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FTIR ANALYSIS OF PHENOLIC COMPOUND AS PANCREATIC LIPASE INHIBITOR FROM INOCULATED *AQUILARIA MALACCENSIS*

(Analisis Sebatian Fenolik daripada *Aquilaria malaccensis* yang di Inokulasi sebagai Perencat kepada Enzim Lipase Pankreas Menggunakan FTIR)

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Abstract

This research aimed to discover the potential of inoculated *Aquilaria malaccensis* extract as a new and safe lipase inhibitor. The phenolic compounds in this plant are expected to promote inhibitory activity towards pancreatic lipase enzyme. Inoculated *Aquilaria malaccensis* was selected for this research, wherein the parts of this species (bark and leaves) were extracted via hydro distillation process. The extracts of this plant which are hydrosol, oil, and leaves were analyzed for phytochemical compound via Fourier Transform Infrared Spectroscopy (FTIR). FTIR analysis of the extracts of inoculated *Aquilaria malccensis* revealed the presence of hydroxyl functional group in both leaves and hydrosol extracts but absence in oil. This validate the presence of phenolic compound in hydrosol and leaves extract. Therefore, the leaves and hydrosol extracts have potential as an anti-obesity agent by inhibiting pancreatic lipase.

Keywords: *Aquilaria malaccensis*, phenolics, essential oil, hydrosol

Abstrak

Kajian ini dijalankan bertujuan mengenal pasti potensi ekstrak *Aquilaria malaccensis* yang diinokulasi sebagai inhibitor enzim lipase pankreas yang baru dan selamat. Sebatian fenolik dalam tumbuhan ini dipercayai akan dapat merencatkan aktiviti enzim lipase pankreas. Bahagian tumbuhan iaitu kayu dan daun telah diekstrak dengan menggunakan kaedah penyulingan hidro. Penyulingan hidro pada bahagian kayu tumbuhan menghasilkan minyak dan hidrosol.Ekstrak daripada kayu dan daun kemudian dianalisis untuk sebatian fitokimia menggunakan Spektrofotometer Inframerah Transformasi Fourier (FTIR). Melalui analisis FTIR, kumpulan berfungsi hidrosil didapati pada sampel ekstrak daun dan hidrosol namun tidak pada ekstrak minyak. Kajian ini mengesahkan kehadiran sebatian fenolik pada ekstrak daun dan hidrosol. Oleh itu, ekstrak daun dan hidrosol mempunyai potensi sebagai agen anti -obesiti dengan merencatkan aktiviti enzim lipase pankreas.

Kata kunci: *Aquilaria malaccensis*, fenolik, minyak, hidrosol

Introduction

Obesity is a major contributor towards chronic diseases and disability including hypertension, stroke, diabetes, cardiovascular diseases and more. Ahn et al. defined obesity as abnormal or excessive fat accumulation with imbalance between levels of energy intake and expenditure [1]. Regarding obesity, The Star Malaysia (2013) reported that Malaysia is ranked at the sixth place among Asia-Pacific countries and tops the list of South-East Asia countries [2]. A review on article of Obesity and Overweight by the World Health Organization, WHO (2003) stated that the worldwide obesity has nearly doubled since 1980 with more than 1.4 billion adults were overweight which about 300 million women and more than 200 million men were obese in 2008 [3].

Obesity could be prevented by inhibiting pancreatic lipase in order to block fat absorption in the small intestine after being hydrolysed by pancreatic lipase. Another way is by disturbing adipocyte differentiation to inhibit fat

accumulation [1]. Pancreatic lipase (PL) is a primary enzyme that hydrolyses dietary fat molecules in digestive system by converting triglyceride to monoglyceride and free fatty acid [4]. The inhibition of PL is the key target to mediate obesity since this reaction reduced fat and nutrient absorption to the body. Orlistat is a drug designed to treat obesity in which it acts as lipase inhibitor. However, orlistat consumption has some serious side effects. Its consumption might damage liver and kidney, reduce the effectiveness of other medications including life-saving cancer treatment and also stimulate the multiplication of cancer cells [4].

