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Effect of extraction solvents on chemical characteristics and DPPH scavenging activity of *Aquilaria Malaccensis* leaves

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Aquilaria malaccensis is locally known as "karas" and belongs to Thymelaeaceae family. This study aims to determine the chemical characteristics and antioxidant activity as well as total phenolic content of extracts of different solvents of *Aquilaria malaccensis* leaves. Four different extraction solvents used in this study were; aqueous (AE), 96% of ethanol (EE96), 70% of ethanol (EE70) and hexane (HE). The analysis carried out included Gas Chromatography Mass Spectrometry (GCMS), Fourier Transform Infrared (FTIR) Spectroscopy, total phenolics content (TPC) analysis and antioxidant activity determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH). As a result, EE70 contained the highest TPC value as well as showed the strongest DPPH radical scavenging activity with values 39.9898 mg GAE/g and IC_{50} 92.7714 mg/mL, respectively. Overall findings indicate strong correlation between TPC and DPPH scavenging activity of all samples. Chemical analysis by GCMS and FTIR showed the presence of many chemical constituents and functional groups that were previously reported to be useful as therapeutic agents. FTIR fingerprints assigned were hydroxyl groups, carbonyl groups, aliphatics and glucosides. Meanwhile, GCMS detected compounds under classes of carbohydrates, sugar alcohols, uronic acids, lipids, fatty acids, terpenes and alkanes. The major chemical compounds identified using GCMS included *myo*-inositol, 2,3-butanediol, glycerol, D-lylose, D-glucitol, D-threitol, D-gluconic acid, butanedioic acid, palmitic acid, oleic acid, eicosapentaenoic acid, phytol, squalene, and β-amyrin. Thus, this data was able to contribute more information on the chemical compounds of *A. malaccensis* towards more understanding of this plant potential medicinal values. The compounds also have potential for purification towards application in pharmaceutical and pharmacological industries.

Keywords: *Aquilaria malaccensis*, chemical characteristics, GCMS, FTIR, total phenolics content, DPPH.

INTRODUCTION

Aquilaria malaccensis is a tropical tree belonging to Thymelaeaceae family and locally known as "karas". *A. malaccensis* is one of species from genus *Aquilaria* which is known as the producer of resin impregnated heartwood (Chakrabarty et al., 1994; Naef, 2011). It is a species that adapts to live in various habitats, including those that are rocky, sandy or calcareous, well-drained slopes, ridges and land near swamps (Wiriadinata, 1995). It naturally grows in Bangladesh, Bhutan, northeast India, Indonesia, Iran, Malaysia, Myanmar, Luzon Philippines, Singapore and southern Thailand (Khalil et al., 2013).

A. malaccensis is one of the major agarwood plant (Barden et al., 2000). It has multiple uses and for usages as medicinal, aromatic and in religious purposes (Lee and Mohamed, 2016). It resin has been used as incense as traditional

sedative, analgesic and digestive medicine in East Asia (Feng et al., 2011). Most of the articles regarding *Aquilaria* sp. are discussing about the value of agarwood resin. However, the chemical compositions and benefits of the *A. malaccensis* leaves part are rarely been studied.

Thus, the aims of this study were to determine the chemical characteristics and the DPPH scavenging activity of extracts of different solvents of *A. malaccensis* leaves by using Gas Chromatography Mass Spectrometry (GCMS), Fourier Transform Infrared (FTIR) Spectroscopy and total phenolics content (TPC) analysis, while antioxidant activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH).

MATERIALS AND METHODS

Sample collection and preparation

The fresh leaves of *A. malaccensis* were collected from Merchang Forest Reserve, Terengganu. The collected leaves were washed and cleaned to remove the dirt and impurities before undergoing drying process by oven at 40˚C for 2-3 days until constant weight was obtained. Then, the leaves were ground coarsely by using warring blander. The ground leaves were stored at -80°C prior extraction.

