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Chemical compounds contained in young and mature leaves of agarwood species *Wikstroemia tenuiramis* and its antioxidant properties

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Abstract. *Batubara R, Hanum TI, Affandi O, Wahyuni HS. 2020. Chemical compounds contained in young and mature leaves of agarwood species Wikstroemia tenuiramis and its antioxidant properties. Biodiversitas 21: 4616-4622.* The genus *Wikstroemia* is one of the genus of agarwood producing trees which has not been widely researched and published. One type of this genus is *Wikstroemia tenuiramis* Miq, which grows naturally in North Sumatra, the leaves are like other types of agarwood leaves can be used as raw material for agarwood leaves tea. A research has been conducted to determine the chemical contents contained in the young and mature leaves of agarwood plants species *W. Tenuiramis.* This research methods applied qualitative analysis using phytochemical screening, quantitative analysis to determine tannin content using titration method, antioxidant activity analysis using 2,2-diphenil-1-picrylhydrazil (DPPH) method, and compound tracing using Py-gas chromatography-mass spectrometry (Py-GC-MS) instruments. The qualitative phytochemical analysis results showed no difference in chemical compounds contained in both the young and mature leaves. Both the young and mature leaves contained flavonoids, triterpenoids, and tannins, and did not contain alkaloids, steroids and saponins. The young contained tannins of 1.079 ± 0.001% and the mature contained tannins of 4.645 ± 0.021%, not significant statistically. Py-GC-MS analysis showed the presence of various 30 bioactive compounds contained in both young and mature leaves. The analysis results also showed that both the young and mature leaves had very strong antioxidant activity. Our findings suggest that the two types of leaves have equal utility value of in term of chemical contents and antioxidant potentials.

Keywords: Agarwood, antioxidants, mature leaves, phytochemicals, py-GC-MS, young leaves, *Wikstroemia tenuiramis*

INTRODUCTION

Agarwood-producing plant is one of the important plants of forest in Indonesia. The aromatic end products of agarwood have been used in several countries, such as India, Singapore, Malaysia, Japan, the Middle East and the United States. The geographical distribution of agarwood species is widespread in Java, Sumatra, Kalimantan, Sulawesi, Maluku, Nusa Tenggara, and Papua, and it can almost be found in various forest habitats and conditions (Sumarna 2002). Taxonomically, agarwood-producing plants belong to the Sub-Class of Archichlamydeae. The plants consist of three families, namely Thymeleaceae, Euphorbiaceae and Leguminoceae with eight genera, namely Aquilaria, Aetoxylon, Dalbergia, Enkleia, Excoecaria, Gonystylus, Gyrinops and Wiststroemia. In Indonesia, there are currently 27 species of agarwood known to have a living form of trees, shrubs, bushes, and/or as vines (Sumarna 2012).

More recently, research and use of agarwood leaf as an antioxidant by serving it as a contemporary herbal tea beverage product have been developed, revealing the pharmacological properties of agarwood tea derived from the leaves of Aquilaria (Thymelaeaceae) (Adam et al. 2017). This potential research theme is beneficial to be expanded to other genera and species, and not only on the well-known Aquilaria genus. Phytochemical screening on water extract of agarwood leaves showed contents of phenolic compounds, flavonoids, and steroids (Parwata et al. 2016). The leaves of *Grynops versteegii* contained secondary metabolites of flavonoids, terpenoids and phenolic compounds (Mega and Swastini 2010). *Aquilaria* spp. as a source of compounds beneficial to health has been reviewed regarding the traditional uses, phytochemicals, and pharmacological properties (Hashim et al. 2016). In Thailand, the leaves of *Aquilaria crassna* have been used traditionally for the treatments of various disorders (Sattayasai et al. 2012).

