GC-MS analysis of young and mature wild agarwood leaves (*Aquilaria malaccensis* Lamk) and its antioxidant potential

R Batubara1*, T I Hanum2 and O Affandi1

1Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Medan, North Sumatra 20155, Indonesia
2Department of Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, North Sumatra 20155, Indonesia

*E-mail: ridwantibb@yahoo.com

Abstract. Agarwood grows wildly in the nature and its leaves may be utilized as raw materials for agarwood tea, especially from *Aquilaria* species. The study was conducted to determine the chemical compounds in the young and mature leaves of wild gaharu (*Aquilaria malaccensis* Lamk). Chemical compounds were detected using pyrolysis gas chromatography mass spectrometry (py-GCMS) while antioxidant capacity of the leaf extracts was assayed using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical method. The results obtained a total of 30 chemical compounds in agarwood leaves extract with different composition between young and mature leaves. The agarwood leaves extracts displayed strong antioxidative capacity with a main compound namely octadecanoid acid or stearic acid.

1. Introduction

Distribution of agarwood-producing trees is relatively wide, including North Sumatra. Mandailing Natal Regency is one of the native region inhabited by agarwood-producing tree species. Existence of agarwood in the area was dominantly produced by *Aquilaria malaccensis* Lamk. Bioprospecting of wild agarwood is focusing on its potential as natural ingredient for herbal raw materials.

Agarwood leaf extract from wild *Wikstroemia tenuiramis* and cultivated *A. malaccensis* had IC50 values of 30.482 and 41.130 µg/mL, respectively and were classified as strong antioxidants [1]. Phytochemical screening of young, mature, and mixture of cultivated agarwood leaves revealed the presence of flavonoids, glycosides, tannins and triterpenoids. Followed by py-GCMS analysis, the chemical compounds in the extracts also displayed potential compounds for pharmaceutical drugs in the form of herbal raw materials [2]. In addition, the chemical content in young and mature leaves of wild *W. tenuiramis* also displayed a very strong antioxidative capacity, containing flavonoids, tannins and triterpenoids [3]. Previous investigation on *A. malaccensis* growing in Mandailing Natal Regency obtained that the leaves extract contained alkaloids, flavonoids, tannins and triterpenoids [4]. The presence of flavonoids, tannins and triterpenoids in a herbal material may indicate a strong antioxidant potential [5].

In this study, we have tried to assess the formulation of a herbal drink based on the agarwood leaves extract. The extracts were a mixture of young and mature wild agarwood leaves to represent the practical utilization of agarwood in other way. The chemical composition of young and mature leaves was determined using py-GCMS followed by antioxidant assay to assess their bioactivity potential.
2. Materials and method

2.1. Plant collection
Plant specimen collected in our study was retrieved from agarwood leaves that grows naturally among rubber stands in the community plantation, Laru Village, North Sumatera. The specimen was authenticated as Aquilaria malaccensis while the young and mature leaves were distinguished based on their coloration (Figure 1).

![Figure 1. Young leave and mature leave](image)

2.2. Sample preparation
Agarwood leaves were cleaned under running tap water. The leaves were placed in a drying cabinet until dry. The dried agarwood leaves were pulverized into simplicia powder using a blender. The simplicia powder is kept in a container with no exposure to sunlight and may be kept for a period of time until extraction process.

2.3. Extraction of agarwood leaves
The extraction procedure refers to [6]. Approximately 200 g of simplicia powder was mixed with 1.5 L of 96% EtOH (v/v) in a 2 L-flask. The flask was covered with aluminium foil and kept for 5 days with no exposure to sunlight and occasionally stirred. After 5 days, the mixture was filtered while the residue was mixed again with 96% EtOH and kept for 2 days. The filtrates were collected as EtOH extracts and concentrated in vacuo (40oC) followed by freeze-drying to obtain dried extracts.

2.4. Py-GCMS analysis
Chemical compounds in young and mature agarwood leaves were determined using an instrument, the pyrolysis GCMS (Shimadzu GCMS-QP 2010, Japan).