Extraction

Ground *Aquilaria malaccensis* leaves were macerated separately with aqueous, hexane, 96% of ethanol and 70% of ethanol solvent. The ratio of dried leaves to solvent used was 1:20. The macerated samples were shaken for 24 hours at room temperature, followed by sonication for 30 minutes. The samples were filtered using Whatman filter paper No.1 in order to separate the leaves residue and filtrate (supernatant). This processes were repeated for three times.

All the extracts of individual solvent obtained from day-1 until day-3 were combined and concentrated using rotary evaporator at 40 °C. The crude extracts obtained were kept at 4 °C. The weights of the dried extracts were recorded in order to calculate the percent yields.

Total extraction yields

Total extraction yield is a measure of amount of extract in mass compare with the original mass of dried raw material. It was calculated as the weight (g) of crude extract gained from raw material and express as percentage. The yields were calculated by using the formulation stated below:

$$\text{Yields (\%)} = (W1/W2)*100$$

Where; W1 is the weight of extract after evaporation of solvent; W2 is the weight of dried raw material.

Total phenolics content

The total phenolic content (TPC) of each extract was determined using Folin–Ciocalteau reagents according the method described by Slinkard and Singleton (1977). Each 20 µL of extracts or gallic acid (standard) and 20 µL of distilled water were added into the 96-well plate. Next, the mixture was added with 100 µL of Folin-Ciacalteau phenol reagent and incubated for 5 minutes at room temperature. After 5 minutes, 80 µL of 7.5% sodium carbonate solution was added to the mixture and mixed well. The mixture was incubated for 30 minutes under dark condition. The absorbance was measured at 760 nanometer (nm) versus prepared blank by using microplate reader (Zulkipli, 2017). The concentration of phenolics in the extract was determined by plotting the absorbance value on a series of gallic acid calibration curve equation. The total phenolics content was expressed as gallic acid equivalents (GAE), in milligram per gram (mg/g) dry sample and calculated using the following formula:

$$C = (c * V) / m$$

Where; C = Total Phenolic Content (mg/g); c = the concentration of gallic acid established from the calibration curve (mg/mL); V = the volume of extract (mL); m = the weight of pure plant extract (g).

Fourier-Transform Infrared spectroscopy

Fourier-Transform Infrared (FTIR) spectra were obtained by using Perkin Elmer Spectrum 400 Infrared spectroscope coupled with air-cooled Deutrated Triglycin Sulphate (DTGS) detector (Shimadzu, Nakagyo-ku, Kyoto, Japan). The infrared measurement was made at 400 to 4000 cm^{-1} with resolution 4 cm^{-1} and 16 inferograms co-added before Fourier transformation (Azemin et al., 2014; Zulkipli, 2017). Approximately 10 mg of *A. malaccensis* crude extract was put on the crystal until it be covered, by using spatula or tweezers. The pressure clump was swung assembly in order to make sure the tip were located at right above the samples. The clamp

was screwed down till the sample was firmly trap between the tip and the opposing base. The analysis was carried out according to the equipment's software.

N,O-Bis (trimethylsilyl) Trifluoroacetamide (BSTFA) derivatization

Methoxyamination reagent was prepared by dissolving methoxyamine hydrochloride (stored in dessicator) in pyridine at 20 mg/mL. This reagent was prepared fresh each day. Ribitol was used as internal standard as control of retention variations and for normalization purposes.

Approximately 10 mg of crude extract was weighed into glass vial and diluted with 1 mL of solvent to obtain a solution of 10 mg/mL. The sample was sonicated for 15 minutes at 37 °C. 100 µL of sample was mixed with 20 µL of ribitol. The mixture was left for 30 minutes at room temperature, allowing them to homogenize and then vortexed. The mixture was dried using nitrogen gas. Later, dried sample was mixed with 900 µL of acetonitrile and sonicated for 15 minutes at 37 °C. Then, 50 µL of methoxyamination reagent was added into the mixture and then added with 50 µL of N,O-Bis(trimethylsilyl)trifluoro-acetamide (BSTFA) solution. The mixture was vortexed for 1 minute to allow well mixing (Duong et al. 2016; Farag et al. 2016).

