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Aquilaria malaccensis Leaf as an Alternative Source of Anti-inflammatory Compounds

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Abstract*— Currently, the long-term consumption of aspirin and non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs) as anti-inflammatory medicines and pain relievers, have been reported to cause various side effects. Thus, natural compounds of several plant species including *Aquilaria malaccensis* have been explored as an alternative therapeutic source for inflammation treatment with regard to their safety and efficacy. Despite the accelerating rate on the research of agarwood leaf, the scientific evidences to elucidate the proclaimed pharmacological activities particularly anti-inflammatory activity are still limited. Therefore, it is the interest of this study to investigate the biological activity relating to the anti-inflammatory activity of *A. malaccensis* leaf extracted using Soxhlet and Supercritical Fluid Extraction (SFE) methods. Results showed that *A. malaccensis* leaf ethanolic soxhlet extract (ALEXB) gave higher yield (mg/g) of 98.33 ± 4.11 (9.83% wt/wt) as compared to soxhlet extract using hexane (ALEXA); 24.04 ± 5.27 (2.40% wt/wt) after 6 hours of extraction. Meanwhile, the supercritical fluid extract (SFEX) gave a relatively low yield of 12.57 ± 0.61 (1.26% wt/wt). The GCMS analysis revealed that 25, 30 and 16 compounds were detected in ALEXA, ALEXB and SFEX respectively with phytol as the major compound in the soxhlet extracts and n-hexadecanoic as a major compound in SFEX. Subsequently, in-vitro study showed that the extracts demonstrated inhibition of protein (albumin) denaturation in a concentration-dependent manner throughout a concentration range of 400-16000 µg/ml tested. Exclusively, the GCMS of leaf SFEX showed a peak of 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene (0.4205%), a tricyclic sesquiterpene that was testified to have potential analgesic and anti-inflammatory activity. Further research is warranted to explore the anti-inflammatory activities of *A. malaccensis* leaf extracts and their mechanism of action as an alternative halal ingredient for nutraceuticals and pharmaceuticals.

***Keywords*— anti-inflammatory; *Aquilaria malaccensis*; halal; SFE; Soxhlet**

I. INTRODUCTION

Inflammation is a complex biological response of the immune system to tissue damage or infection. Although inflammation is a crucial element in maintaining normal body's homeostasis, it plays pivotal role in a number of chronic devastating diseases, including cancer, diabetes, arthritis and cardiovascular diseases [1].

Currently, the long-term consumption of aspirin and non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs) as anti-inflammatory medicines and pain relievers, have been reported to cause various side effects such as kidney toxicity, haemorrhagic stroke, gastrointestinal ulceration and bleeding [2], [3]. Thus, natural compounds of various plant species have been explored as an alternative and halal therapeutic source for inflammation related conditions [4], [5]. Being halal is not only confined to the Islamic concept which means the product is free from any ingredients that is prohibited in Islam, but it is a novel benchmark for safety and quality assurance to ascertain the product is safe, pure and harmless [6], [7].

Plenty of plant extracts have been recognized to demonstrate anti-inflammatory activity, and therefore traditionally used for treatment of inflammation in topical and oral preparations. Several parts of *Aquilaria* species have also been reported to exhibit anti-inflammatory effect in a number of recently published articles [8]-[11].

Agarwood species have been utilized for myriad types of applications mainly as folk medicine, perfume and incense. Agarwood has also been counted as an Ayurvedic,

traditional Chinese medicine as well as traditional Thai medicine [12]. In particular, *Aquilaria malaccensis* is one of the *Aquilaria* species that belongs taxonomically to family Thymelaeaceae. This genus is mainly distributed in Malaysian rainforests. *A.malaccensis* is the main producer of the resin-impregnated fragrant wood (known as agarwood) formed in the trunk of the tree following injuries or infection [13].

Agarwood plant has been mentioned in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora and protected as an endangered species (CITES) following the dwindling number of the wild trees due to irrational cutting to obtain its aromatic resin [14], [15]. In response to the scenario, there is now an increasing number of agarwood plantation that is managed more systemically; with abundant and continuous supply of leaf as source of raw material.

