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The antioxidant activities and chemical compounds of *Aquilaria crassna, Aquilaria microcarpa,* and *Gyrinops versteegii* leaves growing in Langkat, North Sumatra, Indonesia

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Abstract. *Batubara R, Hanum TI, Affandi O, Julianti E, Ulfa M. 2022. The antioxidant activities and chemical compounds of* Aquilaria crassna, Aquilaria microcarpa, *and* Gyrinops versteegii *leaves growing in Langkat, North Sumatra, Indonesia. Biodiversitas 23: 6619-6628.* Agarwood has been cultivated for its wood and leaves in Indonesia. This research aims to determine the potential of the leaves of *Aquilaria crassna*, *A. microcarpa*, and *Gyrinops versteegii* that grow in Langkat as raw materials for antioxidant-rich tea based on their chemical compounds and antioxidant activities. The leaf powder was extracted using ethanol. Bioactive compounds were analyzed by GC-MS, while the antioxidant activity was analyzed by the DPPH method. The results showed that *A. crassna* leaves contained alkaloids, flavonoids, tannins, steroid/triterpenoid dan glycosides, whereas *A. microcarpa* leaves contained alkaloids, tannins, saponins, steroid/triterpenoid dan glycosides alkaloids, tannins, and triterpenoids. In comparison, *G. versteegii* leaves contained flavonoids, tannins, saponins, steroid/triterpenoids, and glycosides. The IC_{50} values of ethanol extract of *A. crassna*, *A. microcarpa*, and *G. versteegii* leaves were 30.0079, 29.3263, and 31.6304 µg/mL, respectively. The highest chemical compounds of *A. crassna*, *A. microcarpa*, and *G. versteegii* were Cyano, -acetic acid, 1,2,3,4-Cyclopentane Petrol, and *Oxacycloheptadec-8-en-2-one Ambrettolide* The three species had high antioxidant activity; therefore they are potential sources of antioxidants.

Keywords: Agarwood, *Aquilaria crassna*, *Aquilaria microcarpa*, antioxidant, chemical compounds, *Gyrinops versteegii*

INTRODUCTION

Agarwood is a resinous tree produced as a defensive system against wounding and fungal infection (Tan et al. 2019). A discoloration symptom on the infected area indicates agarwood formation due to resin accumulation (Rasool and Mohamed 2016). Agarwood has long been utilized in medicinal fragrances, medical preparations, traditional medicines, religious rituals, spiritual supports, incense, perfumes, and other fragrant products, and as an aromatic culinary component (Liu et al. 2013; López-Sampson and Page 2018).

Agarwood is an important non-timber forest product found on the major island of Indonesia (Santoso et al. 2011). The agarwood trees in Indonesia are classified into three families: Thymelaeaceae (25 species), Euphorbiaceae (1 species), and Fabaceae (1 species). There are 33 agarwood species in Asia and 26 species found in Indonesia. The most well-known cultivated host species of agarwood are *Aquilaria crassna*, *A. malaccensis*, *A. filaria*, *A. cumingiana*, *A. beccariana*, *A. microcarpa*, and *Gyrinops versteegii* from the Thymelaeaceae family.

Indonesia was the largest agarwood producer in the world (Turjaman and Hidayat 2017). Agarwood production reached more than 600 tons per year until the end of 1990. However, since 2000 the production has continued to decline due to the destructive and illegal exploitation, as well as the gradual loss of tropical rain forests as their natural environment (Lee and Mohamed 2016; Turjaman and Hidayat 2017). Therefore, the production only meets 10-15 percent of the annual requirement of approximately 300 tons. In addition, since 2004, the export of agarwood from Indonesia has been banned (Sumarna 2012).

Although agarwood has a high commercial value, the producing trees are critically endangered (Rasool and Mohamed 2016). Therefore, to fulfill the enormous market demand while also preventing species, large-scale agarwood tree plantations have been established to ensure long-term agarwood production (Azren et al. 2018). One strategy to improve agarwood value is by utilizing the leaves. Agarwood leaves are generally abundant when pruning activities occur. These leaves are considered waste, but some local people also use them as tea leaves.

Several previous studies on agarwood leave as an antioxidant source have been carried out, including tea

products from agarwood leaves, to improve the value of agarwood leaves. For example, the ethanol extract of *Aquilaria microcarpa* Bail leaves contains phenolic compounds, flavonoids, saponins, tannins, and steroids (Sari et al. 2017; Rahmanto et al. 2018). Phytochemical screening demonstrated that *Gyrinops versteegii* (Gilg.) Domke leaves contain phenolic compounds, terpenoids, and flavonoids (Mega and Swastini 2010). Moreover, ethyl acetate and methanol extract of *G. versteegii* leaves were strongly detected to contain the antioxidants compounds, i.e., p-hydroquinone, tannin, and flavonoid. On the other hand, the n-hexane extract exhibited weak antioxidant compounds with a total phenol of 0.45% (Wahyuningrum et al. 2018).

Batubara et al. (2018) conducted studies on acute and subchronic toxicity tests (Batubara et al. 2016), revealing that agarwood tea was safe for consumption. However, leaf utilization needs further investigation to advance development, especially regarding raw material variations. In addition, basic information is still needed to analyze agarwood leaves chemical components and their potential antioxidant activity so that the leaves can serve as a potential source for antioxidant-rich tea that meets the pharmacology aspects. Therefore, this research aims to determine the chemical compounds and antioxidant potential of *Aquilaria crassna* Pierre ex Lecomte, *A. microcarpa*, and *Gyrinops versteegii* leaves that grow in Langkat.

MATERIALS AND METHODS

Study area

Agarwood leave samples were collected from Pekan Bahorok, Langkat District, North Sumatra, Indonesia (Figure 1). The phytochemical tests were analyzed at the Biochemistry Laboratory, the Mathematics and Natural Sciences Faculty. The antioxidant activity was analyzed at the Research Laboratory, the Faculty of Pharmacy. The moisture content and extraction test were measured at the Laboratory of Forest Product Technology, Faculty of Forestry, Universitas Sumatera Utara, Medan, Indonesia. The experiments were also conducted at the Forestry Research and Development Agency, Bogor, Indonesia.