Next, the glass vial was wrapped with aluminium foil and incubated for 30 minutes at 100 °C. The lid was closed during the heating process to prevent the sample from drying. After 30 minutes, the sample was centrifuged at 120 rpm for 5 minutes to dissolve any precipitation of the sample. The sample was ready for analysis.

Gas chromatography mass spectrometry

Gas chromatography mass spectrometry (GCMS) Agilent 19091S-433UI system was equipped with HP-5ms Ultra inert capillary column (30 m x 250 µm x 0.25 µm). The derivatized samples were injected into GCMS system with operation conditions; the injector and detector temperature at 250 °C and transfer line temperature at 150 °C. The oven temperature was programmed at 60°C (1 min), 120°C (17 min) at the rate of 5°C per minute. Helium was used as carrier gas at 8.2317 Psi pressure. Individual components of sample extracts were identified using NIST4, MPW011, FIEHN PCT 2013 and FIEHN 2013 Mass Spectral Library. The compounds presence in each extract were selected by analyzing the percentage of probability score above 70%.

DPPH scavenging assay

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was conducted according to the method described by Brand-Williams et al. (1995). The assay was performed in 96-well plate. Serial dilutions of extracts were prepared by carrying 2-fold dilution (concentration ranging from 1000 to 7.8 µg/mL). Aliquots of 50 µL of test samples and quercetin (positive control), respectively were placed in each well. This followed by the addition of 100 µL of DPPH methanolic solution (0.004% w/v) was added. The mixture was incubated for 30 min at room temperature in the dark. Reduction of DPPH was measured at 517 nm using a microplate reader (Multiskan GO, Thermo Scientific, Vantaa, Finland). The percentage of inhibition of each sample was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0-(A_1-A_2)) / (A_0)] * 100$$

Where; A_0 = the absorbance of the reagent blank; A_1 = the absorbance of the test sample; A_2 = the absorbance of the blank sample.

Statistical analysis

The statistical significance analysis for all the parameter was carried out and verified by calculated the value of mean and standard deviation. Analysis of T-test and variance One-Way ANOVA were determined to compare test samples with standard or control and to identify the efficiency of different extraction solvents, respectively. A *p*-value of less than 0.05 was considered the data as statistically significance. Statistical analysis was conducted by using Statistical Package for the Social Science software (SPSS).

RESULTS AND DISCUSSION

Extraction of *A. malaccensis* leaves

Extract yields could indicate the solvents and extraction method's efficiency in extracting out specific components from plant matrix (Gurnani et al., 2015). Among the most important parameters that affected the extraction yield were extraction time and temperature followed by solvent and sample amount (Do et al., 2014). In the present work, the yield of aqueous (AE), 96% ethanol (EE96), 70% ethanol (EE70) and hexane (HE) extracts were calculated as the weight (g) of crude

extract obtained from dried leaves and expressed as percentage of yield (%). EE70 was found to contained the highest yield (21.3%), followed by EE96 (14.7%) and AE (13.5%), while HE was the lowest (0.8%). This study was in agreement with Spigno et al. (2007), who reported that the addition of water to ethanol improves extraction of phytochemicals, thus suggested that more water content brought an increase of concomitant extraction of compounds.

Total phenolics content analysis

Each solvent results in different percentages of compounds recovery from the plant sample. In this study, TPC was expressed as mg GAE/g with standard gallic acid curve equation plotted was y= 5.5353x + 0.0812, R^2 = 0.9957. The most amount of TPC was found in EE70 with 39.98 mg GAE/g followed by aqueous extract with 30.76 mg GAE/g, EE96 and HE with 20.14 mg GAE/g and 0.18 mg GAE/g, respectively (Table 1). There were significance differences found in total phenolics content between different extracts at 1 mg/mL concentration ($p<0.05$), whereas TPC value of EE70 was significantly different with EE96 and HE.

Naczk and Shahidi (2006), reported that one of the important parameter in extraction was solvent polarity, in which the higher the polarity, the better the solubility of phenolic compounds.