Another genus of agarwood producing plants is Wikstroemia. Each species of agarwood has a specific area of distribution and ecology (Mulyaningsih and Yamada 2008). *Wikstroemia androsaemifolia* Decne. was found in Sumba. Another species is *Wikstroemia tenuiramis* Miq or in the local languages known as known as “gaharu cengkeh”. The distribution of *W. tenuiramis* is Sumatra, Bangka and Kalimantan (Sumarna 2012).

The species of agarwood *W. tenuiramis* is a species of agarwood-producing plants that has not been widely studied, especially in the utilization of parts of the tree, specifically its leaves. However, the phytochemical contents varied, depending on the location of leaf sampling (Asmaliyah et al. 2016), and were also influenced by growth factors or maturity of the leaves. For example, the mature leaves of *Clausena lansium* have the same utility values as leaf buds and could be used as sustainable and economical sources of bioactive compounds for nutraceutical and pharmaceutical industrial applications (Chang et al. 2018). The leaf age influence their antioxidants and phytochemicals (phenols and flavonoids) properties (Anwar et al. 2017). Therefore, this research aimed to investigate the chemical contents of young and mature leaves of *W. tenuiramis* and its antioxidant potential. We expect the results of the study provide a new knowledge on whether there is difference between young and mature leaves of *W. tenuiramis* in term of chemical contents and antioxidant potential to inform the utility value of the two types of leaves.

MATERIALS AND METHODS

Time and location research

This research was conducted in October 2017-March 2018. Sample materials were collected from Siantona village, Mandailing Natal District, North Sumatra Province, Indonesia. Raw material preparation, determination of moisture content, and ethanol extraction were carried out at the Forest Product Technology Laboratory, Faculty of Forestry, Universitas Sumatera Utara (USU), Medan, Indonesia. Tannin content was analyzed at the Food Technology Laboratory, Faculty of Agriculture, Universitas Sumatera Utara. Phytochemical screening was carried out at the Laboratory of Biological Natural Chemistry, Faculty of Mathematics and Natural Sciences, USU. Meanwhile, the Py-GC-MS analysis was conducted at the Forest Research and Development Center, Bogor.

Research procedure

Sampling and plant identification

The samples used in this research were the leaves of *W. tenuiramis*. The samples of young and mature leaves were collected and sorted by leaves color (Figure 1).

Figure 1. The leaves of *Wikstroemia tenuiramis* Miq. Bar = 5 cm.

Raw material preparation

The agarwood leaf samples were rinsed with flowing water then spread on parchment paper until the water was absorbed. Then, the samples were dried in a drying cabinet with temperature of 400–500ºC until dry and brittle. The drying aimed to get the simplicia not easily rotten, therefore, can be stored for a longer period. Dried leaves were blended into powder using a blender then put into a sun-protected container before extraction and analysis.

Ethanol extraction of agarwood leaves

The extraction procedure used maceration method following the guidance by Ditjen POM (1995) using ethanol 96% as solvent. As much as 200 g of simplicia powder of *W. tenuiramis* leaves, both the young and the mature, were put into a glass container, poured with 1500 mL of ethanol 96%, kept always closed and protected from light, and occasionally stirred. After five days, the mixture was filtered. The residue then was soaked with ethanol 96% sufficiently to obtain 2000 ml of mixture, then transferred into a closed vessel and left in a cool place protected from light for two days, then filtered. The filtrate was concentrated using a rotary evaporator at 40 °C until a thick liquid was obtained and then dried using a freeze dryer to become a dry extract.

Characterization, determination of moisture content and tannin content analysis

Characterization was carried out on ethanol extracts of the young and mature leaves with organoleptic specific parameters of color, taste and aroma. Moisture content was determined using gravimetric method. Meanwhile, tannin content was analyzed using titration method with $KMnO_4$ solution.

Phytochemical screening

Phytochemical screening was carried out using the Farnsworth method (1996) on the ethanol extracts. The screening covered examinations of bioactive compounds contained in young and mature leaves of *W. tenuiramis* agarwood including groups of organic compounds such as alkaloids, glycosides, flavonoids, steroids/terpenoids, tannins and saponins.