Procedures

Plant sampling and identification

Agarwood leaves were collected from three cultivated agarwood species in Langkat District, i.e., *Gyrinops versteegii*, *Aquilaria microcarpa*, and *A. crassna*. *G. versteegii* species growing in Langkat were planted by farmers from seedlings collected from Lombok. *A. crassna* grows from seedlings provided by the Bogor Forestry Research and Development Center. At the research site, *A. microcarpa* seedlings that farmers planted originated from Pekanbaru, Riau, and were provided by the Forestry Office of Langkat District, Indonesia.

Preparation of raw material

The samples were cleaned under tap water and then scattered on parchment paper to absorb the water. Afterward, the samples were placed in the drying cabinet at 40-50°C. Finally, dried samples were ground and stored in sun-free storage.

Tannin content

Titration with KMnO4 solution measured the tannin content in samples (Lowenthal Procter method, MoHRI 2000).

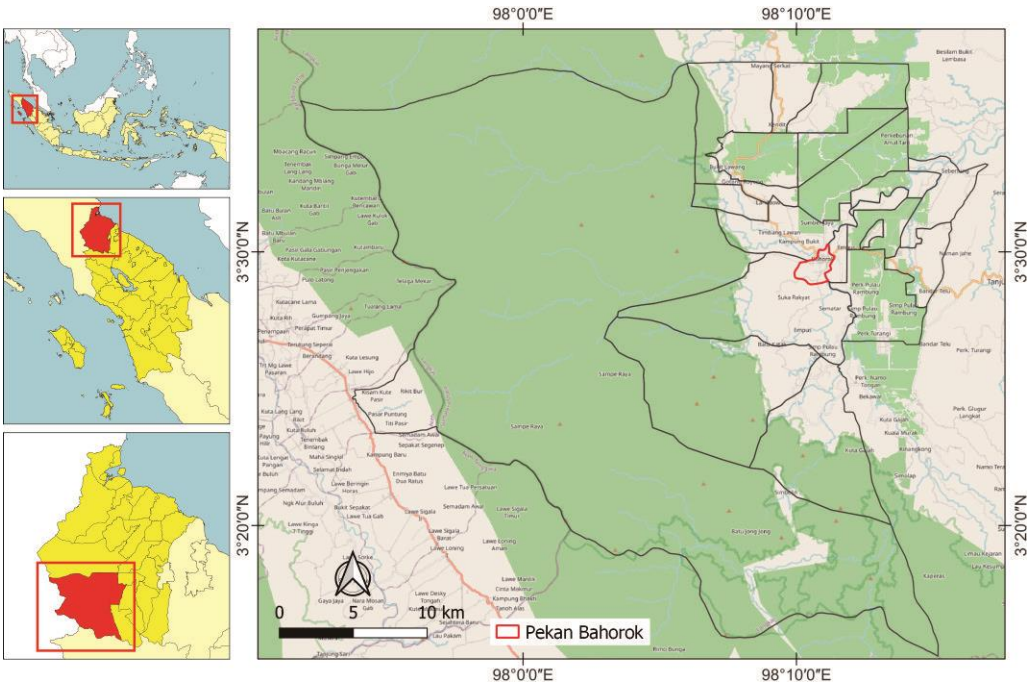

Figure 1. Map of sample collection in Pekan Bahorok, Langkat District, North Sumatra, Indonesia

Phytochemical screening of simplicia

The phytochemical analysis includes alkaloids, saponins, tannins, flavonoids, glycosides, and steroids/ terpenoids. In addition, agarwood leaf simplicia powder and ethanol extracts were subjected to phytochemical screening, referring to Harborne (1987) and MoHRI (2000).

Phytochemical screening of the simplicia

Alkaloid

1 mL of 2N hydrochloric acid and 9 mL of distilled water were mixed with 0.5 g of agarwood leaf simplicia and heated for 2 minutes before cooling and filtering. The filtrate was then used for the alkaloid test. Three test tubes were prepared; each received 0.5 mL of the filtrate with different reagents, i.e., Mayers, Dragendorffs, and Bouchard. If precipitate or turbidity was formed in two or three experiments, it positively contains alkaloids (Ditjen POM, 1995).

Triterpenoid/steroid

Agarwood leaf simplicia (1 g) was macerated for 2 hours in 20 mL of hexane, then filtered and evaporated in a vaporizer cup. The rest was treated with the Lieberman-Burchard reagent. The formation of a blue/greenish-blue color indicates the presence of steroids, while red/pink/purple colors indicate the presence of triterpenoids (Harborne 1987).

Flavonoid

Simplicia (10 g) was dissolved in hot water (100 mL), then boiled for 5 minutes and filtered. Afterward, magnesium powder (0.1 g), concentrated hydrochloric acid (1 mL), and amyl alcohol (2 mL) were added to the water extract and shaken until detached. If the amyl alcohol layer is red, yellow, or orange, it indicates the presence of flavonoids (Farnsworth, 1996).

Tannin

Simplicia (0.5 g) was extracted with 10 mL of distilled water and filtered. First, water was added to dilute the filtrate until it became colorless. Then, 1% iron (III) chloride reagent (1-2 drops) was put into 2 mL of the solution. The blue or blackish-green color indicates the presence of tannins (Ditjen POM, 1995).

Saponin

Agarwood leaf simplicia (0.5 g) was dissolved in hot distilled water (10 mL) and then cooled, followed by shaking for 10 seconds vigorously. Saponins were positively detected by the formation of stable foam, which did not disappear with the 2 N hydrochloric acid addition (Ditjen POM, 1995).

Glycoside

15 mL of 10% HCL was added to the agarwood leaf simplicia, heated for 10 minutes, then filtered. The filtrate was extracted with ether (5 mL) three times. Anhydrous sodium sulfate was added to the filtrate, evaporated at 50°C, then added 2 mL methanol and evaporated again. Afterward, the result was dissolved with water (1 mL) and Molisch (8 drops), then sulfuric acid (1 mL) was carefully added. The purple ring at the liquid boundary indicates the positive result of glycoside (Ditjen POM 1995).

Phytochemical screening of the ethanol extract

Extraction of agarwood leaves

Extraction of agarwood leaves was performed by maceration using 96% ethanol. Two hundred g of agarwood leaves were macerated in 96 % ethanol (1500 mL) for 5 days, covered, and sun-protected, stirring occasionally. The solution was filtered after five days of maceration. Afterward, the residue was macerated with 96% ethanol (2000 mL), let stand for 48 hours, and filtered. A rotary evaporator was used to concentrate the macerate at 40°C. Finally, the dry extract was obtained using a Freeze Dryer. The chemical compounds were screened by adding reagents according to the group of tested compounds.