2.5. DPPH scavenging activity assay
The potential of plant extracts to scavenge DPPH free radicals was specified on the basis of standard methods [7,8]. The percentages of inhibition of the DPPH free radical were compared to a standard antioxidant (ascorbic acids), for given extract concentrations, and were calculated using the formula:

\[
\text{% Inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

Where, Acontrol is the absorbance of control (ascorbic acid), Asample is the absorbance of plant extract. The absorbance was measured at 516 nm. The IC50 value indicated the concentration of plant extract in scavenging 50% of DPPH compound. The IC50 value was estimated through non-linear regression using MS Excel 2013.
3. Results and discussion

3.1. Chemical compounds in the young and mature leaves of wild agarwood

The chemical compounds contained in the young and mature leaves of wild agarwood *A. malaccensis* are listed in Tables 1 and 2. We documented a total of 30 compounds with different composition between young and mature leaves. There were 13 similar compounds with a main compound namely octadecanoic acid (CAS) or stearic acid.

In Table 1, the identified compounds in young agarwood leaves from the highest portion in sequence were represented by Octadecanoic acid (CAS) Stearic acid (17.42%), Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro- (CAS) 1,1-DIBROMO-2-CHLO (10.75%), 9,12-Octadecadienal (CAS) (8.98%), 9-Octadecenoic acid (Z)- (CAS), Oleic acid (6.45%), Heptanoic acid (CAS) Heptoic acid (6.04%), and Carbamic acid, phenyl ester (CAS) Phenyl carbamate (5.65%). Most of the compounds are acidic compounds.

In Table 2, the identified compounds in mature agarwood leaves from the highest portion in sequence were represented by Octadecanoic acid (CAS) Stearic acid (17.44%), Heptanoic acid (CAS) Heptoic acid (11.40%), 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane (10.57%), and Nitrogen oxide (N2O) (CAS) Nitrous oxide (5.53%). The main compounds in mature leaves were similar to the ones in young leaves, dominated by acidic compounds.

| Peak | R. Time | Area       | Conc. % | Name                                              |
|------|---------|------------|---------|--------------------------------------------------|
| 1    | 3.423   | 57663720   | 10.75   | Octadecanoic acid (CAS) Stearic acid             |
| 2    | 12.399  | 30289721   | 5.65    | Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro-CAS  |
| 3    | 13.595  | 7436109    | 1.39    | Phenol, 2-methyl- (CAS) o-Cresol                  |
| 4    | 14.546  | 3257924    | 0.61    | Heptanoic acid (CAS) Heptoic acid                |
| 5    | 15.245  | 20254592   | 3.77    | Benzaldehyde, 4-methyl- (CAS) p-Tolualdehyde     |
| 6    | 15.466  | 13745750   | 2.56    | Decanoic acid (CAS) Capric acid                  |
| 7    | 15.988  | 9729432    | 1.81    | Benzonitrile, 2-methyl- (CAS) 1-Methyl-2-cyanobenzene |
| 8    | 16.319  | 4631233    | 0.86    | Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol |
| 9    | 17.243  | 14717919   | 2.74    | Tetradecane, 1-chloro- (CAS) Myristyl chloride   |
| 10   | 17.645  | 10453320   | 1.95    | Cyclohexane, (3-chloro-1-propynyl)- (CAS) 3-Chloro-1-Cyclohexylpr |
| 11   | 18.033  | 32414978   | 6.04    | Heptanoic acid (CAS) Heptoic acid                |
| 12   | 18.173  | 16243401   | 3.03    | 4-Hydrazinyltoluene                              |
| 13   | 19.063  | 7440478    | 1.39    | Silane, trichloroecysyl-                         |
| 14   | 19.142  | 1923939    | 0.36    | Silane, trichloroecysyl-                         |
| 15   | 19.221  | 4252380    | 0.79    | 3,5-Dodecadiyne, 2-methyl- (CAS) 2-Methylidencyclopentane (CAS) 11-Cyclopentanyldiynoic acid |
| 16   | 19.475  | 18812920   | 3.51    | 11-Tetradecenc1-ol, acetate, (Z)- (CAS) cis-11-Tetradecenyl acetate |
| 17   | 19.625  | 5623235    | 1.05    | Benzoyl cyanide                                  |
| 18   | 19.863  | 6892716    | 1.28    | 11-Tetradecenc1-ol, acetate, (Z)- (CAS) cis-11-Tetradecenyl acetate |
| 19   | 20.164  | 7382226    | 1.38    | 11-Tetradecenc1-ol, acetate, (Z)- (CAS) cis-11-Tetradecenyl acetate |
| 20   | 20.767  | 18462622   | 3.44    | 9,12-Octadecadienal (CAS)                       |
| 21   | 20.889  | 93491480   | 17.42   | Octadecanoic acid (CAS) Stearic acid             |
Table 2. List of chemical compounds in mature agarwood leaves (*Aquilaria malaccensis* Lamk).

