 Phytochemicals and GC-MS of *Aquilaria beccariana* van Tiegh leaves growing naturally and cultivated in North Sumatera

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Abstract. *Aquilaria beccariana* van Tiegh is one of the agarwood (gaharu) species that grows naturally and cultivated. However, the chemical and antioxidant potential of its leaves have not been studied much. This research aimed to examine the chemical compounds and the antioxidant activity of the ethanol extract of the agarwood leaves *A. beccariana* van Tiegh which grows naturally and cultivated. The leaves were processed into simplicia and then extracted using 96% ethanol as solvent. Phytochemical screening agarwood leaves to determine the chemical compounds such as alkaloids, flavonoids, glycosides, steroids/triterpenoids, saponins and tannins. The antioxidant activity test was carried out using the DPPH method to obtain the IC₅₀ (Inhibitory Concentration) value. The compound tracing used GC-MS. The results showed that the simplicia and the extract of the leaves both natural-grown and cultivated contained secondary metabolites of alkaloids, flavonoids, glycosides, steroids/triterpenoids, saponins and tannins. The antioxidant activity test showed that the ethanol extract of the leaves of *A. beccariana* van Tiegh both natural-grown and cultivated was categorized as very strong. The results of the identification of chemical compounds by GC-MS were identified 18 compounds in the natural-grown leaves, while 40 compounds were identified in the cultivated ones.

1. Introduction

Indonesia has 26 types of agarwood hosts that originated from 33 species in Asia at least. These spread across Sumatera, Kalimantan, Nusa Tenggara, Maluku, Sulawesi, Halmahera, Bangka and Papua [1]. In Indonesia, there are 6 types of agarwood recognized from the Aqualaria genus, namely *A. malaccensis*, *A. microcarpa*, *A. beccariana*, *A. hirta*, *A. cumingiana*, and *A. filarial* [2].

The tree of agarwood is useful not only for its sapwood but its stems, bark, roots, and leaves are potentially used as face care treatment and skim moisture [3]. The research and utilization of agarwood leaves have been developed for a long time. The utilization of agarwood leaves requires a series of research for its future development, especially related to variations in raw materials, especially from the aspects of type and growth place.

In addition to *A. malaccensis* Lamk, *A. beccariana* van Tiegh has also been cultivated as agarwood-producing trees from the Aquilaria genus. *A. beccariana* leaves contain phenolic and
flavonoid chemical compounds and have antioxidant potential with IC50 value of 72.25 ± 0.72 ppm [4].

Exploratory, A. beccariana van Tiegh grows naturally in several places and has been cultivated in the North Sumatera area. Based on that, it is deemed necessary to conduct basic research on what chemical components are contained in agarwood leaves, A. beccariana Van Tiegh., which are natural-grown and cultivated as well as their antioxidant potential.

2. Research Methods

2.1. Time and Locations
This research was conducted from October 2018 - June 2019. The sampling locations were in Pekan Bahorok Village, Langkat Regency, and Tanjung Medan Village, South Labuhan Regency. Identification was carried out at the Herbarium Medanense, Faculty of Mathematics and Natural Sciences, and phytochemical screening test was carried out at the Phytochemical Laboratory of the Faculty of Pharmacy, afterwards, the extraction was carried out at the Laboratory of Forest Products Technology, Faculty of Forestry, Universitas Sumatera Utara. Furthest behind, the GCMS test was carried out at the PT. Dadjaja, Surabaya.

2.2. Procedure

2.2.1. Plant Sampling and Identification. The leaves of agarwood trees which grow naturally in Tanjung Medan village, Labuhan Batu Regency, and cultivatived in Pekan Bahorok village, Langkat Regency were used in this research. The variety identification held in Herbarium Medanense was A. beccariana van Tiegh.

2.2.2. Raw Material Preparation. Agarwood leaves were cleaned from sticks with piped water, then parched until the water is absorbed on parchment paper. The leaves dried in a drying cabinet until brittle ones with a temperature 40 °C. The purpose of this drying is to get dry simplicia, so it can be stored for a long time. Then, the dried leaves were grinded using a blender. The simplicia mill was put into a container that was protected from the sun before the extraction process was carried out.

2.2.3. Extraction of agarwood leaves with an ethanol solvent. The extract was made by maceration with 96% ethanol as solvent, 200 g of simplicia powder was put into a beaker, poured with 1500 ml of 96% ethanol, closed, left for 5 days protected from light and occasionally stirred. After 5 days the mixture was filtered. The dregs were washed with sufficient 96% ethanol to obtain 2000 ml, then transferred in a closed vessel and left in a cool place protected from light for 2 days, then poured and filtered. The macerate was concentrated using a rotary evaporator at 40 °C to obtain concentrated macerate and then dried using a freeze dryer.

