Taking essential oil by water distillation and antibacterial activity test of refined water from agarwood plant parts (*Aquilaria malaccensis* Lamk)

M Nurminah1*, R Batubara2, T Ismanelly3 and Albert2

1Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia.
2Faculty of Forestry, Universitas Sumatera Utara, Medan, Indonesia.
3Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia.

E-mail: *miminurminah@usu.ac.id or mimisinaga@yahoo.co.id

**Abstract.** Agarwood distilled water is a by-product produced during water distillation from wood containing resin in *Aquilaria* sp. Distilled water contains a small fraction of aromatic compounds obtained from plants during the distillation process which collects in distilled water. This research is expected to provide information about the potential of agarwood plant parts. The methods were raw material preparation, determination of water content, making distilled water, phytochemical screening and antibacterial activity test. The results were water content (average value 1.98-5.29 %) has met the BPOM RI standards, we met glycosides in leaf, trunk, skinned stems, bark. Parts of agarwood has antibacterial activity against *Streptococcus* mutants bacteria.

1. **Introduction**

Non-timber forest products are one of the forest resources that have a comparative advantage and are in direct contact with forest communities, for example, agarwood (gaharu) [1]. Apart from essential oils, distillates (residual) from the refining process of agarwood have been used by the community. The used waste of aloe that has been distilled is used for incense and materials for religious ceremonies, while the distilled water of agarwood is used for health, beauty, fitness, and drinking (coffee) by the people of Berau Regency [2].

Agarwood distilled water (hydrosol) is a by-product produced during water distillation from wood containing resin in *Aquilaria* sp. This by-product is unique as it has the aroma of essential oils from *Aquilaria* sp. Even after 1 year of storage. Generally, distilled water contains a small fraction of aromatic compounds obtained from plants during the distillation process which collects in distilled water [3].

2. **Materials and methods**

This research was conducted October 2019 to February 2020. Sampling was carried out at the location of agarwood tree planting in Bahorok, Langkat Regency, North Sumatra Province. Sample preparation and measurement of moisture content were carried out at the Research Laboratory, Forest Products Technology Laboratory, Faculty of Forestry. Testing of essential oil yield, manufacture of distillate water, and phytochemical screening of parts of the agarwood plant was carried out at the Phytochemical Laboratory and Research Laboratory, Faculty of Pharmacy, University of North...
Sumatra. Testing for antibacterial activity was carried out at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, University of North Sumatra.

The tools used in this research include laboratory glass tools (measuring cup, funnel glass, Erlenmeyer, test tube, and dropper), glass bottles, zinc scissors, digital balance, an oven, a set of soxhlet extractor, a set of a distilled kettle, Petri dishes, tube racks, blenders, desiccators, evaporative plates, callipers, buckets, hot plates, and sacks. The materials used are parts of the agarwood plant (Aquilaria malaccensis Lamk.) In the form of leaves, stems (agarwood stems formed by inoculation), barkless stems (agarwood stems formed by barking the stems), and bark. Streptococcus mutants culture, Mueller Hinton Agar (MHA) media, 70% alcohol, gloves, and masks. The materials used for extraction with Soxhlet are n-hexane solvent and filter paper, while distillate water for agarwood is used to solvent distilled water.

2.1. Research procedure

2.1.1 Raw material preparation. The raw material was cleaned of dirt, the leaf samples were washed under running water while the other samples were cleaned with a dry cloth. The samples were then dried for ± 4 hours in the sun and then dried indoors for several days. Specifically, the leaf samples were dried in a drying cabinet. The sample to be tested first is cut into pieces and then blended until smooth.

2.1.2 Determination of water content. The empty plates were dried in an oven at 105°C for 15 minutes and cooled in a desiccator and then weighed. 1 gram of simplicia is weighed into a dish, then placed in an oven at 105°C for 24 hours or until the weight is constant. The plate containing the simplicia was cooled in a desiccator and then weighed.

2.1.3 Making distilled water. The manufacture of agarwood distilled water is carried out using the hydrodistillation method or water distillation according to [4], namely by boiling the sample together with water in a closed container. The distillation is carried out with a 1:10 ratio between the raw material and the solvent so that the material is completely submerged in water but not too full, where the solvent used is distilled water. A total of 100 grams of sample to be distilled into the kettle then mixed with 1 litre of distilled water, the kettle is closed, locked, and placed on an electric heater then connected to a condenser. The condenser is then assembled in such a way as to become a cooling system, then the electric heater is set at 100°C. The distillation is carried out until no more distilled water is obtained from the distillate funnel. After finishing the distillate, it is immediately transferred to a black glass bottle that has been cleaned then tightly closed and stored in the refrigerator. Three replications were made for each type of material.

