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Phytochemical and antioxidant activity of gaharu leaf tea (*Aquilaria malaccensis* Lamk) as raw material of tea from middle Tapanuli Regency, North Sumatera Province

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Abstract. Gaharu (agarwood) grows naturally in Sumatera, especially in Middle Tapanuli regency. The development of knowledge and research about gaharu leaf has been used as raw material for brewed beverage or tea. The purposes of this research were to observe the leaf used as raw material of gaharu leaf tea that grows naturally in Middle Tapanuli by looking at the chemical compound contained in the leaf which function as antioxidant and to know the antioxidant activity and tannin content of the leaf. Gaharu leaf from 2 growing sites (Sigiring-giring village and S. Kalangan II village) was identified, then processed into leaf simplicia and extracted with 96% ethanol solvent. Phytochemical screening of alkaloid, glycosides, steroids/triterpenoids, flavonoids, tannins and saponins compounds were conducted. The antioxidant activity test was performed by DPPH method to obtain IC₅₀ (Inhibitory Concentration) value. The results showed that the agarwood leaf from Sigiring-giring contained terpenoids and saponins and from S. Kalangan II contained of tannins and saponins. The results of antioxidant activity test showed that agarwood leaf extract has IC₅₀ value in 56,985 dan 44,382 µg/ml which meant strong and very strong antioxidant activity category.

1. Introduction

The distribution of agarwood trees in Indonesia are Sumatera, Java, Kalimantan, Sulawesi, Maluku, Papua and Nusa Tenggara. Agarwood is a resin obtained from microbial infection of trees from Thymeleacea, Leguminosae and Euforbiaceae families. Gaharu grows naturally in nature and endemic in North Sumatera especially in Middle Tapanuli regency. The experiment of agarwood leaves utilization has been done based on chemical compound content of flavonoids group namely flavone, flavonol and isoflavon. The leaves are also used as a brewed drink that act as an antioxidant.

Reference [1] explained that flavonoids, terpenoids and phenol compounds were estimated to have activity as anti-free radical (antioxidants). Natural antioxidants were scattered in some parts of plant, such as in wood, bark, roots, fruits, flowers, seeds and leaves [2]. And leaves are a source of abundant antioxidants.

Research and utilization of agarwood leaves as a source of antioxidants within tea product has developed. Today, the agarwood harvested from nature is still continuing in several regions. The



research conducted was to try to utilize agarwood leaves from nature as raw material for tea. The purpose of this study was to determine the feasibility of agarwood leaves that growing in Middle Tapanuli regency as raw material for tea by testing the chemical compounds and the antioxidant activity contained in agarwood leaves from two growing sites namely Sigiring-giring village and S. Kalangan II village.

2. Materials and Methods

2.1. Location

The sample used in this study is agarwood leaves from the identification result is a type of *A. malaccensis* Lamk from Sigiring-giring and S. Kalangan II Villages which grows naturally. For phytochemical screening conducted at the Natural Material Chemistry Laboratory, Faculty of Mathematics and Natural Sciences and antioxidant testing were conducted at the Research Laboratory, Faculty of Pharmacy, while the determination of water content and extraction were conducted at Forest Product Technology Laboratory, Faculty of Forestry and the Tannin test were conducted at Food Chemical Analysis Laboratory, Faculty of Agriculture, University of North Sumatra.

2.2. Procedure

2.2.1. Sample collection. The sample used in this study is agarwood leaves from the identification result is a type of *A. malaccensis* Lamk from Sigiring-giring and S. Kalangan II Villages which grows naturally.

2.2.2. Raw material preparation. Gaharu leaves were cleaned of dirt with flowing water, then distribute on parchment paper until the water was absorbed. The plant materials were done by unnatural drying dried in the drying cupboard to dry and brittle the leaves with temperature 40°C-50°C. The purpose was to get the simplicia that was not easily damaged, so it can be stored for a long time. The dried leaves were grinded using a blender and placed shielded from the sun before extraction and testing activities.