Alkaloid

One (1) mL of extract was warmed for two minutes in 2% H_2SO_4 and then added with Dragendorff's reagent. Orange-red precipitate indicates the presence of alkaloids.

Triterpenoid/steroid

Acetic anhydride and H_2SO_4 were put into the extract. The color change to reddish/violet indicated the presence of triterpenoids. The presence of steroids was indicated by changing color from violet to blue/green.

Flavonoid

One (1) mL of extract was dissolved with NaOH and HCl. Changing color from yellow to a colorless solution indicated the presence of flavonoids

Tannin

Pb(CH_3COO)2 1% was put into the extract. Yellow precipitate indicated the presence of tannins.

Saponin

The extract was put in the test tube, and warm distilled water was added. Then vigorously shakes for up to 30 seconds. The stable foam (1 cm height) characterized the presence of saponins.

Glycoside

One (1) mL of extracts was dissolved with water (1 mL) and Molisch reagent (8 drops). Afterward, the sulfuric acid (1 mL) was carefully added. The purple ring at the liquid boundary indicated the positive result of the Molisch reaction or the presence of glycoside.

Antioxidant activity

The antioxidant activity of samples was estimated with the DPPH method, following Molyneux (2004). The absorbance of the sample was measured at 516 nm using A UV-Vis spectrophotometer to determine the IC_{50} value. The antioxidant activity is measured based on the IC_{50} value (Prakash 2001; Andayani et al. 2008). The IC_{50} value was measured based on the linear regression equation that plotted concentration and the reduction percentage of

DPPH. The positive control was ascorbic acid. A UV-Vis spectrophotometer was utilized to measure absorbance at 516 nm to determine the IC_{50} value.

Compounds analysis with GC-MS

The GC-MS analysis was performed at the Forestry Research and Development Agency, Bogor, using Pyrolysis GC-MS.

RESULTS AND DISCUSSION

Phytochemical screening of ethanol extract of agarwood leaves

Phytochemical screening was performed in the simplicia powder and the ethanol extract of agarwood leaves to observe their secondary metabolites. All samples had different phytochemical compounds in the simplicia and extract, although they are in the same species and among species (Table 1).

The results showed that some compounds were present in simplicia but not in the extract, and vice versa. For example, alkaloids were not detected in *A. crassna* simplicia but in the extract. It indicated that the solvent attracted the compound more strongly. On the other hand, alkaloids were detected in *G. versteegii* simplicia but not in the extract, indicating that the compounds were small, making them undissolved in the solvent. Flavonoids were not identified in *G. versteegii* simplicia but were identified in the extract. Saponins were identified in *A. crassna* simplicia but were not in the extract. On the other hand, they were not identified in *A. microcarpa* simplicia but in the extract. Ethanol is a polar solvent. Therefore, polar chemical components are extracted by ethanol. For example, alkaloids are polar compounds, and flavonoid glycosides and aglycones are polar flavonoids.

Mega and Swastini (2010) showed that *G. versteegii* agarwood leaves contained phenolic compounds, flavonoids, and terpenoids. In addition, the study by Wahyuningrum et al. (2018) showed that ethyl acetate and methanol extract of *G. versteegii* agarwood leaves contain antioxidant compounds, i.e., p-hydroquinone, tannin, flavonoid, 3.40% and 4.27% total phenol in the ethyl acetate and methanol extracts, respectively. On the other hand, a weak antioxidant was examined in the n-hexane extract with a total phenol of 0.45% (Wahyuningrum et al. 2018).

Aquilaria crassna is a native to Thailand. It grows primarily in tropical rainforests. Agarwood has been divided into two genera in Thailand, i.e., *Aquilaria* and *Gyrinops*, both belonging to the Thymelaeaceae family (Nimnoi et al. 2011).

Aquilaria microcarpa species grows naturally in Sumatra. The phytochemical screening on *A. microcarpa* leaves showed that the simplicia did not contain saponins, but the extract did. It was because the solvent attracts saponins and the *G. versteegii*. The phytochemical screening of the ethanol extract of *A. microcarpa* leaves revealed that it contained phenolic compounds, flavonoids, steroids, tannins, and saponins (Sari et al. 2017). In addition, in a previous study by Rahmanto et al. (2018), the phytochemical screening demonstrated that ethanol extract of *A. microcarpa* leaves contained flavonoids, phenols, tannins, saponins, and steroids.

Gyrinops versteegii is an endemic and rare species that grow in Lombok (Yelnititis 2014). Agarwood trees in the forest of West Lombok are known as Ketimunan, which have five local varieties, i.e., Beringin, Buaya, Pantai, Madu, and Soyun. The infraspecific category of *G. versteegii* is the classification level name under the species. In forestry, the name of this variety level is known by the term "provenance" (Mulyaningsih et al. 2017).

Phytochemical screening of agarwood leaf extract showed that the extracts contain several secondary metabolites that may have pharmacological importance. For example, in a previous Fatmawati and Hidayat (2016) study, the leaf extract showed dose-dependent cytotoxic activity against cervical cancer. In addition, the leaves of *A. malaccensis* have the potency to improve glucose absorption by enhancing GLUT4 in skeletal muscle (Said et al. 2016).

Table 2 showed that all samples contained tannin with varying levels. Tannins have unique chemical characteristics with a molecular mass of 0.5-3 kDa. In addition, tannins could form complexes with proteins to form copolymers (Adamczyk et al. 2017). Some leaves and unripe fruits (such as rambutan, mango, and sapodilla) contain tannin compounds. They are polyphenolic compounds belonging to the flavonoids group, which have a rough texture in food (Hayati et al. 2010; Mabruroh 2015). Tannins are classified into two types, i.e., hydrolyzable tannins and condensed tannins.

Table 1. Phytochemical compounds of three cultivated agarwood leaves

Compounds	Species					
	Aquilaria crassna		*Aquilaria microcarpa*		*Gyrinops versteegii*	
	Simplicia	Extract	Simplicia	Extract	Simplicia	Extract
Alkaloids	-	+	+	+	+	-
Flavonoids	+	+	-	-	-	+
Tannins	+	+	+	+	+	+
Saponins	+	-	-	+	+	+
Steroids/Triterpenoids	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+

Note: +: present, -: not present.