2.2.4. Phytochemical screening. Phytochemical screening is a qualitative chemical examination compounds contained in extracts and simplicia plant. These compounds are organic compounds, therefore screening is mainly aimed at groups of organic compounds such as alkaloids, flavonoids, tannins, glycosides, steroids/terpenoids, and saponins. The test followed the method of [5]-[6].

2.2.5. Identification by GC-MS (Gas Chromatography-Mass Spectrometry. The type of tool used in the GC-MS test was Shimadzu GC-MS-QP 2010.

2.2.6. Antioxidant Test with DPPH method. The powder of plant extracts to scavenge DPPH free radicals was specified based on [7]-[8] methods.
3. Results and Discussion

3.1. Phytochemical Screening

A phytochemical test showed that there was no difference in chemical content between simplicia and extract of A. beccariana leaves (Table 1). However, there was a difference in content between natural-grown and cultivated. The natural grow of A. beccariana leaves did not contain glycoside compounds, but cultivated did.

Glycosides are complex substances containing sugars found in some plants. Glycosides are usually β-glycosides form in [9]. Many glycoside classification systems have been carried out. Some of these classifications are based on the sugar group and some are based on the aglycone group [10].

| Chemical compound | Natural-grown Leaves Simplicia | Leavess | Cultivated Leaves Simplicia | Leaves Extract |
|-------------------|--------------------------------|--------|----------------------------|--------------|
| Alkaloid          | +                              | +      | +                          | +            |
| Flavonoids        | +                              | +      | +                          | +            |
| Glycoside         | -                              | -      | +                          | +            |
| Saponins          | -                              | -      | -                          | -            |
| Steroids/Triterpenoids | + | + | + | + |
| Tannins           | +                              | +      | +                          | +            |

Description: + = contains the compound being tested, - = doesn’t contain the compound being tested.

3.2. GC-MS Identifications

GC-MS identifications resulted in various chemical compounds contained in agarwood leaves, A. beccariana, both natural-grown and cultivated. There were 18 compounds found in the natural-grown type (Table 2) and 40 compounds found in the cultivated type (Table 3). The compounds are more diverse in the cultivated.

The main compound found in natural-grown of A. beccariana was Neophytadiene with concentration 22.61%. The other two compounds were n-Hexadecanoic acid (11.13%) and Hexadecanoic acid, ethyl ester (11.39%). Hexadecanoic acid, methyl ester and Neophytadiene are the main compounds found in agarwood leaves of A. malaccensis Lamk [11].

| Peak | Retention Time | Concentration (%) | Name of Compounds |
|------|----------------|-------------------|-------------------|
| 1    | 2.584          | 0.56              | Silane, methyl-(CAS) |
| 2    | 2.769          | 2.99              | Ethane, 1,1-dioxy-(CAS) |
| 3    | 2.880          | 4.76              | 1,2,3-Propanetriol (CAS) |
| 4    | 4.531          | 1.08              | Phenylethyl Alcohol |
| 5    | 5.001          | 2.37              | 1,3-Dioxolane, 4-ethyl-(CAS) |
| 6    | 11.977         | 1.69              | (7R,8R)-cis-syn-trans-Tricyclo |
| 7    | 12.844         | 1.81              | Methanesulfonamide, N,N-dimethyl- |
| 8    | 13.537         | 4.69              | Neophytadiene |

Table 2. Identification results of chemical compounds contained in the leaves of A. beccariana van Tiegh natural-grown with GC-MS.
The main compounds found in cultivated A. beccariana species are 1,2,3,4-Cyclopentanetetrol, (1.alpha.,2.beta.,3.beta.,4.alpha.)- (CAS) 1,2,3,4- with a concentration of 8.08%. The other compounds were Oxacycloheptadec-8-en-2-one (CAS) Ambretolide (5, 97 %) and 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0] nonane (5,00 %). A lot of compounds were found in cultivated agarwood leaves, the average concentration of these compounds was low. Neophytadiene compound with a low concentration of 1.70%.