Agarwood leaf generally has a length of 5 to 11 cm and diameter of 2 to 4 cm with an elliptical blades shape [16]. A wide range of functional groups with diverse potential bioactive compounds has been identified in agarwood leaf [17]. It is reported that the agarwood leaf extract possesses various pharmacological effects including anticancer, antiallergic, antiviral as well as anti-inflammatory [17], [18]. In Malaysia, the decoction of *A. malaccensis* leaf has been reported to be applied externally to treat swelling and consumed to treat vomiting [19]. Meanwhile, the medicinal health effects of agarwood tea (the most popular agarwood leaf-based product in the market) have been recorded for many years. Ibrahim (2016) stated that, the herbal tea of *A. malaccensis*, *A. crassna* and *A. sinensis* were found to have antidepressant and anti-aging properties [19].

Despite the accelerating rate on the research of agarwood leaf, the scientific evidences to elucidate the proclaimed pharmacological activities are still limited. Pharmacological actions of crude extracts from various parts of *Aquilaria* species summarized by Hashim *et al.* (2016) showed that anti-inflammatory activities were observed in the ethanolic and methanolic leaf extract of *A. sinensis* (Lour) Gilg. and *A. crassna* respectively [17]. However, to the best of our knowledge, the anti-inflammatory activity from *A. malaccensis* leaf has not been reported. Therefore, it is the interest of the current study to explore the anti-inflammatory activity of *A. malaccensis* leaf extracted using Soxhlet method and Supercritical Fluid Extraction (SFE), intending to reveal the evidence for its folkloric uses as well to initiate further exploitation of halal, safe and effective abundant resources of *A. malaccensis* leaf.

II. The Material and Method

A. Plant Material

Fresh leaves of non-inoculated *A. malaccensis* (agarwood plant which was not being induced or injected with microbial concoction for development of resin) were freshly harvested from local agarwood farm in Bangi, Selangor, Malaysia in February 2018. The leaves were oven-dried at a temperature of 40^{o}C overnight. The sample was then ground to a size of ≤ 200 μm. The powder was kept in the dark in sealed glass bottles at room temperature.

B. Chemicals

Analytical grade reagents and solvents used in this experiment were obtained from Sigma-Aldrich Chemicals (St. Louis, MO, USA) and Merck Chemicals (Darmstadt, FR Germany) unless otherwise stated.

C. Extraction

1) *Soxhlet extraction:* Soxhlet extraction method was performed using 300 ml of hexane and ethanol as the solvent respectively. An amount of 6 grams of the powder was placed in the thimble in the Soxhlet chamber. The extraction was run for 6 hours. Change of colour of solvent in the thimble into its original state indicated that all bioactive compounds have been extracted. The extract was evaporated under reduced pressure at 40^{o}C using a rotary evaporator (Heidolph-instruments, Rotavapor, Germany) to obtain concentrated hexane and ethanolic extracts. The weight of the crude extracts were measured after evaporation of excess solvent and preserved in a petri dish sealed with aluminium foil for further analysis [20]-[21].

2) *Supercritical fluid extraction:* Supercritical fluid extraction was performed in the SFE unit (Supercritical Fluid Centre, Faculty of Food Science and Technology, Universiti Putra Malaysia). The process was done for two batches. The extractor vessel was loaded with 480.5 g (batch 1) and 420 g (batch 2) of dried and ground *A. malaccensis* leaves with particle size ≤ 200 μm. Liquid carbon dioxide flow into the extractor vessel at a flow rate 6-7 kg/hr at a temperature of 50^{o}C and pressure adjusted to 350 bar. The extract was separated at the separator vessel once carbon dioxide changes to gaseous state upon reducing the pressure.

The yields obtained from the two extraction methods were calculated by using the following equation (1) [20]:

$$y = W_2/W_1 \quad (1)$$

Where y: yield of extract (mg/g)
W_1: weight of sample (g)
W_2: weight of agarwood leaf extract (mg)

D. Gas chromatography-mass spectrometry (GCMS) analysis of A.malaccensis leaf extracts

1) *Sample preparation: A.malaccensis* leaf soxhlet extracts were diluted in respective solvent and supercritical fluid extract was dissolved in hexane to obtain 1 % (w/v) extract, respectively. 10 μl of the prepared extract was mixed with 990 μl of the respective solvents into 1 ml microcentrifuge tube and stirred well. After filtration, the mixture transferred into vials for GCMS analysis. All samples were prepared in triplicates [22].