DPPH scavenging activity assay

The ability of the sample to trap DPPH *(1,1-diphenil-2-picrylhydrazil)* as a free radical in methanol solution with an IC_{50} value was used as a parameter to determine the antioxidant activity of the test sample (Prakash 2001). The power of plant extracts to scavenge DPPH free radicals was specified following the methods by Prakash (2001) and Molyneux (2004). The measured absorbance was made at 517 nm, with positive control (ascorbic acid). The percentages of inhibition of the DPPH free radical, as a function of the impact extracts concentrations, were calculated using the equation:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{Test}}{A_{control}},$$

Where: $A_{control}$ = absorbance of control; A_{Test} = absorbance of samples.

The IC50 values indicate the concentration of samples that is needed to trap 50% of DPPH free radical. The IC50 was estimated by nonlinear regression using Excel version 2013.

Py-GC-MS analysis

To identify the bioactive compounds in young and mature leaves of agarwood, the GC-MS analysis was performed using the Py-GC-MS instrument. Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) is an instrumental method that enables a reproducible characterization of intractable and non-volatile macromolecular compounds. It entails application of thermal energy in a helium atmosphere to produce volatile fragments and products from a macromolecule – compounds capable of being analyzed using GC/MS. It differs from GC/MS in the type of sample analysed and the method by which it is introduced to the GC/MS system. Instead of the direct injection of a highly refined organic solution, a few μg of the original natural material is nalysed directly. Thus compounds that are not amenable to analysis by GC and GC/MS are ideal candidates for Py-GC/MS analysis (Sithole et al. 2013). Py-GC/MS is a technique that uses a microscale pyrolysis unit to pyrolyze organic material on a micro-to milligram scale (Ware 2013). Py–GC/MS is an important technique for biomass characterization, because it involves not only the compositional information of the complex component macromolecules, but also the characteristics of volatile pyrolysis products (Gao et al. 2013). The type of tool used is the Shimadzu GCMS-QP 2010.

RESULTS AND DISCUSSION

Extract characterization, phytochemical screening results, and tannin content

Dry concentrated extracts obtained had moisture content of less than 10%. There was no difference between the extract of young and mature leaves (Table 1). Similarly, for the characteristics regarding organoleptic evaluation, there was no difference as well. The difference was only at the yield. The mature leaves yielded more extract than the young ones. However, people commonly consume agarwood leaves regardless of the leaves maturity while mature leaves yield the optimum ability to produce secondary metabolites and therefore the levels of phenolic and flavonoid contents were high (Anwar et al. 2017).

Phytochemical screening was carried out on agarwood leaf extracts to obtain information on secondary metabolite compounds contained in both young leaves and old leaves of *W. tenuiramis*. Table 2 showed the results of the phytochemical screening. Phytochemical screening on the young and mature leaves showed no differences in the groups of chemical compounds.

The quality of tea is affected by the maturity of the leaves and the processing method applied to them. Leaves maturity affects the content and type of polyphenols. Flavonoids and tannins are included in group of polyphenol compounds. Phytochemical screening result obtained provides important information about the chemical compounds contained in the leaves of *W. tenuiramis* agarwood qualitatively. Meanwhile, quantitatively, the mature leaves yielded higher than the young ones. Because the phytochemical results showed that both of them positively contained tannin compounds, further quantitative analysis were carried out on tannins.