2.2.3. Determination of water content. The determination of water content was done by Gravimetri method.

2.2.4. Phytochemical screening. Phytochemical screening is a qualitative chemical examination of biologically active compounds presenting within simplicia and plant extracts, organic compounds. Therefore, screening is primarily intended for groups of organic compounds such as alkaloids, glycosides, flavonoids, steroids/terpenoids, tannins and saponins.

2.2.5. Preparation of ethanol extract of the leaves (*A. malaccensis* Lamk). The preparation of the extract was carried out maceration with 96% ethanol solvent, as much as 200 g of simplicia (dust) inserted into a glass vessel, poured with 1500 ml of 96% ethanol, covered the glass, left for 5 days shielded from light and occasionally stirred. After 5 days, the mixture was filtered. The dregs were washed with 96% ethanol sufficiently to obtain 2,000 ml, then removed in a closed vessel and left in place shielded from light for 2 days, then filtered. The macerate was concentrated using a rotary evaporator device at 40 °C until a concentrated macerate was obtained and then dried using a freeze dryer to obtain a dry extract.

2.2.6. Antioxidant activity test. Antioxidant activity test of leaves water extract was evaluated by Free Radical Scavenger using DPPH as stated in [3] with slight modification. Ascorbic acid was used as positive control. The measurement of absorbance was performed by UV-Vis spectrophotometer at 516 nm wavelength.

3. Results and Discussions

3.1. Determination of water content

Water content is closely related to the quality of simplicia. The determination of water content is useful for presuming the durability or resilience of samples in storage.

The water content of agarwood leaves after drying with direct sunlight for three days and then drying in rack can be seen in Table 1. The result has fulfilled SNI requirement that is not exceeding maximum 12%. The result also meets the POM standard that the water content of the dried leaves do not exceed 10% [4].

Table 1. Water content of agarwood leaves

Gaharu Leaves Location	Water Content (%)
Desa Sigiring-giring	7.942
S. Kalangan II	8.713

In Table 1 the water content of simplicia 7.942 and 8.713%. Drying under direct sunlight was usually fluctuating influenced by the daily temperature. To anticipate the uniform result, after three days of drying in the direct sunlight was followed by drying in the drying rack. Moisture content affected the quality of dry tea because it will affect the storage time. According to [5], the factors that affect to the quality of food products are the water content in the product.

3.2. Determination of tannin content

High-low levels of tannin content were influenced by the amount of extract in water (tea) because the colloid would form if tannin was dissolved into water and made a sour and bitter taste on agarwood leaves. Value of tannin content was seen in Table 2.

Tannin is a polyphenol compound that can be complex with proteins forming copolymers. Tannin is present in leaves, immature fruit. Tannin is an active compound of plants belonging to flavonoid group that has a bitter sense on food [6]. Tannin compounds are belonging to the flavonoid compound [7]. Tannin is a phenol compound that has a large molecular weight. Tannin is consisting of two types of hydrolysed tannins and condensed tannins. Tannins have an acidic and spicy taste, as well as giving color to tea. Tannins cannot crystallize and can precipitate solution proteins [8].

Table 2. Tannin content of agarwood leaves

Gaharu Leaves Location	Tannin Content (%)
Desa Sigiring-giring	3.1335
S. Kalangan II	3.1911

Tannin on *Camellia sinensis* tea was 1.21% and tannin content of two types of agarwood leaves was higher than *C. sinensis*. Fermentation is a process that resulted in chemical changes in tea caused by enzymes [9]. During the fermentation process, the oxidation of cells released during milling with oxygen happened, in the presence of an enzyme that acts as a catalyst. The longer the fermentation done on gaharu leaves, the greater the decrease in tannin content in agarwood leaves. The decrease of tannin was caused by tannin oxidation with polyphenol enzyme involvement. The high levels of tannin in 2 types in this study due to the absence of fermentation treatment performed on the leaves, for example by rolling agarwood leaves.

3.3. Phytochemical screening results

Gaharu (agarwood) leaves research was conducted to increase the benefits of the tree. Phytochemical screening is a preliminary study to find out secondary metabolites within plants. Phytochemical screening was conducted on fresh agarwood leaves simplicia dust to obtain information of secondary metabolite compounds class contained in the leaves. The results of phytochemical screening can be seen in Table 3.