2) *GCMS analysis:* The volatile constituents of the triplicate *A.malaccensis* leaf extract samples were analysed by using gas chromatography system; Agilent 7890A (Agilent Technologies) coupled with Agilent 5975C quadrupole mass spectrometer and autosampler. Hewlett Packard HP-5MS ultra inert silica capillary column (30 m x 0.25 mm; 0.25 μm) was used. The analytical conditions for GCMS are listed in Table 1. The detected peaks from the total ion chromatography (TIC) and mass chromatograms

were identified based on National Institute of Standards and Technology (NIST) 2008 mass spectral library [23].

TABLE 1.
ANALYTICAL CONDITIONS FOR GCMS ANALYSIS BASED ON HASHIM ET AL [23].

				Conditions	
Carrier gas				He	
Gas flow				2 ml/min	
Split Ratio				1:50	
Injection Volume				1 µl	
Mode				Split	
Interface temperature				250 °C	
Electron impact (emission current)				70 eV	
Scan range				32 to 500 amu	
Oven Program		Rate (°C/min)	Value (°C)	Hold time (Min)	Run time (Min)
	Initial	-	80	2	2
	Ramp	10	250	10	29

E. In-vitro anti-inflammatory assay

The assay was conducted following the procedure in [5] with some modification. The reaction mixture consisted of 2 ml of varying concentrations of the test extract (400, 800, 2000, 4000, 8000 and 16000 (µg/ml), 0.2 ml of egg albumin and 2.8 ml phosphate buffer saline (pH 6.4). Equal volume of deionized water served as control. The mixtures were incubated for 15 min at 37°C ± 2°C in a biological oxygen demand incubator and heated afterwards for 5 min at 70°C. After cooling, the absorbance of the test and control solutions was measured at λ=660 nm. The assay was done in triplicates. The percentage of inhibition of protein denaturation was calculated by using the following equation (2) [5]:

$$\%\ Inhibition = 100\ x\ \left[\left(\frac{Vt}{Vc}\right) - 1\right] \qquad (2)$$

Where Vt: absorbance of test sample
Vc: absorbance of control

III. RESULT AND DISCUSSION

A. Yield of Aquilaria malaccensis leaf extracts

Results presented in Table 2 and Table 3 showed that Soxhlet (ALEX) extraction method with ethanol (B) as solvent gave the higher yield (mg/g) of 98.330 ± 4.113 (9.83% wt/wt) as compared to ALEX with hexane (A) as solvent 24.039 ± 5.268 (2.40% wt/wt) after 6 hours of extraction, and (SFEX) obtained by SFE extraction 12.57 ± 0.608 (1.25% wt/wt). Similarly, Hendra *et al.* (2016) reported that the *A. malaccensis* leaf extracted using Soxhlet with different types of solvents had the yield percentage of methanol (9.2%), chloroform (3.4%) and water (1.9%) [24]. Bimakr *et al.* (2011) revealed that the yield percentage of spearmint (*Mentha spicata L.*) leaf extracted using Soxhlet for 6 hours, 3 grams of sample and 150 ml of methanol and ethanol as solvent were 8.91% and 7.27% respectively [25]. Meanwhile, a former study conducted by Muruganandam *et al.* (2017) discovered that the optimum yield of *Piper betle* leaf ethanolic Soxhlet extract was 10.94% with optimum extraction time of 3 hours, temperature of 71.91°C, solvent volume of 281.43 ml and sample weight of 2 grams [26]. The yield obtained in this present study is comparable with the previous studies. Studies showed that the yield of extract is highly influenced by the types of solvent regardless of the solvent volume, extraction time and sample weight. The present and previous studies have shown that the mid-polar solvent such as ethanol and methanol gave the highest yield as compared to non-polar and polar solvent. The higher yield of ALEXB as compared to ALEXA could be due to the existence of hydroxyl (OH) group and ethyl (C_2H_5) group in ethanol that enable the solvent to extract both polar and nonpolar compounds respectively [26]. Unlike hexane, the solvent can only dissolve nonpolar compounds [26]. Eventhough polar and non-polar solvents vary in their abilities and effeciencies in extracting the bioactive compounds, in terms of yield, it can be concluded that polar solvent promoted a higher yield of extract as compared to non-polar solvent [24].

