Chemical Compounds and Antioxidant Potential in Hot Water Extract of Cultivated Agarwood (Aquilaria malaccensis) Lamk Leaves

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Abstract. Agarwood (Aquilaria malaccensis Lamk) leaves has potential as a source of antioxidants because in the hot water extract contains secondary metabolite compounds. This research aims to observe the chemical compounds and antioxidant potential in hot water extract of cultivated agarwood leaves. Agarwood leaves are processed into simplicia, and extracted with hot water. Several tests conducted in this research, including phytochemical screening in order to observe the chemical compounds in hot water extract of agarwood leaves; GC-MS test to observe the chemical constituents, and DPPH method to observe the antioxidant activity. Agarwood leaves hot water extract positively contained of flavonoid, triterpenoid, tannin and glycoside compounds. The identification result of agarwood leaves hot water extract using GC-MS test showed 9 compounds, they are Undecane, Benzene 1,3-dichloro-2-methoxy-, 1.alpha.- (Angeloxy)-6.beta-(isobutyroxy)-9-oxo-10.alpha.Hfuranoeeromiphaline, Methyl (6-Methyl-3-pyradazinyl) Ketone, 1-Heptyl-1H-(1,2,3)-triazole-N-{2'-(hydroxyethoxy)ethylamino] ethyl}-4-carboxamide, 1-Heptyl-1H-(1,2,3)-triazole-N-{2'-(hydroxyethoxy)ethylamino[ethyl]-4-carboxamide, Borane. [2-(dimethylphenylsilyl)-1, ethyl-1-propenyl]diethyl-, (E)-, Hexadecanoic acid, methyl ester, and 1-Phenyl-5,5-dimethyl-4,6-dioxo-5-sila-8-nitrostil-1-ene. The antioxidant activity of agarwood leaves hot water extract is categorized as the very strong antioxidant activity.

Keywords: agarwood leaves, hot water extract, compounds, antioxidant activity

1. Introduction

As a non-timber forest product, agarwood found in nature has a lot of impacts for society, culture, economy, and environment. Now, the potency of agarwood in nature has been decreased and required to develop cultivation [1].

In this sophisticated era, agarwood leaves not only used in perfumery and cosmetics, moreover agarwood also used as herbal medicine to treat various diseases [2]. The beneficial part of agarwood is the leaf. One of agarwood leaves that has been used by community in tea form is A. malaccensis Lamk., that helps to reduce fatigue and get our energy back [3].

Besides delicious taste, agarwood tea is also good to drink in cold or rainy weather which helps to boost our immune. The other benefits are relieve headache, improve man’s stamina, prevent colds, soothe stomachache,
etcetera [4]. The utilization of agarwood leaves as drinking tea is supported by chemical compounds in agarwood leaves [5]. The experiment of agarwood leaves utilization has been done based on chemical compound of flavonoids group namely flavone, flavonol and isoflavone [6]. A number of compounds found in agarwood leaves using phytochemical screening are alkaloid, terpenoid, saponin and tannin [7]. Phytochemical screening on the brew and kombucha of agarwood leaves are positive containing phenolic compound, flavonoid and tannin [8].

Methanol extract and agarwood water extract provide antioxidant activities [9]. The extractive substance in water soluble are sugar, pigment agent, gum, tannin and starch [10]. Tannin existence in the extract result contributes to the agarwood leaves antioxidant activity because tannin is categorized as phenolic compound. The tannin content in agarwood A. malaccensis Lamk from Laru Village, Mandailing Natal Regency North Sumatera Province is 5.62 % [11].

Gaharu wood not only obtained from the direct nature, now can be cultivated in plantation crops. Gaharu A. malaccensis were planted by the community in Langkat and Deli Serdang District Bahorok Sub-district [12], is one of the cultivating area in Langkat. Based on the explanation above, a research is needed to be conducted to observe chemical compounds contained in cultivated agarwood A. malaccensis Lamk leaves from Bahorok, Langkat District and its antioxidant activity, particularly in the agarwood leaves hot water extract. There has been no research until this present time related to the chemical compounds found in hot water extract of cultivated agarwood leaves specifically grown in Pekan Bahorok Village, Bahorok Sub-district, Langkat District. The data obtained is important information which will be used as a reference for future use, especially the antioxidant content. Considering the potential of cultivated agarwood leaves is very abundant.

2. Research Method

2.1. The Time and Place of the Research.
This research had been conducted since February until August 2020. The antioxidant assay at Research Laboratory of Faculty of Pharmacy, Universitas Sumatera Utara (USU), while the extraction at Forest Products Technology Laboratory, Faculty of Forestry, USU and GC-MS assay at Bea Cukai Laboratory Medan.

2.2. The Sample of Plant.
The sample of plant used agarwood leaves cultivated by farmers in Pekan Bahorok Village, The Sub-district of Bahorok, The District of Langkat, North Sumatera.