The results in Table 3 showed that the phytochemical screening test on agarwood leaves from two growing sites has a different content of chemical compounds. Reference [10] showed that the phytochemical screening of simplicia of agarwood leaf from Bahorok Langkat has a class of secondary metabolites, namely flavonoids, glycosides, tannins and steroids / triterpenoids which have the potential as antioxidants. The difference of the chemical content of the agarwood leaves from Middle Tapanuli was caused by the tree grown naturally in nature that has still at the stage of growth poles, not yet formed their secondary metabolites.

The results obtained in Table 3 showed that phytochemical screening test have any differences in chemical compound group content on 2 growing sites of agarwood. Beside, *Grynops versteegii* also contains secondary metabolite compounds of flavonoids, terpenoids and phenolic compounds [1].

Table 3. Phytochemical screening of agarwood leaves

Compounds	Village	
	Sigiring-giring	S Kalangan II
Alkaloids	-	-
Flavonoids	-	-
Tannins	-	+
Saponins	+	+
Triterpenoids	+	-

The result of phytochemical screening gave the important information about the chemical compound contained by gaharu leaves. Screening the chemical compounds would facilitate the determination of usage, especially in the advanced utilization, mainly for treatment. Cytotoxic activity depending on the dose observed in gaharu fraction shows the potential therapeutic usefulness of this medicinal plant against cervical cancer [11]. *A. malaccensis* leaves showed the ability to increase glucose uptake by increasing levels of GLUT4 in skeletal muscle. Further experiment is needed to explore this gaharu leaves as a strong antidiabetic [12].

3.4. IC_{50} (inhibitory concentration) value of tested sample (antioxidant activity)

The amount of antioxidant activity characterized by IC_{50} value was the concentration of the sample solution needed to inhibit 50% of DPPH free radical [13]. IC_{50} values were obtained based on the calculation of the linear regression equation obtained by plotting concentration of tested solution and DPPH scavenger percentage as a parameter of antioxidant activity, where the concentration of tested solution (ppm) as X-axis and the percentage of scavenger as Y-axis. The strength of antioxidant activity which categorized based on the IC_{50} value was referred to [14] which tabulated in Table 4.

The capability of the tested sample in scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) as free radicals in methanol solution with IC_{50} values (concentration of the sample which capable of scavenge free radicals by 50%) was used as a parameter to determine the antioxidant activity of the sample [15]. IC_{50} value of the leaves ethanol extract was listed in Table 5.

Table 4. Strength category of antioxidant activity

Category	Concentrations (mcg / ml)
Very strong	<50
Strong	50 -100
Moderate	101-150
Weak	151-200

Table 5. The IC₅₀ value of agarwood leaves ethanol extract (µg/ml)

Village	Antioxidant Activity
Sigiring-giring	56.985 µg/ml
S. Kalangan II	44.382 µg/ml

The strong or weak antioxidants are determined by several factors, one of which is the chemical composition. The chemical composition is also influenced by its habitat [16]. The main compounds that cause strong antioxidants are phenol group compounds, such as flavonoids. Ethanolic extract of the leaves have classes of secondary metabolites namely flavonoids, glycosides, tannins and steroids/triterpenoids that potential as antioxidants.

The agarwood leaf tea has a very strong antioxidant content compared to others. The results of the analysis of the highest antioxidant activity from ten steeping types of Indonesian black tea quality were obtained by Dust I quality, with IC₅₀ value of 97.00 µg/ml, while for the lowest antioxidant activity with IC₅₀ value of 178.56 µg/ml obtained by BTL quality [17]. This antioxidant content is an important thing that must be considered in processing the agarwood leaf tea.

Reference [18] explains that various plant commonly consumed in Indonesia contain antioxidants. The research result of gaharu leaf tea has high antioxidant content that if consumed then be one source of antioxidant body.

4. Conclusion

The gaharu leaf from Sigiring-giring village contained terpenoid and saponin and the gaharu leaf from S. Kalangan II contained tannin and saponin. The results of antioxidant activity test showed that gaharu leaves extract from Sigiring-giring and S. Kalangan II have IC₅₀ value in 56,985 and 44,382 µg/ml, respectively, with strong and very strong antioxidant activity category.

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