Table 1. Characterization results of *Wikstroemia tenuiramis* leaves ethanol extract

Parameters	**Leaves ethanol extract**	
	Young	**Mature**
Yield (%)	7.40 ± 0.28	8.30 ± 0.14
Moisture content (%)	5.35 ± 0.01	5.13 ± 0.06
Organoleptic properties:		
Color	Greenish brown	Greenish brown
Taste	Chelate	Chelate
Smell	Weak aromatic	Weak aromatic

Table 2. Phytochemical screening results of *Wikstroemia tenuiramis*. leaves ethanol extract

Compounds	**Leaves ethanol extract**	
	Young	**Mature**
Alkaloids	-	-
Flavonoids	+	+
Tannin	+	+
Saponin	-	-
Triterpenoid	+	+

Note: **+:** containing the compound, -: not containing the compound

Table 3. Tannins content of *Wikstroemia tenuiramis* leaves ethanol extract

Type of the leaves	**Tanin content (%)**
Young	1.079 ± 0.001
Mature	4.645 ± 0.021

The tannin compounds contained in the mature leaves of agarwood species *W. tenuiramis* are higher than those in the young leaves (Table 3). These results are in accordance with those from previous study on other plants. For example, the level of phenolic and flavonoids compound contained in mature leaves of *Aquilaria beccariana* was higher than in the young and the older leaves (Anwar et al. 2017). Tea drink made from young leaves of seagrass (*Enhalus acoroides*) has lower flavonoids level of 0.0888%, or in other words, tea drink made from the old leaves has higher level of flavonoids total average value compared to young leaves (Tehubijuluw et al. 2018). Additionally, the level of maturity of the leaves, in general, affects the content and the type of polyphenols contained in the leaves of gambier plants (Pambayun et al. 2007).

In contrast, polyphenols contained in young leaves of tea (*Camellia sinensis*) are higher than the mature ones (Izzreen and Fadzelly 2013). Hasan et al. (2000) noted that the younger the tea leaf, the higher the total phenol and catechin. The polyphenols level contained in the young leaf tea is higher than in the old leaf because the aging led to significantly reduced moisture and total phenolic content, therefore, the phenolic profiles also significantly change as the tea leaf getting mature (Liu et al. 2020).

GC-MS Analysis

The results of Py GC-MS analysis are displayed in the form of chromatograms for the highest Retention Time and Concentration (Figures 2 and 3) and the list of compounds identified as constituents contained in the extract of young and mature leaves of agarwood species *W. tenuiramis* (Table 4 and 5). Both the young and mature leaves contained 30 compounds, but the type of the compounds varied. Among the 30 compounds identified, there were only 13 similar compounds contained in the extract of both the young and mature leaves.

The constituents contained in the young leaves of *W. tenuiramis* (Table 4) consisted 11 compounds with concentrations of more than 3%, and among them the top three main constituents were 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%). The most constituent compounds were acidic compounds.

Meanwhile, the main constituents in the mature leaves (Table 5) consisted of 10 compounds with three main compounds were 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%). As well as in the young leaves, the main constituents of the mature leaves were also dominated by acidic compounds.

For comparison, the main content of chemical compounds contained in the methanol extract of Pangi (*Pangium edule* Reinw) leaves is octadecadienic acid 24.60%, whereas the leaves are classified in noncommercial herbs and only used as vegetables by the local community (Tony et al. 2017). Hence, the chemical compounds contained in the leaves of *W. tenuiramis* need to be studied in terms of their potential and subsequent uses.

The leaf of *W. tenuiramis* contained Heptanoic acid (CAS) Heptoic acid (Table 4 and 5). Heptanoic acid is used in the preparation of esters, such as ethyl heptanoate, which are used in fragrances and as artificial flavors. Heptanoic acid is used to esterify steroids in the preparation of drugs such as testosterone enanthate, trenbolone enanthate, drostanolone enanthate, and methenolone enanthate (Primobolan). The triglyceride ester of heptanoic acid is the triheptanoin, which is used in certain medical conditions as a nutritional supplement (Annaken et al. 2006).