In the current study, SFE yielded relatively small amount of *Aquilaria malaccensis* leaf extract compared to the conventional Soxhlet method using ethanol and hexane. The result is consistent with a similar study in literature that aimed to compare the yields of the Soxhlet and SFE methods of Nettle Root (*Urtica dioica* L.), and it was recognized that the highest yield percentage was attained by Soxhlet extraction with ethanol (14.14%), followed by Soxhlet extraction by n-Hexane (0.768%), while the supercritical extraction has a lower yield (0.537%) [27]. Similarly, 70% ethanol soxhlet extraction showed a higher crude extract yield percentage (8.58%) compared to the SFE extraction (0.2%) in extracting spearmint (*Mentha spicata* L.) leaves [25]. The Extraction of Peanut Skin using ethanol soxhlet extraction a higher yield percentage (36.282%) as compared with supercritical CO2 extraction (15.47%) [28]. Despite its relatively low yield percentage, some studies testified that the extract obtained by SFE featured better quality and greater number of flavonoids [25], as well as higher phenolic content and enhanced radical scavenging activity [29] compared to Soxhlet extraction. SFE was likewise used in extracting Aquilaria species in a number of studies. *A. malaccensis* stem bark was ground to a particle size ≤ 500 µm and extracted by SFE at 50°C, under pressure of 34.5 MPa; with CO2 flow rate ≤ 1ml/min, for 30 min., and the yield obtained was as low as 3.66 g oil/100g sample [19]. It was also observed that the addition of a co-solvent to SFE process increased the yield obtained as reported in a study performed on the dried agarwood oil of *Aquilaria crassna* by Wetwitayaklung *et al.* (2009), which showed that the yield percentage without co-solvent (0.06%) increased to 0.14% upon addition of ethanol co-solvent to the extraction process [30].

TABLE II
YIELD OF *AQUILARIA MALACCENSIS* LEAF CRUDE EXTRACT (ALEX) OBTAINED THROUGH SOXHLET; A: HEXANE, B: ETHANOL: VALUES ARE MEAN ± SD.

Sample	**ALEXA**	**ALEXB**
Weight of sample/run (g)	6.01 ± 0.01	6.01 ± 0.00
Weight of extract (mg)	144.30 ± 31.53	591.00 ± 24.76
Yield (mg/g)	24.04 ± 5.27	98.33 ± 4.11
Yield % (wt/wt)	2.40 ± 0.53	9.83 ± 0.41

*wt: weight

TABLE III
YIELD OF *AQUILARIA MALACCENSIS* LEAF CRUDE EXTRACT OBTAINED THROUGH SUPERCRITICAL FLUID EXTRACTION (SFE)

Sample	SFEX	
	Run 1	**Run 2**
Weight of sample	480.50	420.00
Weight of extract (mg)	6250.00	5100.00
Yield (mg/g)	13.00	12.14
Average yield	12.57 ± 0.61	
Yield % (wt/wt)	1.30	1.21
Average yield %	1.26 ± 0.06	

B. GCMS analysis of Aquilaria malaccensis leaf extracts

Methods of extraction may affect the chemical profile of the leaves. GCMS analysis revealed that the different types of extracts gave different number of peaks and compounds; ALEXA (26 peaks with 25 compounds), ALEXB (40 peaks with 30 compounds) while SFEX (16 peaks with 16 compounds). Phytol was the most abundant compound detected at retention time of 18.26 min which accounted for 34.062% and 29.267% respectively of all ALEXA and ALEXB contents, while Phytol was absent in the SFEX. On the other hand, n-hexadecanoic recorded the highest level (38.93%) in SFEX. Due to the solvent power of CO_2, SFE is proposed to dissolve non-polar hydrophobic compounds, while the extraction of polar hydrophilic components is challenging [31]. Accordingly, the absecnce of phytol (a polar diterpene alcohol) in SFEX is justifiable. However, Phytol was reported earlier in the chemical composition of *Mentha spicata* L. obtained by SFE in the presence of 20% ethanol as a polar co-solvent [31], while it also appeared in SFE extract of *Acalypha indica* without co-solvent and under different extracting conditions (40°C and 60°C, 300 bar, 28.25 g/min) [33]. Nevertheless, n-hexadecanoic acid is soluble in CO_2 due to the prescence of long hydrocarbon chain that increases the non-polar characteristic of the compound. A greater percentage of n-hexadecanoic acid in SFE extract than Soxhlet extract was detected in three different Lamiaceae species [34].