The tannins and flavonoids in tea were used in ancient medicine to cure burns and neutralize insect toxins due to their anti-inflammatory and antibacterial properties. *Gyrinops* tea made from mature and dried *G. versteegii* leaves contains higher tannins than fresh ones (Wangiyana et al. 2018). The tannins content of *W. tenuiramis* and *A. malaccensis* (Batubara et al. 2018a) were 4.95% and 5.62%, respectively, while *Camellia sinensis* contains 1.21% (Rohdiana 2001). Therefore, the tannin content of agarwood is higher than that of *C. sinensis*. The extracted liquid of agarwood leaves was greenish-black, and the drying process produced a black and thick greenish extract. Both used ethanol as a solvent. Therefore, the drying process did not affect the color of the extracts. The percentage of extracts is shown in Table 2.

Antioxidant activity based on IC_{50} (inhibitory concentration)

The average IC_{50} value of *Aquilaria cressna*, *A. microcarpa*, and *G. versteegii* was 30.01 µg/mL, 29.33 µg/mL, and 31.63 µg/mL, respectively, categorized as very strong antioxidant activity (Table 3). Tannins and phenolic compounds possess strong antioxidant activity, and *G. versteegii* had the highest antioxidant and total phenol with IC_{50}<50 µg /mL (Parwata et al. 2016).

Tannins are compounds that contribute to antioxidant activity. The advantage of agarwood leaves tea compared to other teas was its robust antioxidant activity. The chemical compounds of plants are affected by their habitat, and the chemical composition determines the level of antioxidant activity (Firdiyani et al. 2015). A previous study by Sudaryat et al. (2015) showed that the type of Dust I had the most significant antioxidant activity among ten varieties of Indonesian black tea, with an IC_{50} value of 97.00 g/mL, while the type of BTL had the lowest, with an IC_{50} value of 178.56 g/mL. Therefore, the antioxidant content should be considered when processing tea from agarwood leaves. Werdhasari (2014) stated that antioxidants could scavenge free radicals produced through the body's metabolism, including air pollution, food contamination, and sunlight.

A previous study by Hendra et al. (2016) showed methanol extract of mature leaves of *A.malaccensis* had an IC_{50} value of 19.62 ± 1.49 µg/mL, while the fraction with combined solvent of chloroform-methanol (1:3) had an IC_{50} of 17.39 ± 1.43 µg/mL as the highest antioxidant activity (Hendra et al. 2016). Furthermore, Sari et al. (2018) reported that the gel containing ethanolic extract of *A. microcarpa* leaf had IC_{50} values ranging from 26.39-28.94 µg/mL, while the gel containing ethyl acetate fraction had IC_{50} values ranged 22.22-23.05 µg/mL. They demonstrated robust antioxidant activity (Sari et al. 2018). The chloroform extract of young *G. versteegii* leaves has an IC_{50} value of 22.71 ± 1.31 µg/mL that showed potent cytotoxic activity against the HeLa cell line (Nuringtyas 2018). *A. crassna* has been used as herbal tea in Thailand to increase the liver and circulatory system; it also enhances physiological balance and cardiovascular function in Asia (Wisutthathum et al. 2018). *A. crassna* leaves extract possesses a laxative (Kakino 2010) and antioxidant activity (Sattayasai et al. 2012; Kamonwannasit et al.2013). The acute oral toxicity study of *A. crassna* leaves extract showed that the extract is non-toxic in mice (Ghan et al. 2016).

Table 2. The average tannin contents of three cultivated agarwood leaves

Species	Tannin contents (%)
Gyrinops versteegii	3.15 ± 0.07
Aquilaria microcarpa	3.21 ± 0.20
Aquilaria crassna	3.28 ± 0.06

Table 3. The IC_{50} value of three species of agarwood leaves and ascorbic acid

Extracts	Repetition	Regression equation	IC_{50} (µg/mL)	Antioxidant strength category
Aquilaria crassna	1	Y = 0.861X + 24.290	29.86	Very strong
	2	Y = 0.870X + 23.753	30.17	Very strong
	3	Y = 0.860X + 24.205	29.99	Very strong
	Average		30.01	Very strong
Aquilaria microcarpa	1	Y = 0.877X + 24.793	28.74	Very strong
	2	Y = 0.884X + 24.355	30.38	Very strong
	3	Y = 0.876X + 24.726	28.85	Very strong
	Average		29.33	Very strong
Gyrinops verteegii	1	Y= 0.837X + 23.527	31.63	Very strong
	2	Y= 0.837X + 23.525	31.63	Very strong
	3	Y= 0.837X + 23.514	31.63	Very strong
	Average		31.63	Very strong
Ascorbic acid	-	Y= 32.709X + -37.652	2.68	Very strong

GC-MS analysis

GC-MS analysis revealed that each agarwood species contained a different chemical compound. For example, *A.crassna* contained 30 compounds, while *A. microcarpa* contained 40 compounds, and *G. versteegii* contained 40 compounds. In addition, the main compounds in the three species of agarwood were different. For example, the five main compounds with the highest concentration in *A. crassna* were Acetic acid, cyano-Cyano-Acetic Acid (5.74%), Pentanal, n-Pentanal (4.70%), 2,4-HexadienoiccompoundsLimonene acid, 3- methyl-4-propyl-, dimethyl ester, (E, E) - (CAS) (4.61%), Propanal, 2-oxo- Pyruvaldehyde (4.39%) and 9-Octadecenoic acid (Z) - Oleic acid (4.19%). On the other hand, the five main components with the highest concentrations in *A. microcarpa* were 1,2,3,4-Cyclopentane Petrol, (1.alpha., 2.beta., 3.beta4.alpha.) - 1,2,3, 4- (8.08%), Oxacycloheptadec-8-en-2-oneAmbrettolide (5.97%), 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo (4.3.0) nonane (5.00%), Bicyclo [8.2.0] dodecane, 11,11-dimethyl- (4.74%), and 2,3-Dihydro-Benzofuran (4.68%). On the other hand, the five highest concentrations compounds of *G. versteegii* were Oxacycloheptadec-8-en-2-one Ambrettolide (10.63%), Hexadecanoic acid Palmitic acid (9,52%), Cylcopropyl carbonyl (6.14%), Propanal, 2-oxo-pyruvaldehyde (5.81%), and 2,3-Dihydro-Benzofuran (4.62%). The detailed results are presented in Tables 4 to 6.