Antioxidant potentials of the leaves of *Wikstroemia tenuiramis*

In this research, the mature leaves extract of *W. tenuiramis* had stronger antioxidant activity (IC50 value of 25.860 ± 0.721 ppm) than the young leaves (IC50 value of 26.585 ± 0.689 ppm). However, both are in the same interval category which is very strong (IC50 values < 50 ppm), and not significant statisticcally, therefore, has great potential as sources of antioxidants. Indeed, having strong capacity to act as powerful antioxidants are due to the content of phenolic and flavonoids compounds contained in both the young and the mature leaves are shown in the Tables 2 and 3, where the level of phenolic compounds (i.e. tannins) contained in the mature leaves is higher than in the young. Therefore, it can be infered that the phenolic and flavonoid compounds contained in the *W. tenuiramis* leaves contribute to the antioxidant activity. For comparison, the mature leaves of agarwood species *A. beccariana* that were suggested as the potential antioxidant had IC50 value of 72.25+0.72 ppm (Anwar et al. 2017).

Leaf's extract of the naturally grown *W. tenuiramis* from Mandailing Natal District, Indonesia had IC50 value of 30.482 (Batubara et al. 2019). This is comparable to the IC50 value of *A. mallacensis* Lamk cultivated in the same district with 41.130 μg/ml (Batubara et al. 2019). These suggest that the extract of the leaf of *W. tenuiramis* has the potential to developed into alternative tea beverage which is rich in antioxidants. Moreover, chemical content analysis on the two agarwood species mentioned above showed that the leaves positively contained alkaloids, flavonoids, steroids/triterpenoids, and tannins (Batubaral et al. 2018). Antioxidant activity of ethanol extract and water extract of *W. tenuiramis* is very strong with IC50 values of 27.78 and 28.80 μg/ml in the fresh leaves in ethanol solvent and hot water, respectively, and IC50 values of 25,35 and 28,59 μg/ml for the rotten leaves in ethanol solvent and hot water, respectively (Surjanto et al. 2019).

The results of Py GC-MS analysis also support the antioxidant analysis results since the major compounds contained in both the young and the mature leaves were identified as acid compounds, namely Octadecanoic acid (CAS) Stearic acid. Batubara et al. (2020) noted that the primary compound with the highest concentration in agarwood (*A. malaccensis*) leaves from Laru Village is also Octadecanoic acid (CAS) Stearic acid, contributing to the great role in strong antioxidant capacity of agarwood leaves.

Contrary to the *W. tenuiramis* leaves, the young leaves of *Vernonia amygdalina* possess higher antioxidant and antidiabetic potentials than the old leaves, and differ in their phytochemical compositions and also in pancreatic and hepatic regenerational abilities (Asante et al. 2016). Pomegranate leaves are also an important source of potentially healthy bioactive compounds and antioxidants. Total levels of phenolics and flavonoids decreased significantly in the early stages of leaf growth. However, concentrations of total alkaloids increased during leaf growth and development. Antioxidant activities in pomegranate leaves were significantly correlated with the level of total phenolics and flavonoids. All bioactive compounds measured from pomegranate leaves increased during leaf growth and development (Zhang et al. 2010).