Similarly, GCMS analysis conducted by Lee *et al*. (2016) on *A. malaccensis* leaf essential oil extracted using Soxhlet method with isopropanol as solvent revealed that the oil contained n-hexadecanoic acid (76.3%), octadecatrienoic acid (30.0%), squalene (32.8%) and phytol (28%) [21]. Phytol, the major bioactive compounds of ALEXA and ALEXB was reported to be a precursor of synthetic vitamin E and K [21]. Phytol was also reported to have properties of anticancer, antimicrobial and anti-inflammatory [22], [35]-[37]. On the other hand, n-hexadecanoic acid which was present in the three extracts and predominant in SFEX is reported to have antiandrogenic, antioxidant, antimicrobial and anti-inflammatory properties [35], [37].

Exclusively, the GCMS of SFEX showed a peak of 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene (0.4205%), a tricyclic sesquiterpene that was reported to have potential analgesic and anti-inflammatory effect [38]. 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene (also known as Aromadenderene), is presumed to be the biomarker compound of Agarwood [39], and plays an important role in grading Agarwood quality [40]. Since temperature affects the extraction of chemical compounds, Aromadendrene, considered a volatile odor-active component [41] was probably lost during heating in Soxhlet extraction.

The presence of the phthalic acid esters is noticeable in SFEX and to a lesser extent in ALEXA, due to their comparable ability to dissolve non-polar compounds. It is worth to mention that Phthalic acid is an aromatic dicarboxylic acid with a distinguishable odor and is thought to be an adulterant in agarwood oil [42].

The relative contents expressed in the form of percentage of all compounds detected in ALEXA, ALEXB and SFEX analysed by GCMS are listed in Table 4. Meanwhile, the chromatograms of ALEXA, ALEXB and SFEX are presented in Fig. 1

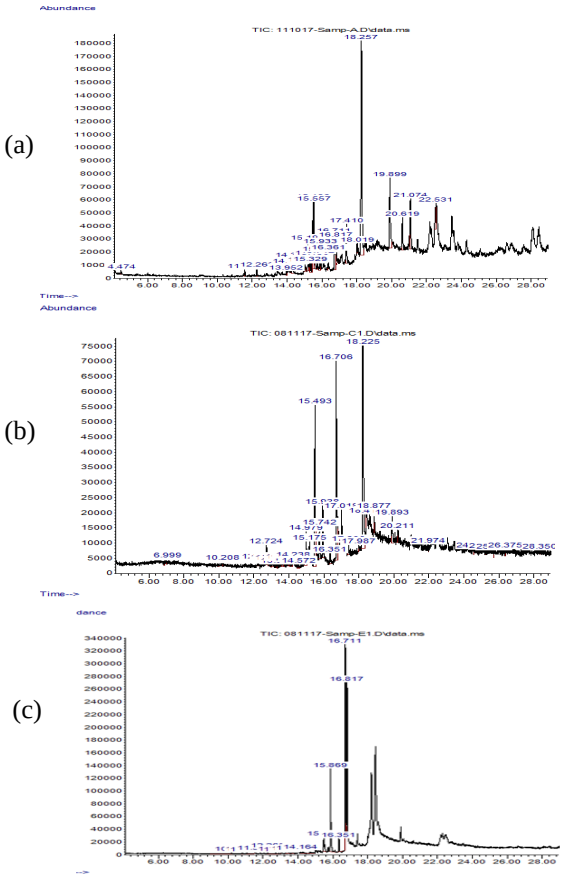

Fig. 1 GCMS Chromatogram of *A.malaccensis* leaf extract (a) Soxhlet; A: Hexane, (b) Soxhlet; B: Ethanol and (c) Supercritical Fluid Extraction

C. In vitro anti-inflammatory activity of Aquilaria malccensis leaf extracts

Protein denaturation is among the manifestations associated with inflammation [43]-[45]. Consequently, agents that able to inhibit protein denaturation would be an alternative for the development of anti-inflammatory drug [5]. Hence, in-vitro study was performed as a preliminary screening to investigate the presence of anti-inflammatory activity prior to in-vivo study.