At present, natural resources have been utilized as medicinal agent. Natural medicines are recognized as cost effective and safe with zero side effects at plausible dosage. It is found that natural resources have become one of the most vital approaches to treat obesity since it is rich with bioactive compounds [5]. Phytochemicals are natural bioactive compound that found in plants that work together with minerals, vitamins and fibre to promote good health. Currently, there are many researches are carried out to study the potential of phytochemical for anti-obesity treatment using various natural resources. According to Yun (2010), scientists have identified many phytochemicals that have potential to be used in obesity treatment by inhibiting PL. The phytochemicals identified are polyphenols, flavonoids, and saponin [4]. These compounds can be extracted from wide variety of plants such as soybean, grape seed extract, black tea, green tea, oak, broccoli, apple and many more [6]. In addition, Tucci et al. mentioned that polyphenol extract from plant such as apple or grape seed is able to inhibit PL. For example, polyphenol extracted from apple inhibits PL with IC_{50} value of 1.4 μg/ml where IC_{50} is defined as the sample concentration causing 50% inhibition of the pancreatic lipase activity [7]. This research will be focusing on identification of phenolic compound in inoculated *Aquilaria malaccensis* due to their significance on inhibiting PL.

Aquilaria spp. which also known as agarwood or 'gaharu' is one of the valuable forest products due to its high demand in various industries such as perfumery and pharmaceuticals. *Aquilaria malaccensis* is one of the Malaysian treasures that rich in phytochemicals contents in its resin. Hence, this research was attentive to discover the miracles of its phytochemicals content since at this present time there is no detailed documentation of phytochemicals compound presence in *Aquilaria spp.* [8]. In this research, inoculated *Aquilaria malaccensis* extracts are used as research materials to discover the new and safe pancreatic lipase inhibitor. The extracts of the plant which are hydrosol, oil and leaves taken from the hydro distillation process of inoculated *Aquilaria malaccencis*. Hydro distillation technique is used for extraction of phytochemical compound of inoculated *Aquilaria malaccencis* because it is safe to operate, eco-friendly and cost effective [9]. This study focused on the identification of phenolic compounds from inoculated *Aquilaria malaccensis* extract by using FTIR as potential PL inhibitor.

Materials and Methods

Collection of the plant materials

The bark, stem and leaves for compound extraction via hydrodistillation was collected from inoculated *Aquilaria malaccensis* planted in Jalan Kebun, Shah Alam, Selangor.

Pre-treatment of inoculated *Aquilaria malaccensis* leaves

Inoculated *Aquilaria malaccensis* leaves collected were separated prior to washing. The leaves were very well selected where the damaged and diseased one were excluded. Then, those selected were washed and cleaned to remove the dirt and impurities before undergoing drying process. The drying process was conducted at 60 ^{o}C for 24 hours inside an oven. Finally, the dried leaves were cooled at room temperature before they were ground and sieved into smaller particle size between 600 μm to <300 μm.

Hydro distillation process of inoculated *Aquilaria malaccensis* leaves

The leaves sample was boiled with the distilled water in a round bottom flask where the temperature was maintained at 70 ^{o}C. The water vapor from the hydro distillation process was collected. The water vapor consists of steamed and volatile compound that rises from the extractor and pass through to the condenser. The sample was then evaporated in rotary evaporation before undergoing the analysis for chemical compound presence via FTIR.

Soaking and drying of grinded inoculated *Aquilaria malaccensis* bark and stem

The grinded inoculated *Aquilaria malaccensis* was soaked in distilled water for 7 days during the pre-treatment process. This treatment was carried out to promote the enlargement of the pore size [10]. This will enhance the

releasing of the phytochemicals compound from the wood. The soaking ratio of inoculated *Aquilaria malaccensis* to distilled water was 1:2 (kg/litre). This soaking method was a modification from A. Hakim et.al, (2013) [9]. The soaked samples were dried at outdoor condition.

Hydro distillation of grinded inoculated *Aquilaria malaccensis* bark and stem

The soaked inoculated *Aquilaria malaccensis* chips were boiled in hydro distiller vessels. Aluminium vessel was used for hydrodistillation. The hydro distillation process occurred at constant temperature of 100 ^{o}C at atmospheric pressure. This process was carried out for five days. The resultant steam contained plant volatile compound is condensed and captured. The vapour consists of steam and volatile compounds rose from the extractor and went through the condenser. Two forms of heterogeneous liquid mixture were collected which are hydrosol (aromatic water) and essential oil. The liquid mixture was separated and hydrosol was collected for further analysis. This extraction method was obtained and modified from A.Hakim et al. [9].