| Peak | R. Time | Area   | Conc. % | Name                                                                 |
|------|---------|--------|---------|----------------------------------------------------------------------|
| 1    | 3.554   | 26039247 | 5.53    | Nitrogen oxide (N2O) (CAS) Nitrous oxide                               |
| 2    | 12.477  | 20964642  | 4.45    | Cyclohexene, 1-methyl-4-(1-methylethenyl)-(CAS) 1-P-Mentha-1,8-Diene   |
| 3    | 13.61   | 4827651   | 1.02    | Phenol, 2-methyl-(CAS) o-Cresol                                        |
| 4    | 15.1    | 4895402   | 1.04    | 2-Propenoic acid, 2-methyl-est (CAS) Ethyl methacrylate               |
| 5    | 15.281  | 6424353   | 1.36    | Benzaldehyde, 4-methyl-(CAS) p-Tolualdehyde                            |
| 6    | 15.475  | 6850635   | 1.45    | Heptanoic acid (CAS) Heptolic acid                                     |
| 7    | 16.013  | 7099888   | 1.51    | 7-Cyano(15N)-Cycloheptatriene                                           |
| 8    | 16.918  | 4022748   | 0.85    | Dodecane (CAS) n-Dodecane                                              |
| 9    | 17.092  | 7116438   | 1.51    | 1-Nonyne (CAS) 1-C9H16                                                |
| 10   | 17.243  | 3648251   | 0.77    | Tetradecane (CAS) n-Tetradecane                                        |
| 11   | 18.058  | 53702258  | 11.40   | Heptanoic acid (CAS) Heptolic acid                                     |
| 12   | 19.063  | 9356735   | 1.99    | 1-Dodecene (CAS) Adacene 12                                            |
| 13   | 19.4    | 6450392   | 1.37    | 9,12-Octadeadienal (CAS)                                               |
| 14   | 19.473  | 5771671   | 1.22    | Tetradecanoic acid (CAS) Myristic acid                                |
| 15   | 19.893  | 5981886   | 1.27    | Silane, trichloroicosil- Propanodinitril, (2-methylcyclohexidyldiene)- (CAS) |
| 16   | 20.083  | 7741182   | 1.64    | Octadecanal (CAS) Stearaldehyde                                        |
| 17   | 20.166  | 11742461  | 2.49    | 2-Butanan, 4-cyclohexyl-(CAS) 4-Cyclohexyl-2-butanal                  |
| 18   | 20.36   | 10647947  | 2.26    | 9,15-Octadecadienoic acid, methyl ester (CAS) Methyl 9,15-Octadecad    |
| 19   | 20.761  | 14416817  | 3.06    | Octadecanoic acid (CAS) Stearic acid                                  |
| 20   | 20.881  | 82165185  | 17.44   | 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane                  |
| 21   | 20.992  | 49814886  | 10.57   | 9,12-Octadeadienal (CAS)                                               |
| 22   | 20.072  | 21040321  | 4.46    | 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane                  |
| 23   | 21.133  | 7074034   | 1.50    | 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane                  |
| 24   | 21.325  | 22934401  | 4.87    | 5,10-Diethoxy-2,3,7,8-Tetrahydro-1H,6H-Dipyrolo[1,2-A:1',2'-D]P     |
| 25   | 21.475  | 3970761   | 0.84    | 2,4,6-Trimethyl-1-nonene                                              |
| 26   | 22.112  | 20888851  | 4.43    | 9-Octadecenoic acid (Z)-(CAS) Oleic acid                              |
| 27   | 22.258  | 9566847   | 2.03    | Silane, trichloroicosil- trans-Farnesol                               |
| 28   | 29.927  | 7026893   | 1.49    | Heptadecane, 2-methyl-(CAS) 2-Methylheptadecane                      |
| 29   | 31.055  | 7455224   | 1.58    | Tricosane, 2-methyl-(CAS) 2-Methyltricosane                           |
3.2. Antioxidant capacity of agarwood leaves extract

In this study, the mature agarwood leaf extract displayed the strongest antioxidant activity ($IC_{50} 25.860 \pm 0.721$ ppm) compared to young leaves ($IC_{50} 26.585 \pm 0.689$ ppm). However, both were in the same level, classified as very strong scavengers, $IC_{50} < 50$ ppm. Strong or weak antioxidants were determined by several factors, one of which was the chemical composition contained in the leaves [9].