Table 1; Total phenolics content different extracts of *A. malaccensis* leaves.

Extract	Total Phenolics Content (mg GAE/g)
AE	30.7608 ± 7.6297[a]
EE96	20.1484 ± 2.6944[b]
EE70	39.9898 ± 11.9526[c]
HE	0.1837 ± 0.0629[d]

Values were given as mean ± STD (n=4). Different superscripts indicates significant different ($p<0.05$). AE: Aqueous extract, EE96: 96% of ethanol extract, HE; Hexane extract, and EE70: 70% of ethanol extract.

Polar solvents are frequently used for recovering polyphenols from plant matrices. Ethanol has been known as a good solvent for polyphenol extraction and was safe for human consumption while methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols, whereas aqueous acetone was good for extraction of higher molecular weight flavanols (Turkmen et al., 2006). This study revealed that the most effective solvent for phenolics extraction was the mixture of aqueous and ethanol solvents which corresponded to some previous reports. The aqueous mixtures containing polar organic solvents such as ethanol, methanol, and acetone were proved in yielding extracts containing high composition of phenols (Mohsen and Ammar, 2008; Dai and Mumper, 2010).

Fourier-Transform Infrared (FTIR) spectroscopy analysis

A. malaccensis leaves of different extracts were analyzed using FTIR to identify the functional components of chemicals in each sample. Figure 1 show six important areas (A1 to A6) with different chemicals fingerprint presence in four different extracts of *A. malaccensis* leaves. Table 1 showed the list of assignment of FTIR analysis in *A. malaccensis* leaves extracts.

The broad peaks in A1 region (3000 cm^{-1} to 3600 cm^{-1}) indicated the characteristics of hydroxyl group (O-H bonds) of water molecules, alcohols, phenolics, carbohydrates or peroxides compounds or N-H bonds of proteins (Easmin et al., 2016). The hydroxyl group presence in these extracts suggests the presence of phenolic compounds. Meanwhile, A2 (2800 cm^{-1} to 3000 cm^{-1}) indicated the presence of stretching vibrations of aliphatic C-H bonds in $-CH_3$ and $-CH_2$ groups in lipids and methoxy derivatives (Azemin et al., 2014). The sharp peaks of HE spectrum in A2 were at wavelengths 2914.44 cm^{-1} and 2846.94 cm^{-1}.

Spectra fingerprints in A3 (1450 cm^{-1} to 1750 cm^{-1}) was assigned to C=O stretching of aldehydes, ketones and esters, carboxyl groups of free acids or ester (Arockia, 2015), C=C stretching associated with aromatic skeletal (Pramila, 2012) as well to N-H bending and C-N stretching vibrations of amino acids (Azemin et al., 2014). Thus, AE, EE96 and EE70 were suggested to contain aromatic constituents (1598, 1616 and 1600, respectively). Meanwhile, signals in A4 (1200 cm^{-1} to 1450 cm^{-1}) suggests that all sample extracts contain the fingerprints corresponds to C-C stretching of phenyl group, O-H bending of alcoholic group and C-O and C-N stretching and N-N bending of amide vibrations (Easmin et al., 2016).

Strong signals in A5 region (950 cm^{-1} to 1150 cm^{-1}) indicates the presence of C-O stretching

vibrations of glucoside bonds (Rashid et al. 2009). The strongest fingerprints at 1043 cm^{-1} were found to be present in AE and EE96. Lastly, only AE, EE96 and EE70 contained fingerprints in A6 region (700 cm^{-1} to 900 cm^{-1}) that correspond to the presence of C=C and C-C compounds (Pramila, 2012).

