic test was conducted to find out the respondent's opinion on the color, taste, and aroma of gaharu leaf tea. Test results can be seen in Table 6. Based on Table 6, It can be seen that the result of the respondent to gaharu leaf tea based on the type (fresh and rotten) that gaharu leaf tea most preferred by the community is gaharu leaf tea species *W. tenuiramis* Miq from rotten leaves both in terms of color, and taste with a favorite scale  $> 3$  (like). Based on this, it is known that the preferred level of the community prefers the tea mixture leaves of gaharu leaf *W. tenuiramis* Miq, can be seen from the tannin content of gaharu leaf tea. Gaharu leaf tea with high tannin content has a lower favorite than tea with a low tannin content.

Unlike the case if separated between fresh leaves and rotten leaves, then on the results of hedonic test for fresh leaves the community prefers rotten. On rotten leaves, results of hedonic test for community prefer the rotten leaves type *W. tenuiramis*, the excellence the type *W. tenuiramis* is the taste side. Food taste is one of the important parameters that affect the people's acceptance of a food product. According to [7] taste which is produced influenced by the components present in the foodstuff and the process it undergoes. Taste is a crucial factor in the consumer's final decision to refuse or accept a food. Although the rating parameters in terms of color and aroma are good, but if the food taste dislike, then the product will be rejected by consumers or community. The most preferred taste parameter by the panelist in Table above is gaharu leaf rotten  $3,40 \pm 0,90$ . This happens because of the low taste of tightening contained in tea. According to [12], preferences for drinking tea occur since childhood in the family. In this study, the tested tea is a new product that has not been consumed by the consumer for a long time.

**Table 6.** Results of Hedonic Test Survey Level Of Community Preferences to Gaharu Leaf Tea

Part	Color	Taste	Aroma
Fresh leaves	$3,18 \pm 0,71$	$3,38 \pm 0,86$	$3,45 \pm 0,74$
Rotten leaves	$3,18 \pm 0,76$	$3,40 \pm 0,90$	$3,43 \pm 0,74$

\*Scale: 1 = Dislike Very Much, 2 = Dislike, 3 = Not sure, 4 = Like, 5 = Like Very Much

Color is physical attributes assessed first in determining the quality of food and sometimes can be used as a measure to determine taste, texture, nutritional value and microbiological properties. According to [13] attractive food colors can affect and arouse an appetite for the community, even the color of the food can be a clue to the quality of food produced.

The aroma produced by gaharu leaf tea is due to the essential oil contained in the gaharu leaf. According [14] said that the aroma of tea is composed of volatile compounds (essential oil) where the aroma of tea originated in the plantation and partly developed during the tea making process. The aroma of food becomes an indicator the delicacy of a food. The aroma produced by volatile compound contained in foodstuffs. Aroma can arise naturally and because of processing [7].

#### 4. Conclusions

1. Fresh leaves and Rotten leaves of gaharu species *Wikstroemia tenuiramis* Miq can be used as an alternative tea, with a water content lower than 10%, and tannin content below 5%
2. Activity of antioxidant from ethanol extract and water extract of gaharu leaf type *Wikstroemia tenuiramis* Miq is very strong with IC50 values, fresh leaves and rotten leaves in ethanol solvent and hot water respectively : 27,78 and 28,80; 25,35 and 28,59µg/ml.
3. The level of community preferences for gaharu leaf tea species *Wikstroemia tenuiramis* Miq are on a 3-4 scale or are on not sure - like hose, feasible to be developed as an alternative tea rich in antioxidant.

#### 5. Recommendation

Given that gaharu today are increasingly scarce, but potentially exploited more widely, especially the leaves, is expected to intensify gaharu cultivation, especially the species *W.tenuiramis* Miq.

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