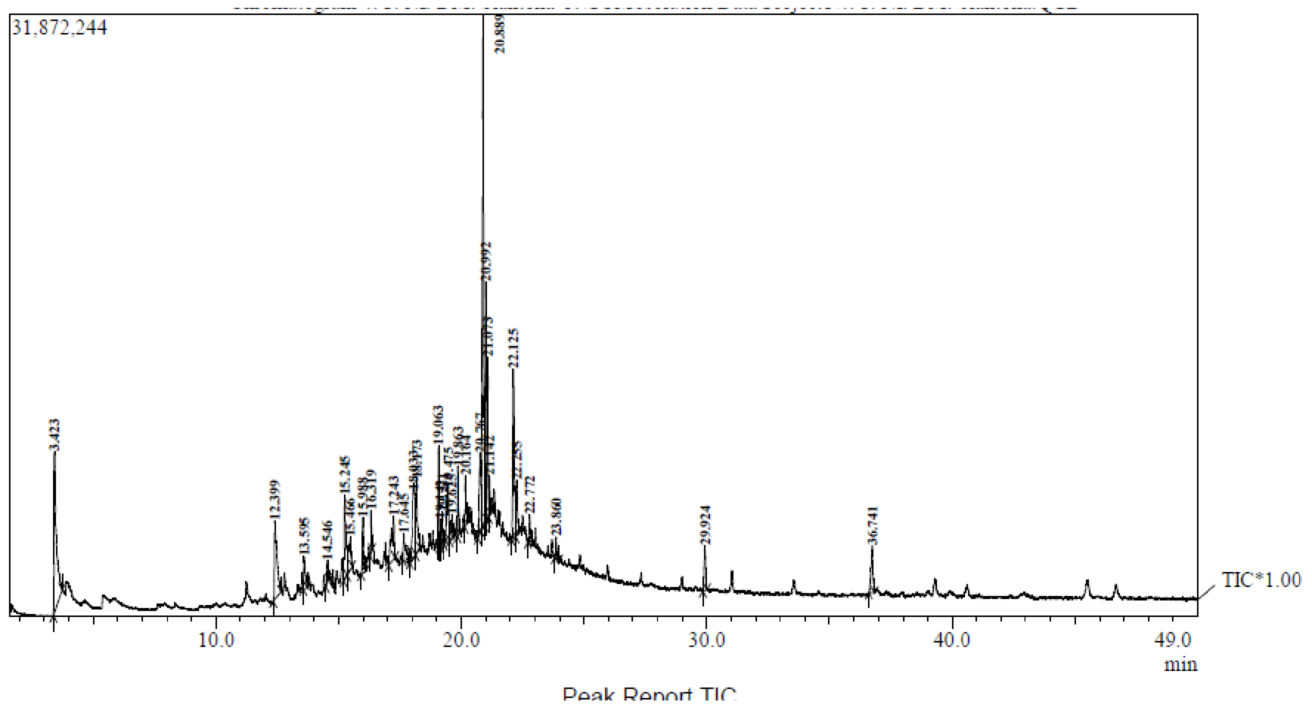

Figure 2. GC-MS chromatogram of ethanol extract from the young leaves of *Wikstroemia tenuiramis*

Table 4. Chemical constituents of ethanol extract from the young leaves of *Wikstroemia tenuiramis* as detected by GC-MS

Peak	Retention time (Rt)	Concentration (%)	Name of chemical constituent
1	3.423	10.75	Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro-(CAS) 1,1-DIBROMO-2-CHLO
2	12.399	5.65	Carbamic acid, phenyl ester (CAS) Phenyl carbamate
3	13.595	1.39	Phenol, 2-methyl-(CAS) o-Cresol
4	14.546	0.61	Heptanoic acid (CAS) Heptoic acid
5	15.245	3.77	Benzaldehyde, 4-methyl-(CAS) p-Tolualdehyde
6	15.466	2.56	Decanoic acid (CAS) Capric acid
7	15.988	1.81	Benzonitrile, 2-methyl-(CAS) 1-Methyl-2-cyanobenzene
8	16.319	0.86	Phenol, 2,6-dimethoxy-(CAS) 2,6-Dimethoxyphenol
9	17,243	2,74	Tetradecane, 1-chloro-(CAS) Myristyl chloride
10	17.645	1.95	Cyclohexane, (3-chloro-1-propynyl)-(CAS) 3-CHLORO-1-CYCLOHEXYLPR
11	18.033	6.04	Heptanoic acid (CAS) Heptoic acid
12	18.173	3.03	4-Hydrazinyltoluene
13	19.063	1.39	Silane, trichloroeicosyl-
14	19.142	0.36	Silane, trichloroeicosyl-
15	19.221	0.79	3,5-Dodecadiyne, 2-methyl-(CAS) 2-METHYLDODEC-3,5-DIYNE
16	19.475	3.51	Cyclopentaneundecanoic acid (CAS) 11-CYCLOPENTANYLUNDECANOIC
17	19.625	1.05	Benzoyl cyanide
18	19.863	1.28	11-Tetradecen-1-ol, acetate, (Z)-(CAS) cis-11-Tetradecenyl acetate
19	20.164	1.38	11-Tetradecen-1-ol, acetate, (Z)-(CAS) cis-11-Tetradecenyl acetate
20	20.767	3.44	9,12-Octadecadienal (CAS)
21	20.889	17.42	Octadecanoic acid (CAS) Stearic acid
22	20.992	8.98	9,12-Octadecadienal (CAS)
23	21.073	3.54	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane
24	21.142	0.70	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane
25	22.125	6.45	9-Octadecenoic acid (Z)-(CAS) Oleic acid
26	22.255	2.55	Silane, trichloroeicosyl-
27	22.772	0.93	2-Undecanethiol, 2-methyl-(CAS) 2-Methylundecyl-2-thiol
28	23.860	0.72	1-Nonene, 4,6,8-trimethyl-(CAS) 4,6,8-TRIMETHYLNONENE-1
29	29.924	1.64	trans-Farnesol
30	36.741	2.72	Tricosane, 2-methyl-(CAS) 2-Methyltricosane