Antioxidant activity is related to the concentration of phenolic and flavonoid compound. In W. tenuiramis Miq, the leaves contained phenolic compounds in the form of tannins with a higher concentration in mature leaves than young leaves [3]. Based on previous research, it was revealed that the phenolic and flavonoid compounds contained in the EtOH extract of A. malaccensis leaves also contributed to the antioxidant activity [4].

The mature leaves of A. beccariana displayed an $IC_{50}$ value of 72.25 ± 0.72 ppm [11]. The young leaves of Vernonia amygdalina was reported to possess chemical compounds with higher antioxidant and antidiabetic potentials as compared to the mature leaves which was different in composition, pancreatic and hepatic regeneration properties [12]. The results of the py-GCMS analysis also supported the results of the antioxidant test, where the dominant compound in both mature and young leaves was an acidic compound, namely Octadecanoic acid (CAS) or Stearic acid which played a prominent role in generating strong antioxidant properties in agarwood leaves [4].

Accumulation of phenolics and flavonoids in the early stage of leaf growth was less than in mature leaves followed by the increasing level of alkaloids. Hence, the mature leaves may contain more bioactive compounds as revealed from a study of pomegranate [12].

4. Conclusion

We documented a total of 30 compounds in young and mature leaves of wild agarwood A. malaccensis growing in Laru Village with 13 different chemicals. The dominant compounds in both type of leaves were Octadecanoic acid (CAS) or Stearic acid. The $IC_{50}$ value of the mature leaves was 25.860 ± 0.721 ppm while the young leaves were 26.585 ± 0.689 ppm, both classified as very strong antioxidants.

Acknowledgement

The authors would like to express their gratitudes to the Ministry of Research, Technology and Higher Education, for funding this research in 2018 under scheme of Basic Research Scheme (PDUPT).

References

[1] Batubara R, Nurminah M, Hanum TI and Surjanto 2019 Potency of Gaharu Leaves that Grows Naturally and Cultivately as Raw Material of Antioxidant-rich Tea (Indonesia: Proceedings of ICEASD & ICCOSED 2019)
[2] Batubara R, Wirjosentono B, Siregar AH, Harahap U and Tamrin 2021 Rasayan J. Chem. 14 751
[3] Batubara R, Hanum TI, Afandi O and Wahyuni HS 2020 Biodiversitas 21 4616
[4] Batubara R, Surjanto, Hanum TI, Handika A and Afandi O 2020 Biodiversitas 21 1588
[5] Maharani R, Fernandes A, Turjaman M, Lukmandaru G and Kuspradini H 2016 Int. J. Pharmacog. Phytochem. Res. 8 1576
[6] Directorate General of Drug and Food Control 1995 Materia Medika Indonesia. National Agency of Drug and Food Control of Indonesia (Jakarta, Departemen Kesehatan RI)
[7] Prakash A 2001 Anal. Prog. 19 2
[8] Molyneux P 2004 Songklanakarin J. Sci. Technol. 26 212
[9] Batubara R, Hanum TI and Risnasari I 2018 Antioxidant Activity and Preferences Test of Agarwood Leaves Tea (Aquilaria malaccensis Lamk) Based on Leaves Drying Methods.
(Indonesia: Proceedings of BROMO Conference 2018)

[10] Anwar K, Triyasmono L, Rizki MI, Halwani W and Lestari F 2017 *Res. J. Pharm. Biol. Chem. Sci.* **8** 129

[11] Asante DB, Effah-Yeboah E, Barnes P, Abban HA, Ameyaw EO, Boampong JN, Ofori EG and Dadzie JB 2016 *J. Diabetes Res.* **2016** 8252741

[12] Zhang L, Gao Y, Zhang Y, Liu J and Yu J 2010 *Scientia Horticulturae* **123** 543