FTIR analysis

The identifications of the active functional groups presence in inoculated *Aquilaria malaccensis* oil, hydrosol and leaves extracts was conducted by the aid of FTIR spectroscopy (Perkin Elmer Spectrum 2000). The investigation was performed within the wavelength ranging from 4000 to 400 cm^{-1} where the spectrum takes about two minutes to be recorded. This range was selected because phenolic compounds functional groups will be identified at this wavelength. Lastly, comparison between the resultant spectrums with the standard for entirely functional groups was conducted [8].

Results and Discussion

Pancreatic lipase is the enzyme that responsible for hydrolysis of 50-70% of triglycerides into monoglycerides and fatty acid, thus pancreatic lipase inhibition is a valuable pathway towards the treatment of obesity [11]. Several studies had reported the potential of phenolic compounds as pancreatic lipase inhibitor. Ahn et al. reported that flavonoid from *Nelumbo nucifera* is able to inhibit pancreatic lipase where the flavonoids, flavones without glucose inhibit pancreatic lipase whereas flavone glycosides did not [1].

FTIR was used for the identification of functional group presence in the leaves, hydrosol and oil inoculated *Aquilaria malaccensis*. Figure 1 - 3 show FTIR spectrum of leaves, hydrosol and oil extracts taken from inoculated *Aquilaria malaccensis* respectively. The functional groups which are present in the inoculated *Aquilaria malaccensis* extracts are further described in details [12].

O-H bond of hydrogen bonded alcohol/phenol (3600-3200 cm^{-1}) group frequency was present in leaves and hydrosol extract with 3323.61 cm^{-1} and 3312.65 cm^{-1} respectively. O-H bond of alcohol/ phenol functional group was absent in oil extract. A broad spectrum can be observed from Fig. 1 and Fig. 2 represent the availability O-H bond in the extracts. Both leaves and hydrosol extracts has strong H-bond since the lower the frequency, the stronger the H bond. This is the important part because presence of O-H bond indicates the existance of phenolic compound in the extracts. This finding is in agreement with Khalil et al. (2013) where presence of alcohol/phenol functional group was identified in Agarwood leaves with frequency of 3388 cm^{-1} and 3384 cm^{-1} for before and after inoculation process respectively[8]. Besides, Ahn et al., (2013) had reported *Nelumbo nucifera* leaves that consist IR spectrum of hydroxyl group (3338 cm^{-1}) was able to inhibit pancreatic lipase [1].

C-H bond of alkanes bonded with -$COCH_3$ (3100-2900 cm^{-1}) group frequency was identified in hydrosol extract with frequency of 2970.73 cm^{-1}. C-H bond of alkanes (2970-2850 cm^{-1}) group frequency was only exist in oil extract at frequency of 2853.03 cm^{-1} assigned to the presence of unfunctionalised C-H stretching band of methylene (-CH2) and methyl (-CH3) groups. C-H bond of aromatic ring (690-900 cm^{-1}) group frequency was present in oil extract with frequency of 887.43 cm^{-1} and 721.53 cm^{-1}. C≡C bond of Alkyne group frequency of 2260-2150 cm^{-1} was present in both leaves and hydrosol extract with frequency of 2160.24 cm^{-1} and 2154.55 cm^{-1} respectively.