Adam et al. (2017) reported that leaves of Aquilaria contained phenolic acids, flavonoids, pyranones, xantonoids, benzophenones, steroids, terpenoids, quinones, nucleosides, and 2-(2-phenylethyl) chromones. The ethanol extract of *A. crassna* and *A. sinensis* leaves analyzed with SGCC, and LC-MS contained genkwanin, mangiferin, and iriflophenone glycosides (Wang et al. 2008; Qi et al. 2009; Feng et al. 2011; Ito et al. 2012; Yu et al. 2013; Wang et al. 2015). The main components of *Aquilaria* leaves were hexadecanoic acid and squalene (Adam 2017). Stearic acid is the major component of agarwood leaves from Laru Village, while the highest concentration of agarwood leaves from Huta Nabolon Village is cyclopropyl carbinol (Batubara et al. 2020). These compounds possess antioxidant activity.

Table 4. The identified chemical compounds of *A. crassna* leaves by GC-MS analysis

Peak	Retention Time	Area	Concentration %	Name
1	5.563	99190616	5.74	Cyanoacetic Acid
2	6.046	16302636	0.94	Nitrogen oxide (N_2O)
3	6.217	10602079	0.61	Carbon dioxide
4	6.550	11415075	0.66	2-Propionic acid
5	8.652	75853605	4.39	2-oxo-Propanal, Pyruvaldehyde
6	13.919	54115669	3.13	Cyclohexanone Anon
7	14.492	11185306	0.65	di-Limonene
8	14.901	35943027	2.08	Phenol), Izal
9	15.201	28599961	1.66	2-Cyclopenten-1-one, 2-hydroxy-3-methylCorylon
10	15.767	13383101	0.77	Phenol, 2-methoxy-, Guaiacol
11	16.115	81300337	4.70	Pentanal, n-Pentanal
12	16.767	10438153	0.60	2H-Pyran-3($H)-one, dihydro-, Tetrahydropyran-3-one
13	17.042	15507600	0.90	Trans-2,3-Epoxynonane
14	17.421	22826858	1.32	Xanthosine, n-Pental
15	17.716	38829592	2.25	2,3-Dihydro-Benzofuran
16	18.349	40984734	2.37	Indolizine, Indolizin
17	18.567	28330415	1.64	Phenol, 2,6-dimethoxy, 2,6-Dimethoxyphenol
18	18.942	28188241	1.63	-
19	19.167	30096700	1.74	Cyclooctane, 1-(Diethyl Boryl),
20	19.377	27217589	1.58	Benzene, 1,2,3-trimethoxy-1,2,3-Trimethoxybenzene (CAS)
21	20.001	70799633	4.10	(R)-2-Benzyl Proline methyl amide
22	20.427	62109562	3.59	4-Methyl-2,5-Dimethoxybenzaldehyde
23	20.642	79527617	4.61	2,4-Hexadienoic acid, 3-methyl-4-propyl-, dimethyl ester, (E, E)-
24	21.148	44502424	2.58	1-Dodecanol, 3,7,11-trimethyl-, Hexahydro Farnesol
25	21.689	72383130	4.19	9-Octadecenoic acid (Z)-, Oleic acid
26	22.053	65782482	3.81	Neophytadiene
27	22.395	43257539	2.50	Neophytadiene
28	22.567	17070305	0.99	13-Heptadecene-1-ol, 1-Hydroxy Heptadec-13-YNE
29	22.768	32374940	1.87	
30	22.992	25344875	1.47	Cyclopentanol, 2,4,4-Trimethyl-

Table 5. The identified chemical compounds of *A. microcarpa* leaves by GC-MS analysis

Peak	Retention Time	Area	Concentration %	Name
1	5.603	113522094	4.40	Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro, 1,1-Dibromo-2-Chloro-2-fluoro cyclopropane
2	6.100	16599795	0.64	Carbon dioxide
3	6.267	11124977	0.43	Nitrogen oxide (N_2O)
4	6.449	8782092	0.34	Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro, 1,1-Dibromo-2-Chloro-2-fluoro cyclopropane
5	6.617	6696510	0.26	2-Propionic acid
6	8.417	9587707	0.37	Acetic acid, Ethylic acid
7	13.871	78565344	3.04	Cyclohexanone, Anon
8	14.517	10128693	0.39	di-Limonene
9	14.859	44611638	1.73	Phenol, Izal
10	15.167	31633789	1.23	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-, Corylon
11	15.767	17900176	0.69	Phenol, 2-methoxy-, Guaiacol
12	16.099	93429819	3.62	Cyclopropyl carbonyl
13	17.023	35976916	1.39	Oxarine, hexyl-
14	17.392	35303109	1.37	Xanthosine, Xanthine riboside
15	17.577	120921238	4.68	2,3-Dihydro-Benzofuran
16	18.173	93545746	3.62	Phenol, 4-ethyl-2-methoxy-
17	18.517	82247577	3.19	Phenol, 2,6-dimethoxy-(2,6-Dimethoxyphenol
18	18.932	42401908	1.64	2,3-Dimethylcyclohexanol 4
19	19.346	110196299	4.27	.alpha.-L-Mannopyranoside, methyl 6-deoxy-3-O-methyl-,diacetate. ME
20	19.667	61424960	2.38	-
21	19.989	84788938	3.28	Ethanone, 1-(-2,6-dihydroxy-4-methoxyphenyl)- (CAS), 2,6-Dihydroxy-4-metho
22	20.427	208451758	8.08	1,2,3,4-Cyclopentanetetrol, (1.alpha.,2.beta.,3.beta4.alpha.)- (CAS), 1,2,3,4-
23	21.167	104035094	4.03	Acetic Acid 3,7,11,15- Tetramethyl-Hexadecyl Ester
24	21.669	122245685	4.74	Biocyclo[8.2.0]dodecane, 11,11-dimethyl- (CAS),
25	22.105	73609292	2.85	2-Hexadecene, 3,7,11,15-tetramethyl- [R-[R*,R*-(E)]]
26	22.217	41476657	1.61	Benzaldehyde, 4-[[4-(acetyloxy)-3,5-dimethoxyphenyl]methoxy]-3-methoxy-
27	22.417	43954257	1.70	Neophytadiene
28	22.702	71288202	2.76	6,11-Undecadiene, 1-ACetoxy-3,7-Dimethyl-
29	23.017	35016442	1.36	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo (4.3.0)nonane
30	23.289	117806283	4.56	Hexadecanoic acid, Palmatic acid
31	23.574	154055849	5.97	Oxacycloheptadec-8-en-2-one, Ambrettolide
32	24.042	129112495	5.00	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo (4.3.0)nonane
33	25.042	78011592	3.02	Octadecane, 1-chloro- 1-Chlorooctadecene
34	25.717	53842359	2.09	12-Hydroxy-dodecanoic acid, lactone
35	26.017	27496623	1.07	Docosane, 7-hexyl- 7-n-Hexyldocosane
36	26.697	65495819	2.54	Tricosane, n-Tricosane
37	27.042	14992684	0.58	Cyclohexane, decyl-, n-Decylcylohexane
38	35.804	95933254	3.72	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- ,Squa
39	37.317	8400323	0.33	Nonacosane, n-Nonacosane
40	45.467	26692974	1.03	Tetratetracontane, n- Tetratetracontane
		2581308967	100.00	