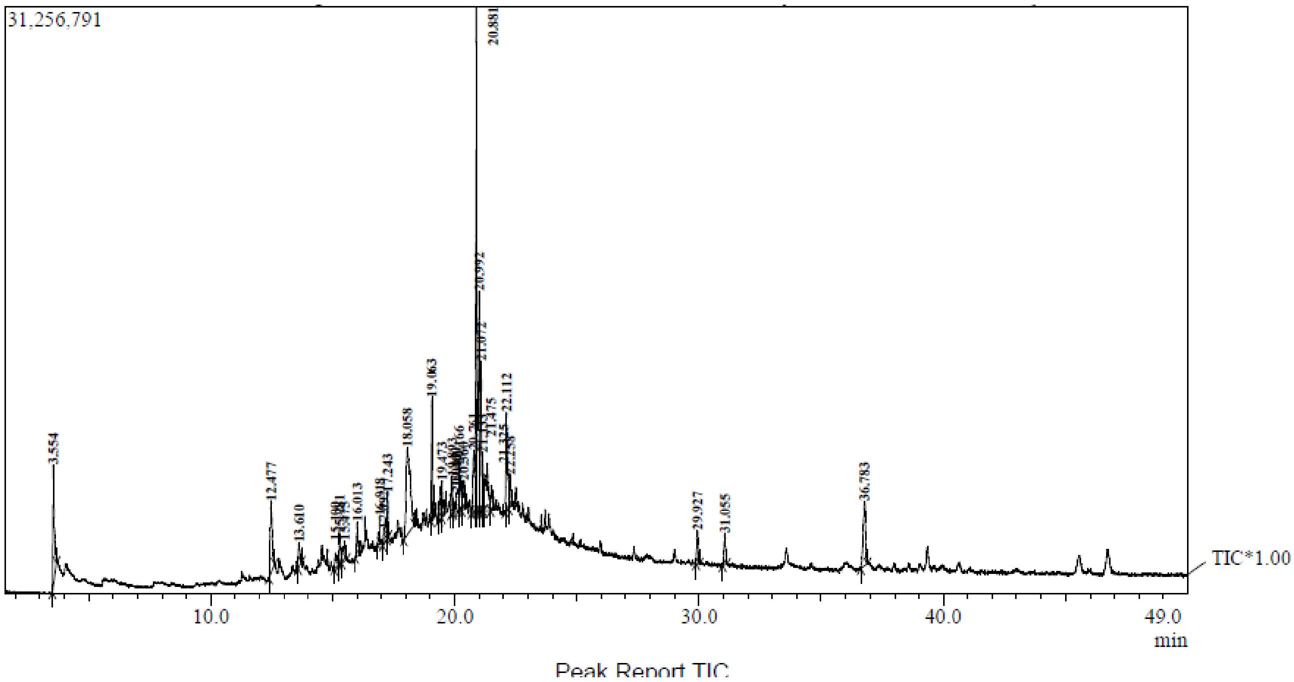

Figure 3. GC-MS chromatogram of ethanol extract from the mature leaves of *Wikstroemia tenuiramis*