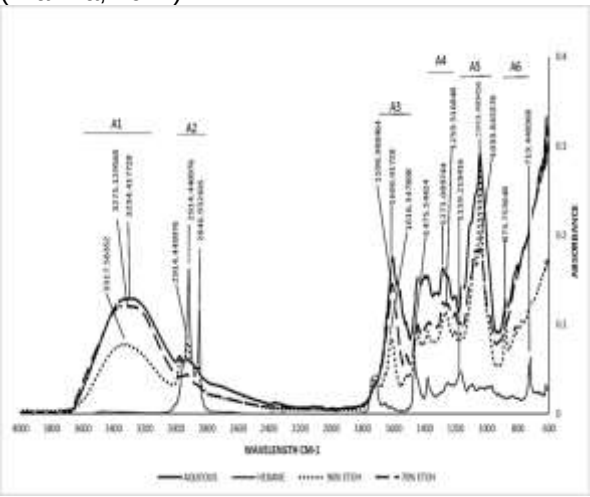

Figure 1; Fourier transform infrared (FTIR) spectroscopy fingerprints of different extracts of *A. malaccensis* leaves.

Table 2; List of assignment of FTIR analysis for biochemical functional groups of *A. malaccensis* leaves extracts.

Fingerprints (cm^{-1})	**Biochemical Components**
Area 1 (3000-3300 cm^{-1})	O-H stretching (water molecules, alcohols, phenolics, carbohydrates, peroxides) or N-H stretching (proteins) vibrations.
Area 2 (2800-3000 cm^{-1})	C-H stretching (aliphatics bonds in $–CH_3$ and CH_2 groups) vibrations.
Area 3 (1450-1750 cm^{-1})	C=O stretching (aldehydes, ketones, esters and carbonyl), C=C stretching (aromatic skeletal), N-H bending, C-N stretching (amino acids) vibrations.
Area 4 (1290-1450 cm^{-1})	C-C stretching (phenyl group), O-H bending (alcoholic group) C-O, C-N stretching and N-N bending (amide) vibrations.
Area 5 (990-1100 cm^{-1})	C-O stretching vibrations (glucoside bonds).
Area 6 (770-800 cm^{-1})	C=C and C-C bending vibrations.

In conclusion, the spectra show all *A. malaccensis* leaves extracts contain chemical fingerprint in A1, A3 and A5 regions except HE. Meanwhile, only HE contained the intense signals in A2 while, EE96 showed moderate intensity. All extracts also contain chemical fingerprint in A3 except HE, thus revealing HE to contain lipophilic, while AE, EE96 and EE70 have high hydrophilic compounds.

Gas chromatography mass spectrometry (GCMS) data analysis

In this study, gas chromatography mass spectrometry (GCMS) analysis was conducted using split mode (2:1) injection. In order to ensure that compounds were volatile and thus accessible for analysis by GC, modification of polar functional groups was necessary.

Thus, derivatization was carried out using reagent, N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) that was capable of reacting with a wide range of classes of compounds (Katona et al. 1999). The total ion chromatogram (TIC) profile shows the peaks of compounds presence in the extracts (Figure 2). Ribitol which was used as internal standard was detected at minute 25.98. The compounds present in the extracts decrease accordingly: EE70 (14 compounds) > EE96 (11 compounds) > AE (10 compounds) > HE (8 compounds). A total of 30 known compounds were identified belonging to different chemical classes including carbohydrates, sugar alcohol, uronic acid, lipids, fatty acids, terpenes and alkanes (Table 3).

Based on the analysis, five major components detected in AE were mostly large amount of sugar alcohols (myo-inositol, 2,3-butanediol and glycerol), a fatty acid (butanedioic acid) and a carbohydrate (D-lylose). Meanwhile, EE96 contains mostly sugar alcohols (D-glucitol and D-threitol), fatty acids (glycerol and eicosa-pentaenoic acid) and a carbohydrate ether (D-psicofuranose,pentakis(trimethylsilyl)ether). EE70 contains large amount of an uronic acid (D-gluconic acid) as well as sugar alcohols (glycerol and D-glucitol), a carbohydrate ether (D-fructofuranose pentakis (trimethylsilyl) ether) and a fatty acid (quinic acid). On the other, the nonpolar HE contains large amount of fatty acids compounds including palmitic acid, oleic acid as well as terpenes including phytol, β-amyrin and squalene (Table 3).