Table 6. The identified chemical compounds of *G. versteegii* leaves by GC-MS analysis

Peak	Retention Time	Area	Concentration %	Name
1	5.496	148188579	13.80	Carbon dioxide
2	5.974	23915716	2.23	1,1-dibromo-2-chloro-2-fluoro- cyclopropane
3	6.154	14294728	1.33	Carbamic acid, monoammonium salt, ammonium carbamate
4	6.325	12508889	1.16	Oxirane, epoxyethane
5	6.492	9072121	0.84	2-propinioc acid
6	8.386	62442760	5.81	Propanal, 2-oxo-, pyruvaldehyde
7	13.807	33323602	3.10	1,2-cyclopentanedione
8	14.392	9969896	0.93	9-hydroxy-linalool
9	14.611	46578600	4.34	Phenol, izal
10	15.092	25206973	2.35	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-(CAS), corylon
11	15.698	7390369	0.69	Phenol, 4-methoxy-, HQMME
12	16.040	65924520	6.14	Cyclopropyl carbonyl
13	16.929	6448181	0.60	Phenol, 3-ethyl-(m-ethylphenol
14	17.400	12394802	1.15	Uridine
15	17.606	49611910	4.62	2,3-dihydro-benzofuran
16	18.158	13589228	1.27	Phenol, 4-ethyl-2-methoxy-
17	18.259	18181231	1.69	1H-Indole, Indole
18	18.492	24593643	2.29	Phenol, 2,6-dimethoxy-, 2,6-dimethoxyphenol
19	18.641	15806233	1.47	1,4-benzenediol, hydroquinone
20	19.058	19988991	1.86	D-Glucose, 4-O[3-acetyl-1-(trimethylsilyl)-1H-indodyl]-2,3,5,6-tetrakis-O-(trime
21	19.326	11094401	1.03	Benzene, 1,2,3-trimethoxy-, 1,2,3-trimethoxybenzene
22	19.642	11644749	1.08	Pyrimidine, 4,6-diamino-5-formylamino-
23	19.951	13117590	1.22	Benzeethanamine, 3,4,5-trimethoxy-, mescaline
24	20.370	15787440	1.47	4-Methyl-2,5-dimethoxybenzaldehyde
25	20.595	8013025	0.75	2,4-Hexadienedioic acid, 3-methyl-4-propyl-, dimethyl ester (E, E)-
26	21.470	10290694	0.96	Phenol, 2,6-dimethoxy-4-(2-propenyl)-, 4-Allyl-2,6-dimethoxyphenol
27	21.608	9957865	0.93	2,4-Dioxaspiro[5,5]undecane, 7,9,11-trimethyl-
28	22.012	22531302	2.10	Neophytadiene
29	22.355	1773110	1.65	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-R*,R*,(E)]]-, Phytol
30	22.725	11585276	1.08	Hexadecanoic acid, methyl ester, Methyl palmitate
31	23.207	102271735	9.52	Hexadecanoic acid, Palmitic acid
32	23.474	114206815	10.63	Oxacycloheptadec-8-en-2-one, Ambrettolide
33	23.775	14082563	1.31	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane
34	23.958	15766586	1.47	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane
35	25.382	5579051	0.52	Hexadecanamide, Amide 16
36	26.006	25272479	2.35	Tetratetracontane n-Tetratetracontane
37	28.106	7629586	0.71	2,2,4A,6A,8A,9,12B,14A-Octamethyl-1,2,3,4,4A,5,6,6A,6B,7,8,8A,9,12,12
38	35.598	16064730	1.50	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-
39	45.167	16056121	1.50	Tetratetracontane
40	49.369	5778533	0.54	Stigmast-5-EN-3-OL, oleat

Based on the results, the leaves of *A. crassna*, *A. microcarpa*, and *G. versteegii* collected from Langkat contain different chemical compounds. However, they contain tannins and flavonoids and have potent antioxidant activity with IC_{50} values less than 50.00 µg/mL. Therefore, it can be concluded that these leaves have the potential to be used as raw materials for antioxidant-rich tea due to their detectable chemical compounds and antioxidant activities.

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REFERENCES

Adam AZ. 2017. Chemical composition, antibacterial and toxicity activities of *Aquilaria* leaves from three commonly planted species in Malaysia. [Thesis]. Universiti Putra Malaysia, Malaysia.

Adam AZ, Lee SY, Mohamed R. 2017. Pharmacological properties of agarwood tea derived from *Aquilaria* (Thymelaeaceae) leaves: an emerging contemporary herbal drink. J Herb Med 10: 37-44 DOI: 10.1016/j.hermed.2017.06.002.

Adamczyk BJ, Simon V, Kitunen, Adamczyk A, Smolander. 2017. Tannins and their complex interaction with different organic nitrogen compounds and enzymes: old paradigms versus recent advances. Chem Open 6 (5): 610-614. DOI: 10.1002/open.201700113.