2.1.4 Phytochemical screening. Alkaloid [5], Triterpenoid [6], Flavonoid [7], Tannin [5], Saponin [5] and Glycoside examination. Distilled water (0.5 ml) was weighed, added 1 ml of 2 N hydrochloric acid and distilled water (9 ml), heated over a water bath (2 minutes). After that, the mixed material cooled and then filtered. The next was alkaloid test, that there were 3 test tubes, then into each test tube, 0.5 ml of filtrate was added.
   a. In tube I, 2 drops of Mayer’s reagent are added, a white or yellow clotted precipitate will form.
   b. In tube II, 2 drops of Dragendorff reagent are added, a brown or brownish-orange precipitate is formed.
   c. In tube III, 2 drops of Bouchard at reagent are added, a brown to blackish sediment will form. We could say alkaloids are called positive if there is sedimentation or turbidity in two or three above experiments.
2.1.5 Antibacterial activity test. The preparation stages include making *Streptococcus mutans* bacterial suspensions, making paper discs, negative control preparations, positive control preparations, sterilizing tools and media, making Mueller Hinton Agar test media, and making concentration series, namely concentrations of 50% and 100% where the 100% concentration is distilled water entirely while the 50% concentration is a mixture of distilled water and distilled water with a ratio of 1:1. The antibacterial activity test used was the Disc Diffusion method (Kirby-Bauer test). The 20 μL suspension of the tested bacteria was put on the media in Petri then rubbed with a sterile cotton swab over the test media. Sterile cotton swabs were twisted several times. Paper discs with a diameter of 6 mm which have been immersed in distilled water with a certain concentration, Positive control in the form of 50 μg ciprofloxacin, and 20% DMSO negative control were placed on the surface of the media according to the desired position. The media was then incubated at 37°C for 24 hours, then measured the diameter of the inhibition zone with the sliding term expressed in millimetres [8].

3. Results and discussion

3.1 Determination of water content

Determination of water content is carried out to determine the water content of the raw materials that will be used for the next stage. Water content has a close relationship with the quality of simplicia produced from raw material. The method used is gravimetric, namely by determining the weight of the sample lost after being placed in the oven for a certain time. Raw materials that have been dried beforehand are blended to taste so that they become simplicia. The average water content of raw material simplicia can be seen in table 1.

| Material Type      | Average (%) | Standard Deviation (SD) |
|--------------------|-------------|-------------------------|
| Leaf               | 1.98        | 0.02                    |
| Trunk              | 3.99        | 1.015                   |
| Skinless Stems     | 5.296       | 0.557                   |
| Bark               | 2.586       | 0.560                   |

The highest simplicia water content is in the stem without bark samples with an average value of 5.296% and the lowest in the leaf samples with an average value of 1.98%. Meanwhile, the samples in the form of stems and bark had an average moisture content of 3.99% and 2.94%, respectively. The water content of all simplicia has met the BPOM RI standards [9], where the simplicia water content must be ≤10%. This is useful for slowing the growth of destructive organisms and for maintaining the quality and longevity of natural materials.

3.2 Phytochemical screening

Phytochemical screening is a preliminary test in determining the class of secondary metabolite compounds that have biological activity from a plant. Plant phytochemical screening is used as initial information in knowing the groups of chemical compounds contained in a plant [10]. The results of the phytochemical screening of distilled water and parts of the agarwood plant (*A. malaccensis* Lamk) can be seen in table 2.
Table 2. Results of phytochemical screening of distilled water from parts of the agarwood plant (*A. malaccensis* Lamk).

| Compound   | Leaf | Trunk | Skinned Stems | Bark |
|------------|------|-------|---------------|------|
| Alkaloids  | -    | -     | -             | -    |
| Flavonoids | -    | -     | -             | -    |
| Glycosides | +    | +     | +             | +    |
| Tannins    | -    | -     | -             | -    |
| Saponins   | -    | -     | -             | -    |
| Triterpenoids | -  | -     | -             | -    |

Description: (+): contains compounds
(-): does not contain compounds

3.3 Antibacterial activity test
The distilled water antibacterial activity test was carried out to determine the antibacterial activity of distilled water from parts of the agarwood plant (*Aquilaria malaccensis* Lamk). The method used is the disc diffusion method (Kirby-Bauer Test) where distilled water from the parts of the agarwood plant will be tested against one of the gram-positive bacteria, namely *Streptococcus mutans*. The results of the test results for the antibacterial activity of distilled water from parts of the agarwood plant (*A. malaccensis* Lamk) against *Streptococcus mutans* bacteria can be seen in Table 3.