Table 5. Chemical constituents of ethanol extract from the young leaves of *Wikstroemia tenuiramis* as detected by GC-MS

Peak	Retention time (Rt)	Concentration (%)	Name of chemical constituent
1	3.554	5.53	Nitrogen oxide (N2O) (CAS) Nitrous oxide
2	12.477	4.45	Cyclohexene, 1-methyl-4-(1-methylethenyl)-(CAS) 1-P-MENTHA-1,8-DIENE
3	13.610	1.02	Phenol, 2-methyl-(CAS) o-Cresol
4	15.100	1.04	2-Propenoic acid, 2-methyl-, ethyl ester (CAS) Ethyl methacrylate
5	15.281	1.36	Benzaldehyde, 4-methyl-(CAS) p-Tolualdehyde
6	15.475	1.45	Heptanoic acid (CAS) Heptoic acid
7	16.013	1.51	7-CYANO(15N)-CYCLOHEPTATRIENE
8	16.918	0.85	Dodecane (CAS) n-Dodecane
9	17.092	1.51	1-Nonyne (CAS) 1-C9H16
10	17.243	0.77	Tetradecane (CAS) n-Tetradecane
11	18.058	11.40	Heptanoic acid (CAS) Heptoic acid
12	19.063	1.99	1-Dodecene (CAS) Adacene 12
13	19.400	1.37	9,12-Octadecadienal (CAS)
14	19.473	1.22	Tetradecanoic acid (CAS) Myristic acid
15	19.893	1.27	Silane, trichloroeicosyl-
16	20.083	1.64	Propanedinitrile, (2-methylcyclohexylidene)-(CAS)
17	20.166	2.49	Octadecanal (CAS) Stearaldehyde
18	20.36	2.26	2-Butanone, 4-cyclohexyl-(CAS) 4-Cyclohexyl-2-butanone
19	20.761	3.06	9,15-Octadecadienoic acid, methyl ester (CAS) METHYL 9,15-OCTADECAD
20	20.881	17.44	Octadecanoic acid (CAS) Stearic acid
21	20.992	10.57	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane
22	21.072	4.46	9,12-Octadecadienal (CAS)
23	21.133	1.50	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane
24	21.325	4.87	5,10-DIETHOXY-2,3,7,8-TETRAHYDRO-1H,6H-DIPYRROLO[1,2-A;1',2'-D]P
25	21.475	0.84	2,4,6-Trimethyl-1-nonene
26	22.112	4.43	9-Octadecenoic acid (Z)-(CAS) Oleic acid
27	22.258	2.03	Silane, trichloroeicosyl-
28	29.927	1.49	trans-Farnesol
29	31.055	1.58	Heptadecane, 2-methyl-(CAS) 2-Methylheptadecane
30	36.783	4.58	Tricosane, 2-methyl-(CAS) 2-Methyltricosane

In conclusion, the ethanol extract from both young and mature leaves of agarwood species *W. tenuiramis* contains the same three phytochemical compounds, namely flavonoids, triterpenoids, and tannins. Futhermore, GC-MS analysis was able to detect the presence of 30 various bioactive compounds contained in both leaves including the same 13 compounds with Octadecanoic acid (CAS) Stearic acid as the highest constituent. These chemical contents, therefore, contribute to the strong antioxidant activity of the ethanol extracts from both young and mature leaves with the IC50 value of 26.585 ± 0.689 ppm and 25.860 ± 0.721 ppm, respectively.

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