2.3. The Making of Simplicia.
Agarwood leaves were washed with flowing water until it is properly clean. Drain it well, then dry it with oven drying at 40°C. After that, the simplicia is powdered using a blender. Store it into a clean container and close tightly.

2.4. The Extraction of Agarwood Leaves with Hot Aquadest Solvent by Applying Maceration Method.
The extraction was processed by maceration method [13] with hot water solvent. Fill the glass container with 200g of powder simplicia, then pour 1500 ml of hot aquadest, close the lid tightly. Leave it on for 5 days, keep it away from the lights and stir it once in a while. After 5 days, filter the mixture. Wash the dregs with hot aquadest until it reaches 2000 ml, then move it into closed vessel and place it in cool temperature, keep it dark for a couple days. After 2 days, pour and filter it. Maceration is concentrated using rotary evaporator at 40°C until it forms concentrated maceration, then dry it with freeze dryer until the extract is completely dry.

2.5. Phytochemical Screening.
Phytochemical screening conducted by the [14] [15]. Screening was done on the hot water extract, including the experiment on the biological active compounds and the organic compounds, such as alkaloid, glycoside, flavonoid, steroid/terpenoid, tannin and saponin.
2.6. The Identification of Active Compounds in Extracts with GC-MS Test.
The compounds identification used Gas Chromatography-Mass Spectrometry (GC-MS) to be done to the hot water extract of agarwood leaves. There was 0.5 gram of extract injected to the GC-MS system. The instrument type was GCMS 7890B.

2.7. The Analysis of Antioxidant Activity Using DPPH Assay.
Experiment procedures referred to [16] [17] methods, including: making 1 mM DPPH solution, determining the maximum wavelength, determining the optimum incubation time, making blank solution, making Vitamin C calibration standard (positive control), making testing solution and antioxidant test with 2,2-diphenil-1-pikrilhidrazil (DPPH). The following step is calculating the percentage of IC$_{50}$ value (Inhibition Concentration 50) by obtaining the line between 50% of the inhibitor and the concentration axis with the equation $y = ax + b$, $y = 50$ and $x$ is the concentration of dilute solution that capable inhibiting 50% of free radical solution 2,2-diphenil-1-pikrilhidrazil (IC$_{50}$).

3. Result and Discussion

3.1. The Phytochemical Screening Result
Phytochemical screening towards agarwood leaves hot water extraction conducted to obtain the information refers to secondary metabolite compounds contained in the extraction. The results of phytochemical screening towards cultivated agarwood *A. malaccensis* Lamk in Table 1.

| Compound       | Hot Water Extract |
|----------------|-------------------|
| Alkaloid       | -                 |
| Flavonoid      | +                 |
| Tannin         | +                 |
| Saponin        | -                 |
| Triterpenoid/steroid | +       |
| Glycoside      | +                 |

Details: + = positively contain compound, - = no compound found

Agarwood leaves hot water extraction contains flavonoid, tannin, triterpenoid/steroid and glycoside. Screening technique is capable to assist phytopharmacology stages that denotes the initial selection of the plant examination to prove the existence of particular chemical compounds of the plant and appertained to its biological activity [15]. Agarwood leaves tea contains phenolic compound, flavonoid and tannin, with total phenolic level 28.524±0.359 mgGAE/gram [8]. Phytochemicals screening towards agarwood that all of leaf types extracted with both of ethanol and water extract were indicated presence of alkoloids and carbohydrate [18].

3.2. The Compound Identification Using Gas Chromatography Mass Spectrometry (GC-MS)
The compound identification contained in agarwood leaves hot water extraction referred to the spectrogram data obtaining fragmentation patterns of each compound (Figure 1). The identified compound of agarwood leaves hot water extraction are 9 compounds, compound names, formula, and structures are stated in Table 2.
The identified compounds are categorized as phenolic compound based on the structures. Furthermore, the identified acid group is hexadecanoic acid compound. This compound denotes as dominant compound found in cultivated agarwood *A. malaccensis* Lamk leaves. The acidic compound in agarwood *A. malaccensis* Lamk naturally grown in Laru District is octadecanoic acid (CAS) stearic acid [19].