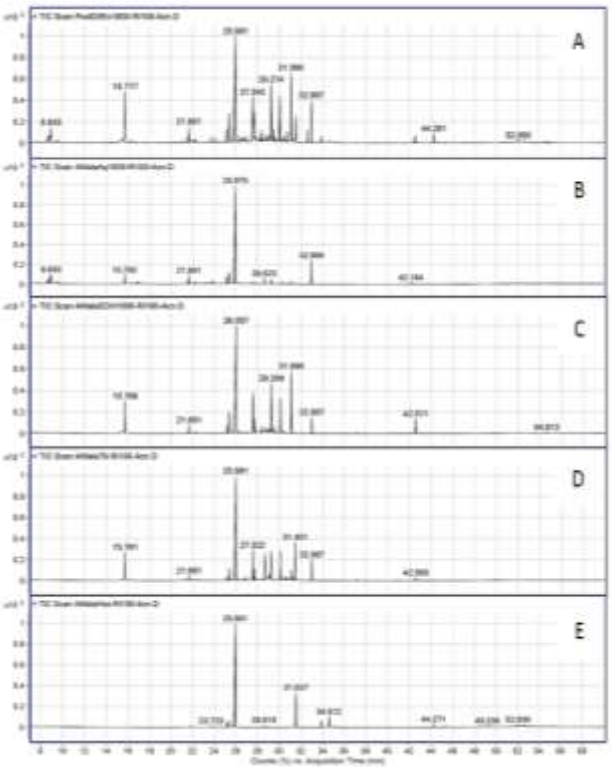

Figure 2; Total ion chromatogram of *A. malaccensis* leaves extracts; A) Mixture of all extracts, B) Aqueous extract, C) 96% ethanolic extract, D) 70% ethanolic extract and E) Hexane extract.

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

DPPH assay was considered as one of the simple colorimetric methods for the evaluation of antioxidant properties (Cheng et al. 2006). DPPH assay was based the principle that DPPH radical accepts an electron donated by an antioxidant compound and then decolorized, which would be quantitatively measured from the changes of absorbance (Raquibul Hassan et al. 2009). In this study, EE96 and EE70 showed the strongest DPPH scavenging activity compared to other extracts (Table 4). The result of increasing DPPH scavenging activity at 1 mg/mL concentration of extracts are; HE (55.5%) < AE (86.5%) < EE96 (92.5%) < EE70 (92.770%) (Table 4).

The extracts concentration used to inhibit 50% of activity (IC_{50}) were accounted to determine the extracts treatment effectiveness Do et al., (2013). The lower the value, the higher the extract treatment capability (Mishra et al., 2012). EE70 and EE96 of *A. malaccensis* leaves showed stronger antioxidant activity with IC_{50} values 0.0424 and 0.049 mg/mL, respectively, compared to other samples. The respective IC_{50} values of AE and HE were 0.05 and 0.92 mg/mL. Meanwhile, antiradical activity was used to define antioxidant action of an antioxidant and also known as reciprocal of IC_{50} (Mishra et al., 2012). Decreasing in the IC_{50} value would result in increase of the antiradical potential of extract. Among all *A. malaccensis* leaves extracts, the strongest antiradical activity was shown by EE (Table 4).

Correlation between chemical components and antioxidant activity

The chemical analyses revealed the presence of many phytochemicals in *A. malaccensis* leaves extracts. In this study, the components of plant macromolecules including carbohydrates, sugar alcohols, uronic acids, lipids, and fatty acids identified using GCMS were *myo*-inositol, 2,3-butanediol, glycerol, D-lylose, D-glucitol, D-threitol, D-gluconic acid, butanedioic acid, palmitic acid, oleic acid, eicosapentaenoic acid. These chemicals play important roles in growth and development of plant (Kuo et al. 2014). Chemicals such as glycerol have also been naturally isolated from crops plants which are maize, pumpkin and bean (Gerber et al. 1988). The of compounds in sugar alcohol classes that were present in AE, EE90 and EE70 extracts corresponded to the presence strong signal of hydroxyl groups (A1) and C-O stretching of glucoside bonds (A5) identified by FTIR analysis.