Andayani R, Maimunah, Lisawati Y. 2008. Antioxidant activity, total phenolic and lichen levels in tomatoes (*Solanum lycopersicum* L). Jurnal Sains dan Teknologi Farmasi 13 (1): 31-37. [Indonesian]

Azren PD, Lee SY, Emang D, Mohamed R. 2018. History and perspectives of induction technology for agarwood production from cultivated Aquilaria in Asia: a review. J For Res 30: 1-11. DOI: 10.1007/s11676-018-0627-4.

Batubara R, Surjanto, Sihombing TM, Ginting H. 2016. The safety of tea agarwood (*Aquilaria malaccensis*) from tree induction through the test of toxicity subchronic oral 90 days. Biofarmasi 14 (2): 69-76. DOI: 10.13057/biofar/f140205.

Batubara R, Hanum TI, Surjanto. 2018a. Phytochemical and tannin content in two species of agarwood leaves from Mandailing Natal Regency North Sumatera Province. AIP Conf Proc 2049 (1): 030009. DOI: 10.1063/1.5082510.

Batubara R, Surjanto, Purba M. 2018b. The safety of agarwood tea (*Aquilaria malaccensis* Lamk) from induction tree against oral toxicity. Wahana Forestra: Jurnal Kehutanan 13 (1): 1-11. DOI: 10.31849/forestra.v13i1.1399. [Indonesian]

Batubara R, Surjanto, Hanum TI, Handika A, Afandi O. 2020. The screening of phytochemical and antioxidant activity of agarwood leaves (*Aquilaria malaccensis*) from two sites in North Sumatra, Indonesia. Biodiversitas 21 (4): 1588-1596. DOI: 10.13057/Biodiv/d210440.

Fatmawati, Hidayat R. 2016. Anti-cancer activity of *Aquilaria malaccensis* leaves on human cervical cancer cells. Eur J Pharm Med Res 3(1): 46-49.

Feng J, Yang XW, Wang RF. 2011. Bioassay guided isolation and identification of αglucosidase inhibitors from the leaves of *Aquilaria sinensis*. Phytochemistry 72 (2-3): 242-247. DOI: 10.1016/j.phytochem.2010.11.025.

Firdiyani F, Agustini TW, Ma'ruf WF. 2015. Extraction of bioactive compounds as natural antioxidants on fresh *Spirulina platensis* with different solvents. Jurnal Pengolahan Hasil Perikanan Indonesia. 18 (1): 28-37. DOI: 10.17844/jphpi.v18i1.9561. [Indonesian]

Ghan SY, Chin JH, Thoo YY, Yim HS, Ho CW. 2016. Acute oral toxicity study of *Aquilaria crassna* and α-tocopherol in mice. Intl J Pharm. Sci Drug Res 7: 1456-1461. DOI: 10.13040/IJPSR.0975-8232.7(4).1456-61.

Harborne JB. 1987. Phytochemical Methods. Chapman and Hall, London.

Hayati EK, Fasyah AG, Sa'adah L. 2010. Fractionation and identification of tannin compounds on Belimbing Wuluh (*Averrhoa bilimbi* L.) leaves. Jurnal Kimia 4 (2): 193-200.

Hendra H, Moeljopawiro S, Nuringtyas TR. 2016. Antioxidant and antibacterial activities of agarwood (*Aquilaria malaccensis* Lamk.) leaves. AIP Conf Proc 1755 (1): 140004. DOI: 10.1063/1.4958565.

Ito T, Kakino M, Tazawa S, Oyama M, Maruyama H, Araki Y, Hara H, Iinuma M. 2012. Identification of phenolic compounds in *Aquilaria crassna* leaves via liquid chromatography-electrospray ionization mass spectroscopy. Food Sci Technol Res 18 (2): 259-262. DOI: 10.3136/fstr.18.259.

Kakino M, Tazawa H, Maruyama K, Tsuruma, Araki Y, Shimazawa M, Hara H. 2010. Laxative effects of agarwood on low-fiber diet-induced constipation in rats. BMC Complement Altern Med 10: 1-8. DOI: 10.1186/1472-6882-10-68.

Kamonwannasit S, Nantapong N, Kumkrai P, Luecha P, Kupittayanant S, Chudapongse N. 2013. Antibacterial activity of *Aquilaria crassna* leaf extract against *Staphylococcus epidermidis* by disruption of cell wall. Ann Clin Microbiol Antimicrob 12: 1-7. DOI: 10.1186/1476-0711-12-20.

Lee SY, Mohamed R. 2016. The origin and domestication of Aquilaria, an important agarwood-producing genus. In: Mohamed R (eds.). Agarwood. Tropical Forestry. Springer, Singapore. DOI: 10.1007/978-981-10-0833-7_1.

Liu Y, Chen H, Yang Y, Zhang Z, Wei J, Meng H. 2013. Wholetree agarwood-inducing technique: an efficient novel technique for producing high-quality agarwood in cultivated *Aquilaria sinensis* trees. Molecules 18: 3086-3106. DOI: 10.3390/molecules18033086.

López-Sampson A, Page T. 2018. History of use and trade of agarwood. Econ Bot 72: 107-129. DOI: 10.1007/s12231-018-9408-4.

Mabruroh AI. 2015. Test of antioxidant activity on the tannins extract of Rumput Bambu (*Lopatehrum gracile* Broungn) leaves and its identification. [Thesis]. University of Maulana Malik Ibrahim, Malang. [Indonesian]

Mega IM, Swastini DA. 2010. Phytochemical screening and anti-free radical activity on methanol extract of agarwood leaves (*Gyrinops versteegii*). J Kimia 4 (2): 187-192.

Ministry of Health Republic of Indonesia (MoHRI). 2000. Parameter Standar Umum Tumbuhan Obat. MoH RI, Jakarta. [Indonesian]

Molyneux P. 2004. The use of the stable free radical diphenyl-picrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 26: 212-219.

Mulyaningsih TD, Marsono, Sumardi, Yamada I. 2017. Infraspecific diversity of agarwood (*Gyrinops versteegii* (Gilg.) Domke) on the western Lombok island. Jurnal Penelitian dan Konservasi Alam 1: 57-66. DOI: 10.20886/jphka.2017.14.1.57-67. [Indonesian]

Nimnoi P, Pongsilp N, Lumyong S. 2011. Actinobacterial community and diversity in rhizosphere soils of *Aquilaria crassna* Pierre ex Lec assessed by RT-PCR and PCR-DGGE. Biochem Syst Ecol 39: 509-519. DOI: 10.1016/j.bse.2011.07.010.