TABLE IV
OVERALL BIOACTIVE COMPOUNDS IDENTIFIED IN ALEX; A:HEXANE AND B:ETHANOL AND SFEX: CO2 ANALYSED BY GCMS

No.	RT	Library/ID	Peak Area (%)		
			ALEXA	ALEXB	SFEX
1	4.47	1,6:3,4-Dianhydro-2-deoxy-.beta.-d-lyxo-hexopyranose	0.388	-	-
2	6.88	D-erythro-Pentose, 2-deoxy-	-	1.457	-
3	10.00	Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid	-	0.863	-
4	10.20	D-erythro-Pentose, 2-deoxy-	-	-	1.645
5	10.21	Butanoic acid, 3-oxo-, propyl ester	-	0.243	-
6	10.82	Acetic Acid	-	-	0.32
7	11.076	Methyl N-(thioformyl)dithiocarbamate	-	-	0.4925
8	11.505	Alpha,alpha,4-trimethylbenzyl carbanilate	-	-	0.2596
9	11.57	4(1H)-Pteridinone, 2-amino-6-methyl-	0.586	-	-
10	12.26	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	0.755	-	1.5893
11	12.71	D-erythro-Pentose, 2-deoxy-	-	-	0.5692
12	12.72	Decane, 1-fluoro-	-	1.470	-
13	13.00	Octanal	-	0.223	-
14	13.009	Acetic Acid	-	-	0.2804
15	13.305	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	-	-	0.4205
16	13.31	.alpha.-D-Mannopyranoside, methyl 3,6-anhydro-	-	1.045	-
17	13.480	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-,(E)-	-	-	0.9932
18	13.70	Tetraacetyl-d-xylonic nitrile	-	0.205	-
19	13.724	(3,7,7-Trimethyl-bicyclo[2.2.1]hept-2-yl)-methanol	-	-	1.1163
20	13.95	Sulfurous acid, 2-propyl undecyl ester	0.398	-	-
21	13.97	Piperidine, 3,5-dimethyl-	-	0.332	-
22	14.163	8-Dodecenol	-	-	2.3618
23	14.17	Carane, 4,5-epoxy-, trans	0.706	-	-
24	14.17	Cyclodecanol	-	0.601	-
25	14.32	3-Nonene	1.572	-	-
26	14.98	Behenic alcohol	-	3.802	-
27	15.05	Eicosane, 9-octyl-	0.584	-	-
28	15.18	5-Methyl-1,3-diazaadamantan-6-one	-	2.910	-
29	15.19	2-Propanol, 1-chloro-, phosphate (3:1)	2.387	-	-
30	15.27	5-Methyl-2-pyrazinylmethanol	0.447	-	-
31	15.33	1-Pyrazolidinethiocarboxanilide, 4-ethyl-3-propyl-	0.858	-	-
32	15.43	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	7.630	-	-
33	15.49	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1.alpha.,2.beta.,5.alpha.)]-	6.427	15.966	5.212
34	15.74	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.230	-	-
35	15.74	1,2-Dihexylcyclopropene	-	3.027	-
36	15.868	Phthalic acid, 8-chlorooctyl isobutyl ester	-	-	17.508
37	15.87	Phthalic acid, butyl hexyl ester	1.378	-	-
38	15.93	Bicyclo[10.8.0]eicosane, cis-	1.999	-	-
39	15.94	1,4-Eicosadiene	-	4.417	-
40	16.35	17-Octadecynoic acid, methyl ester	-	0.893	-
41	16.3506	Phtalic acid, butyl cycloheptyl ester	-	-	2.2429
42	16.36	Hexadecanoic acid, methyl ester	1.594	-	-
43	16.71	n-Hexadecanoic acid	4.906	21.449	38.93
44	16.81	Phthalic acid, butyl hexyl ester	-	-	26.05
45	16.82	Phthalic acid, dodecyl ethyl ester	2.547	-	-
46	17.02	Fumaric acid, 2-chloropropyl tridecyl ester	-	3.119	-
47	17.40	Tetradecanal	-	1.181	-
48	17.41	Isoheptadecanol	3.788	-	-
49	17.99	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)-	-	0.214	-
50	18.02	Ditetradecyl ether	0.915	-	-
51	18.26	Phytol	34.062	29.267	-
52	18.42	10-Methyl-E-11-tridece-1-ol acetate	-	0.510	-
53	18.88	Fumaric acid, 2-decyl tridecyl ester	-	1.444	-