Table 4; DPPH free radical scavenging activity of different extracts of *A. malaccensis* leaves.

Extract	Maximum inhibition (%)	IC_{50} value (mg/mL)	Antiradical activity, $1/IC_{50}$
AE	86.5 ± 3.1[a]	0.0595 ± 0.0181[a]	18.1142 ± 5.9242
EE96	92.5 ± 0.5[b]	0.0497 ± 0.0046[b]	20.2400 ± 1.9333
EE70	92.7 ± 2.0[b]	0.0424 ± 0.0031[b]	23.6583 ± 1.6216
HE	55.5 ± 2.9[c]	0.9196 ± 0.0130[c]	1.0875 ± 0.0154

*Values were given as mean ± STD (n=4). Different superscripts in column indicates significant different ($p<0.05$). AE: Aqueous extract, EE96: 96% of ethanol extract, HE; Hexane extract, and EE70: 70% of ethanol extract

Table 3; The major compounds detected in extracts of *A. malaccensis* leaves derivatized in acetonitrile solution and analyzed by gas chromatography mass spectrometry (GCMS).

Group	Compounds	RT (min)	MF	RA (%)			
				AE	EE96	EE70	HE
Carbohydrates	L-rhamnose	23.78	$C_{18}H_{44}O_5Si_4$	3.34	ND	ND	ND
	D-2-deoxyribose	25.11	$C_{14}H_{34}O_4Si_3$	ND	4.37	ND	ND
	D-Lyxose	25.11	$C_{17}H_{42}O_5Si_4$	8.36	ND	ND	ND
	D-(-)-fructofuranose, pentakis (trimethylsilyl)ether	27.52	$C_{21}H_{52}O_6S_{i5}$	ND	ND	12.79	ND
	D-psicofuranose, pentakis (trimethylsilyl)ether	27.68	$C_{21}H_{52}O_6Si_5$	ND	8.02	4.57	ND
	L-sorbopyranose	27.74	$C_{21}H_{52}O_6Si_5$	ND	ND	1.21	ND
	D-fructose,1,3,4,5,6-pentakis-O-(trimethylsilyl)-O-methyloxime	28.16	$C_{22}H_{55}NO_6Si_5$	ND	2.03	ND	ND
	1,2,3,5,6-pentakis-O-(trimethylsilyl)-β-D-galactofuranose,	28.34	$C_{21}H_{52}O_6Si_5$	ND	3.09	ND	ND
	D-xylose	29.24	$C_{17}H_{42}O_5Si_4$	5.22	ND	ND	ND
	1,2,3,5-Tetrakis-O-(trimethylsilyl)-α-D-arabinofuranose	31.18	$C_{17}H_{42}O_5Si_4$	ND	ND	1.46	ND
Sugar Alcohols	2,3-butanediol	8.93	$C_{10}H_{26}O_2Si_2$	15.13	ND	ND	ND
	Erythritol	21.68	$C_{16}H_{42}O_4Si_4$	6.98	3.01	1.37	ND
	D-2-deoxyribose	25.11	$C_{14}H_{34}O_4Si_3$	ND	ND	ND	5.90
	D-threitol	25.36	$C_{16}H_{42}O_4Si_4$	ND	14.46	5.89	8.63
	Ribitol	25.98	$C_{20}H_{52}O_5Si_5$	IS	IS	IS	IS
	D-glucitol	30.06	$C_{24}H_{62}O_6Si_6$	ND	22.18	13.52	ND
	Myo-inositol	32.99	$C_{24}H_{60}O_6Si_6$	29.25	9.15	9.79	ND
Uronic Acid	D-gluconic acid	31.45	$C_{24}H_{60}O_7Si_6$	ND	ND	19.56	ND
Lipids	Glycerol	15.75	$C_{12}H_{32}O_3Si_3$	11.03	20.03	13.54	ND
	Diglycerol	25.11	$C_6H_{14}N_2O_5$	ND	ND	1.59	ND
Fatty acids	Lactic Acid	9.53	$C_9H_{22}O_3Si_2$	3.34	ND	ND	ND
	Butanedioic acid	16.91	$C_{10}H_{22}O_4Si_2$	11.07	ND	ND	ND
	Quinic acid	28.62	$C_7H_{12}O_6$	6.27	2.37	11.54	ND
	2,3,4-Trihydroxybutyric acid tetrakis	30.64	$C_{16}H_{40}O_5Si_4$	ND	ND	1.48	ND
	Palmitic acid	31.56	$C_{19}H_{40}O_2Si$	ND	ND	ND	49.92
	Oleic acid	34.61	$C_{21}H_{42}O_2Si$	ND	ND	ND	12.70
	Eicosapentaenoic acid	42.57	$C_{20}H_{30}O_2$	ND	11.30	1.70	ND
Terpenes	Squalene	44.27	$C_{30}H_{50}$	ND	ND	ND	5.23
	β-amyrin	52.04	$C_{33}H_{58}OS_i$	ND	ND	ND	5.82
	Phytol	33.94	$C_{20}H_{40}O$	ND	ND	ND	7.89
Alkane	Nonadecane	45.41	$C_{19}H_{40}$	ND	ND	ND	3.91