Table 3. Data on the antibacterial activity of distilled water from parts of the agarwood plant (*A. malaccensis* Lamk) against *Streptococcus mutans* (mm).

| Material Type | Concentration of Distilled Water | Control (+) | Control (-) |
|---------------|----------------------------------|-------------|-------------|
|               | 50% Standard Deviation (SD)      | 100% Standard Deviation (SD) |            |
| Leaf          | 9.801 0.602                      | 9.89 0.335  | 16.283      |
| Trunk         | 9.06 1.02                        | 8.538 0.394 | 12.35       |
| Bark          | 9.15 0.408                      | 9.331 0.564 | 15.65       |
| TK rod        | 8.785 0.425                     | 8.015 0.753 | 11.716      |

It can be seen that distilled water from parts of the agarwood plant (*Aquilaria malaccensis* Lamk) has antibacterial activity against *Streptococcus mutans* bacteria. In the treatment given, the highest yield was obtained in distilled water from leaf samples with 100% concentration with an average value of 9.89 mm, while the lowest was found in distilled water from stem samples without bark at 100% concentration, which was 8.015 mm.

Referring to the Clinical Laboratory Standard Institute [11], the effect of a type of bacteria on antibacterial substances is said to be resistant (resistant) if the obtained inhibition zone diameter is ≤ 14 mm, medium (intermediate) if the inhibition zone diameter is 15-18 mm and susceptible if the inhibition zone diameter is ≥ 19 mm. Based on the above criteria, it can be seen that the response of *Streptococcus mutans* bacteria to distilled water from parts of the agarwood plant (*Aquilaria malaccensis* Lamk) can all be categorized as resistant. For control (+) in the form of chloramphenicol, the diameter varies from being categorized as resistant to susceptible to *Streptococcus mutans* bacteria.

4. Conclusions
The results were water content (average value 1.98-5.29 %) has met the BPOM RI standarts, we met glycosides in leaf, trunk, skinned stems and bark. Distilled water from parts of the agarwood plant (*Aquilaria malaccensis* Lamk) has antibacterial activity against *Streptococcus mutans* bacteria.
References

[1] Pramana DB, Jumani and Emawati H 2012 Growth of Gaharu (Aquilaria sp.) Plants in Giri Agung Village, Sebulu District, Kutai Kartanegara Regency, East Kalimantan Province 17 August 1945 (Samarinda: University Samarinda)

[2] Siran SA and Turjaman M 2010 Development of Community empowerment-based Agarwood Production Technology (Bogor: Research and Development Center for Forest and Nature Conservation)

[3] Catty S 2001 Hydrosols: The Next Aromatherapy (Canada: Healing Art Press) pp 9-10

[4] Sastrohamidjojo H 2004 Essential Oil Chemistry (Yogyakarta: Gadjah Mada University Press)

[5] Directorate General of POM 1995 Material Medika Indonesia VI (Jakarta: Ministry of Health of the Republic of Indonesia) pp 321-326, 333-337

[6] Padmawinata K and Soediro I Harborne JB 1987 Metode fitokimia [Phytochemical Methods] (Bandung: ITB)

[7] Farnsworth NR 1996 Biological and phytochemical screening of plants Journal of Pharmaceutical Sciences 55 3 p 263

[8] Sari R, Muhani M and Fajriaty I 2017 Antibacterial activity test of gaharu leaf ethanol extract (Aquilaria macrocarpa Baill.) against staphylococcus aureus and proteus mirabilis bacteria PSR 4 3 p 146

[9] National Food and Drug Administration Agency of the Republic of Indonesia 2014 Regulation of the Head of the Food and Drug Administration of the Republic of Indonesia Number 12 of 2014 concerning Quality Requirements for Traditional Medicines (Jakarta: The agency of drug and food control)

[10] Nainggolan M, Ahmad S and Pertiwi D 2019 Guidance and Report on Phytochemical Practicum. Phytochemical Laboratory of the Faculty of Pharmacy (Medan: Sumatera Utara University)

[11] Clinical and Laboratory Standard Institute 2015 Performance Standard for Antimicrobial Disk Susceptibility Tests (USA: CLSI)