54	19.89	[1,2,4]-Triazolo[4,3-a][1,3,5]-triazine, 5,7-diacetylamino-3-methyl-	-	2.892	-
55	19.90	cis-9-Hexadecenoic acid	9.996	-	-
56	20.21	Ethanone, 2-(2-benzothiazolylthio)-1-(3,5-dimethylpyrazolyl)-	-	1.055	-
57	20.62	4,8,12,16-Tetramethylheptadecan-4-olide	3.953	-	-
58	21.07	Hexanedioic acid, bis(2-ethylhexyl) ester	7.912	-	-
59	21.97	1,1,1,3,5,5,5-Heptamethyltrisiloxane	-	0.219	-
60	22.53	13-Tetradecen-1-ol acetate	2.983	-	-
61	24.59	Silicic acid, diethyl bis(trimethylsilyl) ester	-	0.233	-
62	25.27	1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethylamide	-	0.219	-
63	25.70	9-Borabicyclo[3.3.1]nonane, 9-[3-(dimethylamino)propyl]-	-	0.261	-
64	26.37	(5-Isopropyl-2-methylphenoxy)trimethylsilane	-	0.275	-
65	28.35	1,4-Bis(trimethylsilyl)benzene	-	0.211	-

The in-vitro study showed that the extracts demonstrated inhibition of protein (albumin) denaturation in a concentration-dependent manner throughout a concentration range of 400-16000 µg/ml as depicted in Fig. 2. The three extracts showed similar trend of inhibition with ALEXB showing higher percentage of inhibition (70.045 ± 7.22) at concentration 16000 µg/ml as compared to ALEXA and SFEX. In the meantime, it can be seen in Fig. 2 that the extracts gave almost 50% inhibition of protein denaturation around 8000 µg/ml. The findings are consistent with another study where crude ethanolic extract of *A. agallocha* leaves was reported to inhibit Bovine Serum Albumin (BSA) denaturation (34.09%, 36.95% and 43.13%) at different concentrations (100, 250 and 500 µg/ml) of the extract [46].

In more detail, phytol; an acyclic diterpene alcohol (the major compound of ALEXA and ALEXB) identified by GCMS analsysis has been reported for being able to reduce neutrophil migration, cytokine levels and oxidative stress in acute inflammation models [36]. Maruthupandian and Mohan (2011) stated that phytol was detected in Sneeze wort (*Wattakaka volubilis*) ethanolic leaf extract constitute a promising novel therapeutic agent for rheumoid arthritis and other related chronic inflammatory diseases [37]. Meanwhile, n-hexadecanoic acid, which was found to be the major compound of SFEX (38.93%) and also found to a lesser extent in ALEXA (4.2%) and ALEXB (21.5%), was indicated to have potential anti-inflammatory and anti-arthritic properties [37]. Aparna *et al.*, (2012) reported that hexadecanoic acid controls inflammation through inhibition of phospholipase A2 enzyme by binding to its active site [47]. In addition, 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene, is a tricyclic sesquiterpene that was found solely in SFEX and
was previously testified to have potential analgesic and anti-inflammatory activity [38]. Therefore, the anti-inflammatory activity of ALEX may be attributed to the presence of phytol and n-hexadecanoic acid, while the anti-inflammatory activity of SFEX may be due to the occurrence of n-hexadecanoic acid and 1H-Cycloprop[e]azulene, a biomarker and a chemical distinguisher of Agarwood [39].

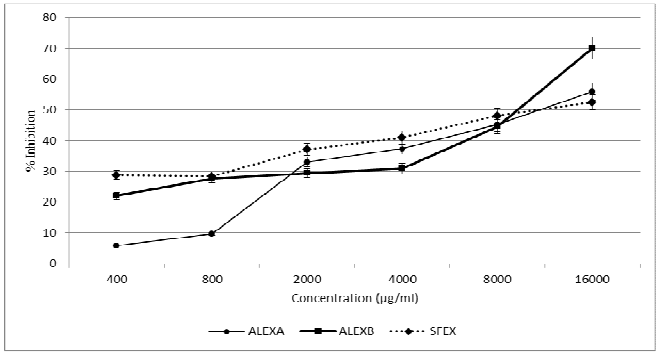

Fig. 2 Anti-inflammatory activity of *Aquilaria malaccensis* leaf extracts. Percentage of protein denaturation inhibition against concentration range of 400-16 000 µg/ml. Experiments were conducted in triplicates.

IV. Conclusion

In conclusion, the present study revealed the anti-inflammatory properties of *Aquilaria malaccensis* leaf extracted using Soxhlet with polar and non-polar solvents, and Supercritical Fluid Extraction respectively. The extracts were able to inhibit more than 50% of the heat-induced protein denaturation with ethanolic Soxhlet extract (ALEXB) showing the highest anti- inflammatory activity. Further research is warranted to explore the anti-inflammatory activities of *A. malaccensis* leaf extracts and their mechanism of action.

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