Table 3. Identification results of chemical compounds contained in the leaves of A. beccariana cultivated type with GC-MS

| Peak | Retention Time | Concentration % | Name                                           |
|------|----------------|-----------------|------------------------------------------------|
| 1    | 5.603          | 4.40            | Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro-   |
|      |                |                 | (CAS) 1,1-Dibromo-2-Chloro                      |
| 2    | 6.100          | 0.64            | Carbon dioxide (CAS) Dry ice                   |
| 3    | 6.267          | 0.43            | Nitrogen oxide (N2O) (CAS) Nitrous oxide       |
| 4    | 6.449          | 0.34            | Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro-   |
|      |                |                 | (CAS) 1,1- Dibromo-2-Chloro                     |
| 5    | 6.617          | 0.26            | 2-Propynoic Acid                               |
| 6    | 8.417          | 0.37            | Acetic acid (CAS) Ethylic acid                 |
| 7    | 13.871         | 3.04            | Cyclohexanone (CAS) Anon                       |
| 8    | 14.517         | 0.39            | dl-Limonene                                    |
| 9    | 14.859         | 1.73            | Phenol (CAS) Izal                              |
| 10   | 15.167         | 1.23            | 2-Cyclopenten-1-one, 2-hydroxy-3-methyl-       |
|      |                |                 | (CAS) Corylon                                  |
| 11   | 15.767         | 0.69            | Phenol, 2-methoxy- (CAS) Guaiacol              |
| 12   | 16.099         | 3.62            | Cyclopropyl carbinol                           |
| 13   | 17.023         | 1.39            | Oxirane, hexyl                                 |
| 14   | 17.392         | 1.37            | Xanthosine (CAS) Xanthine riboside            |
| 15   | 17.577         | 4.68            | 2,3-Dihydro-Benzofuran                        |
| 16   | 18.173         | 3.62            | Phenol, 4-ethenyl-2-methoxy-                  |
| 17   | 18.517         | 3.19            | Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol |
3.3. Antioxidant Activity of Ethanol Extracts of A. beccariana van Tiegh. Leaves
The choice of the DPPH method in determining antioxidant activity was due to the ease and availability of tools and materials for the test. The choice of method was also based on previous research. The results of the antioxidant activity test showed that the natural-grown and cultivated agarwood leaves of A. beccariana species had very strong antioxidant activity, similar to the control in this case ascorbic acid (Table. 4).
Table 4. Antioxidant activity of ethanol extract of agarwood leaves (ppm) type A. beccariana van Tiegh has grown naturally and cultivated and ascorbic acid

| Materials                | IC$_{50}$ (ppm) Value | Antioxidant Activity Strength Categories |
|--------------------------|------------------------|----------------------------------------|
| A. beccariana (Cultivatively) | 27,1780                | Very strong                            |
| A. beccariana (Naturally)   | 27,6491                | Very strong                            |
| Ascorbic acid              | 2,5779                 | Very strong                            |

The potential of antioxidant compounds can be predicted from the phenolic, flavonoid and alkaloid groups which are polar compounds [12]. Most of the antioxidant compounds derived from plant sources have wide variations and chemical properties [13]. Phytochemical screening of agarwood leaves type A. beccariana which grown naturally and cultivatively contained flavonoid compounds. The flavonoid group has the ability to transform to produce compounds with higher activity with antioxidant activity [14]. The higher the flavonoid content, the higher the antioxidant potential [15]. In addition to agarwood leaf compounds of the type A. beccariana which grow naturally and cultivatively, it also contain tannins and steroid/triterpenoid compounds that play a role in determining antioxidant activity.

The main compound in agarwood leaves of the type A. beccariana that grows naturally is the terpenoid group, namely Neophytadiene [16]. The main compounds found in cultivated agarwood species were 1,2,3,4-Cyclopentanetetrol, (1.alpha.,2.beta.,3.beta.,4.alpha.)- (CAS) 1,2,3,4- was a cyclic compound with a hydrogen bond donor potential of a count of 4.

Antioxidants can scavenge free radicals in the body, which are obtained from body metabolism, air pollution, food contamination, and sunlight, and so on [17]. Various plants commonly consumed in Indonesia contain antioxidants, the results of research on agarwood leaves tea type A. beccariana which grow naturally and cultivatively have a high antioxidant content so that if consumed, it becomes one of the body's sources of antioxidants.

Various plants commonly consumed in Indonesia are sources of antioxidants [18] such as tannins, saponins, and triterpenoids [19].

4. Conclusion

Agarwood leaves of the type A. beccariana that grow naturally and cultivated contain compounds that have the potential as antioxidants, namely flavonoids, triterpenoids, and tannins. The main compounds in A. beccariana that grow naturally are Neophytadiene compounds and compounds 1,2,3,4-Cyclopentanetetrol, (1.alpha.,2.beta.,3.beta.,4.alpha.)- (CAS) 1,2,3,4- in A. beccariana that grow cultivated.

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