Nuringtyas TR, Isro, Marina R, Hidayati YSPL, Wijayantiand N, Moeljopawiro S. 2018. The antioxidant and cytotoxic activities of the chloroform extract of agarwood (*Gyrinops versteegii* (Gilg.) Domke) leaves on HeLa cell lines. AIP Conf Proc 2002 (1): 020067. DOI: 10.1063/1.5050163.

Parwata A, Manuaba P, Yasa S, Bidura IGNG. 2016. Characteristics and antioxidant activities of Gaharu (*Gyrinops Versteegii*) Leaves. J Biol Chem Res 33 (1): 294-301.

Prakash A. 2001. Antioxidant activity. Anal Prog 19 (2): 1-4.

Qi J, Lu JJ, Liu JH, Yu BY. 2009. Flavonoid and a rare benzophenone glycoside from the leaves of *Aquilaria sinensis*. Chem Pharm Bull 57 (2): 134-137. DOI: 10.1248/cpb.57.134.

Rahmanto B, Halwany W, Lestari F, Anwar K, Triyasmono L, Rizki MI, Turjaman M. 2018. Characterization of ethanol extract from agarwood (*Aquilaria microcarpa* Bail.) leaf. J Jamu Indones 3 (2): 68-74. DOI: 10.29244/jji.v3i2.56.

Rasool S, Mohamed R. 2016. Understanding agarwood formation and its challenges. In: Mohamed R (eds.). Agarwood. Tropical Forestry. Springer, Singapore. DOI: 10.1007/978-981-10-0833-7_3.

Rohdiana D. 2001. Capture activities of radical polyphenol in tea leaves. Tea and Quinine Research Center, Bandung. [Indonesia]

Said F, Kamaluddin MT, Theodorus. 2016. Efficacy of the *Aquilaria malaccensis* leaves an active fraction in glucose uptake in skeletal muscle on diabetic Wistar rats. Intl J Health Sci Res 6 (7): 162-167.

Santoso E, Subiakto A, Turjaman M. 2011. Silviculture and cultivation technique of agarwood producing plants. Science and Technology V Forestry Book Series. Research and Development Center of Conservation and Rehabilitation, Bogor. [Indonesia]

Sari DI, Rahmawaty D, Apriana D, Amelia R. 2018. Antioxidant activity of prepared gel containing ethanol extract and ethyl acetate fraction of *Aquilaria microcarpa* leaves. Proc Natl Wetl Environ Semin 3 (1): 111-115.

Sari R, Muhani M, Fajriaty I. 2017. Test of the antibacterial activity on ethanolic extract of agarwood (*Aquilaria microcarpa* Baill.) leaves against *Staphylococcus aureus* and *Proteus mirabilis* bacteria. Pharm Sci Res 4 (3): 143-154. DOI: 10.7454/psr.v4i3.3756.

Sattayasai J, Bantadkit J, Aromdee C, Lattmann E, Airarat W. 2012. Antipyretic, analgesic and anti-oxidative activities of *Aquilaria crassna* leaves extract in rodents. J Ayurveda Integr Med 3 (4): 175-179. DOI: 10.4103/0975-9476.104427.

Sudaryat Y, Kusmiati M, Pelangi CR, Rustamsyah A, Rohdiana D. 2015. Antioxidant activity of ten grades of Indonesian black tea (*Camellia sinensis* (L.) O. Kuntze) liquor. Indones J Tea Cinchona Res 18 (2): 95-100. DOI: 10.22302/pptk.jur.jptk.v18i2.70.

Sumarna Y. 2012. Cultivation of agarwood-producing tree species. Forestry Research and Development Agency, Bogor. [Indonesia]

Tan CS, Isa NM, Ismail I, Zainal Z. 2019. Agarwood induction: current developments and future perspectives. Front Plant Sci 10: 122. DOI: 10.3389/fpls.2019.00122.

Turjaman M, Hidayat A. 2017. Agarwood-planted tree inventory in Indonesia. IOP Conf Ser: Earth Environ Sci 54: 012062.

Wahyuningrum M, Sari RK, Rafi M. 2018. Antioxidant and sunscreen activity of *Gyrinops versteegii* extract. Jurnal Ilmu Teknologi Kayu Tropis 16 (2): 141-149. [Indonesian]

Wang H, Zhou M, Lu J, Yu B. 2008. Antitumor constituents from the leaves of *Aquilaria sinensis* (Lour.) Gilg. Linchan Huaxue Yu Gongye. Chem Ind For Prod 28 (2): 1-5.

Wang SC, Wang F, Yue CH. 2015. Chemical constituents from the petioles and leaves of *Aquilaria sinensis*. Biochem Syst Ecol 61: 458-461. DOI: 10.1016/j.bse.2015.07.022.

Wangiyana IGAS, Triandini IGAAH, Putradi D, Wangiyana W. 2018. Tannin concentration of *Gyrinops* tea from leaves of juvenile and mature agarwood trees (*Gyrinops versteegii* Gilg. (Domke)) with different processing methods. J Chem Pharm Res 10 (10): 113-119.

Werdhasari A. 2014. The role of antioxidants for health. Jurnal Biotek Medisina Indonesia 3 (2): 59-68.

Wisutthathum S, Kamkaew N, Inchan A, Chatturong U, Paracha TU, Ingkaninan K, Wongwad E, Chootip K. 2018. Extract of Aqu*ilaria*

crassna leaves and mangiferin are vasodilators while showing no cytotoxicity. J Tradit Complement Med 9 (4): 237-242. DOI: 10.1016/j.jtcme.2018.09.002.

Yelnititis. 2014. The shoot propagation of *Gyrinops versteegii* (Gilg.) Domke. Jurnal Pemuliaan Tanaman Hutan 8 (2): 108-120. DOI: 10.20886/jpth.2014.8.2.108-120.

Yu Q, Qi J, Yu HX, Chen, LL, Kou JP, Liu SJ, Yu BY. 2013. Qualitative and quantitative analysis of phenolic compounds in the leaves of *Aquilaria sinensis* using liquid chromatography-mass spectrometry. Phytochem Anal 24 (4): 349- 356. DOI: 10.1002/pca.2416.