*RT: Retention time, RA: Relative area, MF: Molecular formula, IS: Internal standard, AE: Aqueous extract, EE96: 96% of ethanol extract, HE; Hexane extract, and EE70: 70% of ethanol extract.

In this study, secondary metabolites including phytol, squalene, and β-amyrin were previously attributed as bioactive component. Phytol compound is an important diterpene that possesses antimicrobial, antioxidant, and anticancer activity (Raman et al., 2012). Similarly, the compound β-amyrin was known to possess antidiabetic, anti-inflammatory, antiarthritic and anticancer activities (Ghosh et al., 2015). Other than that, squalene was a triterpene that reported to act as natural antioxidants and possess various pharmacological importance (Kim and Karadeniz, 2012).

The presence of various antioxidant compounds have different solubility in a particular solvent. These compounds protect plants, fruits, and vegetables from oxidative damage and have been used as antioxidants by humans. Finding new and safe antioxidants from natural sources was of great interest for applications as natural antioxidants, functional foods, and nutraceuticals (Do et al., 2013). It has been proven that the antioxidant effect of plant extracts could mainly attributed by phenolic compounds such as flavonoids, polyphenols, tannins and phenolic terpenes (Shahidi et al., 1992). In the current study, strong correlation of total phenolics content have the antiradical of DPPH scavenging activity was detected (data not shown). Thus, this prove that plant antioxidants include of a broad range of different chemicals such previously reported which includes ascorbic acid and tocopherols, polyphenolic compounds or terpenoids. Terpenes which were identified by GCMS in this current study, are the main components of essential oils, and could function as allelopathic agents, attractants in interactions of plant-plant or plant-pathogen/herbivore or repellants (Grassmann, 2005).

CONCLUSION

In this study, chemical analysis conducted on *A. malaccensis* leaves extracts by using GCMS and FTIR showed that those extracts contained variety of chemical constituents and important functional groups. GCMS analysis had revealed the presence of alcohol, sugar alcohol, ester, ether, amine, aldehyde, lipid, fatty acids, phenolics, and carbohydrate chemical classes. It corresponded to FTIR analysis of *A. malaccensis* leaves extracts that exhibit important functional group such hydroxyl groups, carbonyl groups, aliphatics and glucoside compounds. The presence of bioactive compounds in the sample extracts detected by GCMS corresponded to the antiradical properties of extracts determined by DPPH scavenging activity. Based on this data, further studies by isolation and purification steps were strongly recommended to be done in order to identify and characterize each bioactive compound present in this plant.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

ZMR designed the experiment. NACZ and ZMR performed the experiment and analyzed the data. NACZ, ZMR, WNWA and AKY wrote the article. ZMR and WNWA provided supervision and research facilities. NHM provided intellectual inputs and financial support via grant.

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