**Figure 1.** The Chromatogram of GC-MS Test towards Agarwood Leaves Hot Water Extract

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**Table 2.** The Compound Identification Result of Cultivated Agarwood *A. Malaccensis* Lamk Hot Water Extract

| No. | Retention Time (Rt) | Compound Name | Formula | Structure |
|-----|---------------------|---------------|---------|-----------|
| 1.  | 3.216               | Undecane      | C₁₁H₂₄  | ![Undecane](undecane.png) |
| 2.  | 5.858               | Benzene, 1,3-dichloro-2-methoxy- | C₇H₆Cl₂O | ![Benzene](benzene.png) |
| 3.  | 6.431               | 1.alpha-(Angeloxy)-6.beta- (isobutyroxy)-9-oxo-10.alpha.Hfuranoeremophilane | C₂₆H₃₀O₆ | ![Furanophenol](furanophenol.png) |
| 4.  | 7.631               | Methyl (6-Methyl-3-pyridazinyl) Ketone | C₇H₈N₂O | ![Ketone](ketone.png) |
5. 7.742  1-Heptyl-1H-(1,2,3)-triazole-N-[[2'-(hydroxyethoxy)ethylamino]ethyl]-4-\text{-} Carboxamide  \text{C}_{14}\text{H}_{27}\text{N}_{5}\text{O}_{2}

6. 9.238  1-Heptyl-1H-(1,2,3)-triazole-N-[[2'-(hydroxyethoxy)ethylamino]ethyl]-4-\text{-} Carboxamide  \text{C}_{14}\text{H}_{27}\text{N}_{5}\text{O}_{2}

7. 10.384  Borane, [2-(dimethylphenylsilyl)-1-ethyl-1-propenyl]diethyl, (E)-  \text{C}_{17}\text{H}_{29}\text{Bsi}

8. 10.698  Hexadecanoic acid, methyl ester  \text{C}_{17}\text{H}_{34}\text{O}_{2}

9. 11.843  1-Phenyl-5,5-dimethyl-4,6-dioxo-5-silaocta-8-nitrooct-1-ene  \text{C}_{13}\text{H}_{19}\text{NO}_{4}\text{Si}

3.3. Antioxidant Activity Assay with DPPH
Vitamin C is the standardization of doing the research. The antioxidant activity determined by the compound ability in agarwood leaves extract which leads to decrease the intensity of radical purple DPPH on the maximum wavelength. The decreasing of this DPPH purple intensity due to the lack of chromophore or compound conjugated double bonds caused by the existing extract acts as radicals fighter that will donate the H atom to DPPH into reduced yellowish DPPH-H [20]. The yellow pigment formed after the addition of DPPH used by the hydrogen
atom donated by the compound in agarwood leaves ethanol extract, so that affects the reduced DPPH molecule followed by the missing of purple pigment in DPPH solution.

The result of Vitamin C antioxidant measurement is stated in Table 3. The value in Table 3 shows that vitamin C and agarwood leaves hot water extract have powerful antioxidant activity because of its IC$_{50}$ value less than 50 μg/ml.

Table 3. IC$_{50}$ Value (μg/ml) of Ascorbic Acid and Agarwood Leaves Hot Water Extract

| Ingredient                        | Antioxidant Activity (μg/ml) |
|-----------------------------------|------------------------------|
| Ascorbic acid                     | 2.580 ± 0.020                |
| Agarwood Leaves Hot Water Extract | 27,907 ± 0.629               |

The antioxidant activities are categorized very strong because they have active compounds as antioxidant. The compounds which potential acts as antioxidant can be predicted as phenolic group, flavonoid, and alkaloid (polar compound). Flavonoids are reductors which act as hydrogen donator to free radicals [21]. According to [22] that phenolic compound has its own antioxidant activity. This antioxidant character related to the location of phenolic cluster that donates the hydrogen atom to particular free radical so that it is no longer reactive. The appropriate result on the phytochemical test shows that agarwood leaves hot water extract contains flavonoid, tannin, triterpenoid and glycoside. Tannin and flavonoid are the compounds containing phenolic clusters. The result of phytochemical test towards agarwood A. malaccensis is the content of six compounds including alkaloid, terpenoid, flavonoid, steroid, saponin and tannin [9].

The very strong antioxidant activities are supported by GCMS assay from the nine identified dominant compounds as phenolic group. This result is also supported by the existence of acidic compound, hexadecanoic acid contributing to its antioxidant activity.

Antioxidant activity for all types of Borneo Agarwood leaves extract with hot water extraction (400x dilution) showed a higher percentage than its ethanol. It is mainly due to the fact that boiling water could completely activate the degradative enzymes as against the ethanol solvent. The antioxidant activity correlated with active compounds phytochemicals. Ethanol and water extracts of fresh leaves showed that it may account for its antioxidant in Borneo Agarwood (A. malaccensis Hull). Generally, all of types of Borneo Agarwood leaves (young leaves and mature leaves) extracted with hot water were indicated higher in antioxidant activity than ethanol [18].

4. Conclusion

According to the result of the research, it can be concluded that chemical compounds contained in agarwood leaves hot water extract play a big role for its very strong antioxidant activity with IC$_{50}$ value 27,907 ± 0.629 μg/ml. The result using GC-MS assay showed that cultivated agarwood leaves hot water extraction contains 9 biological active compounds.

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