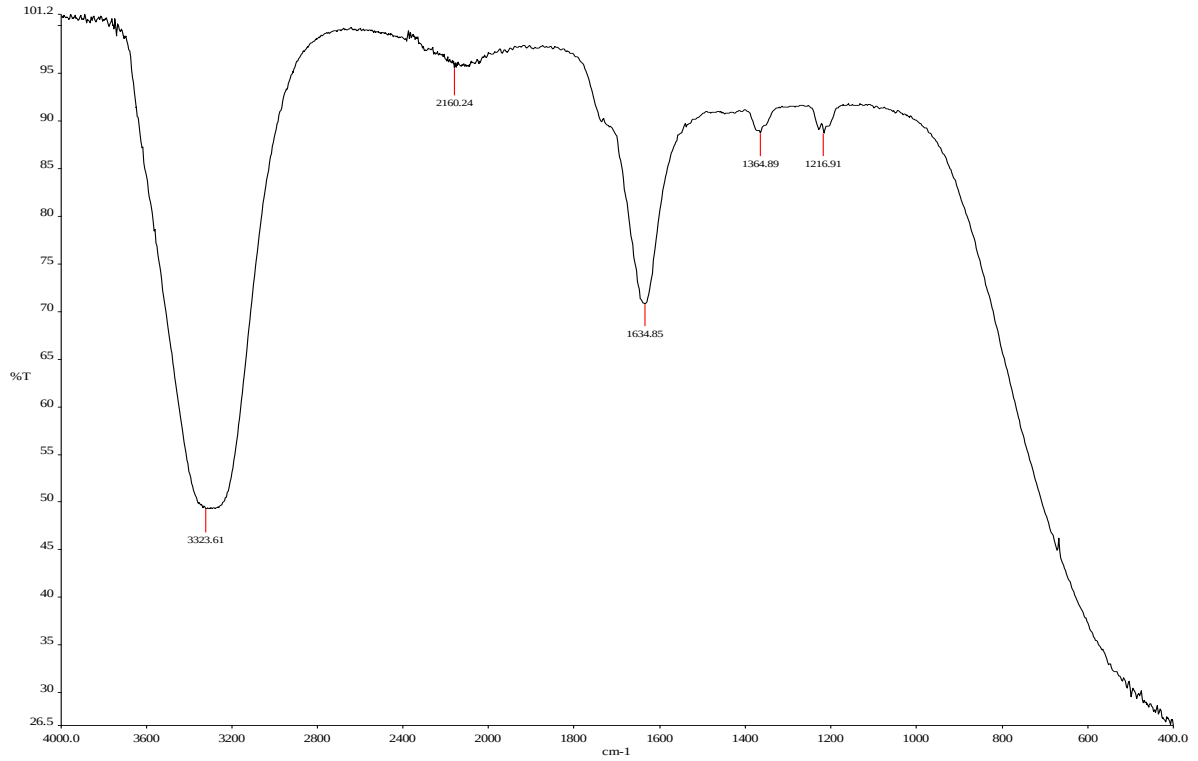

Figure 1. FTIR spectrum of inoculated *Aquilaria malaccensis* leaves extracts

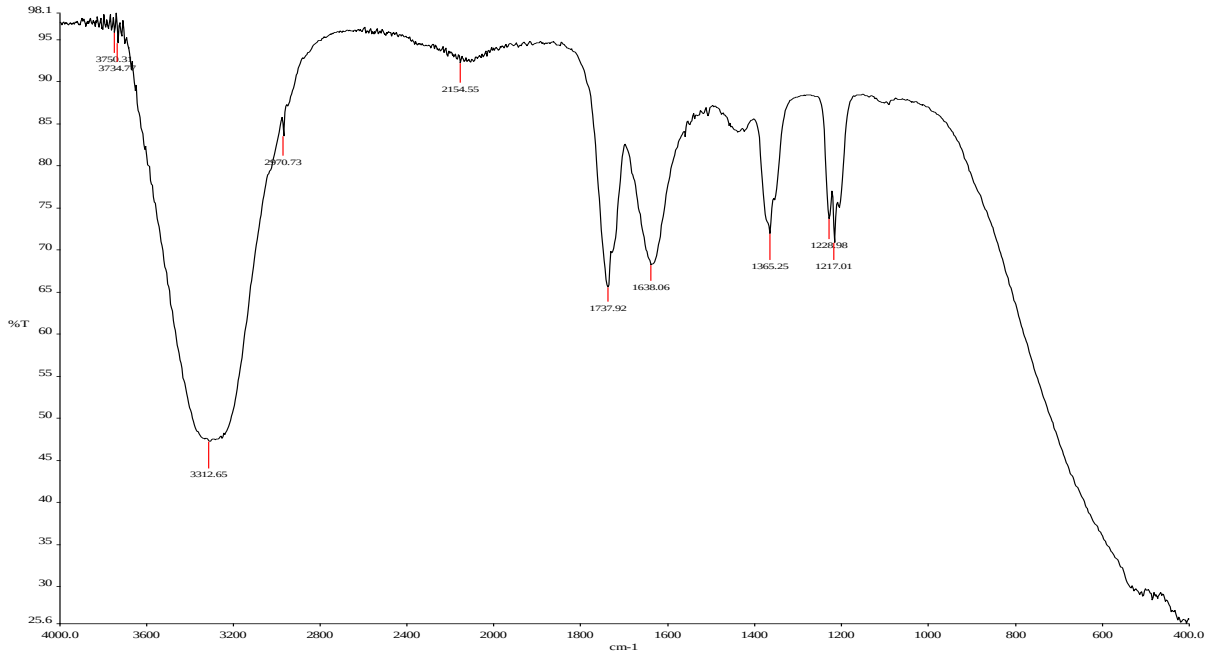

Figure 2. FTIR spectrum of inoculated *Aquilaria malaccensis* hydrosol extracts

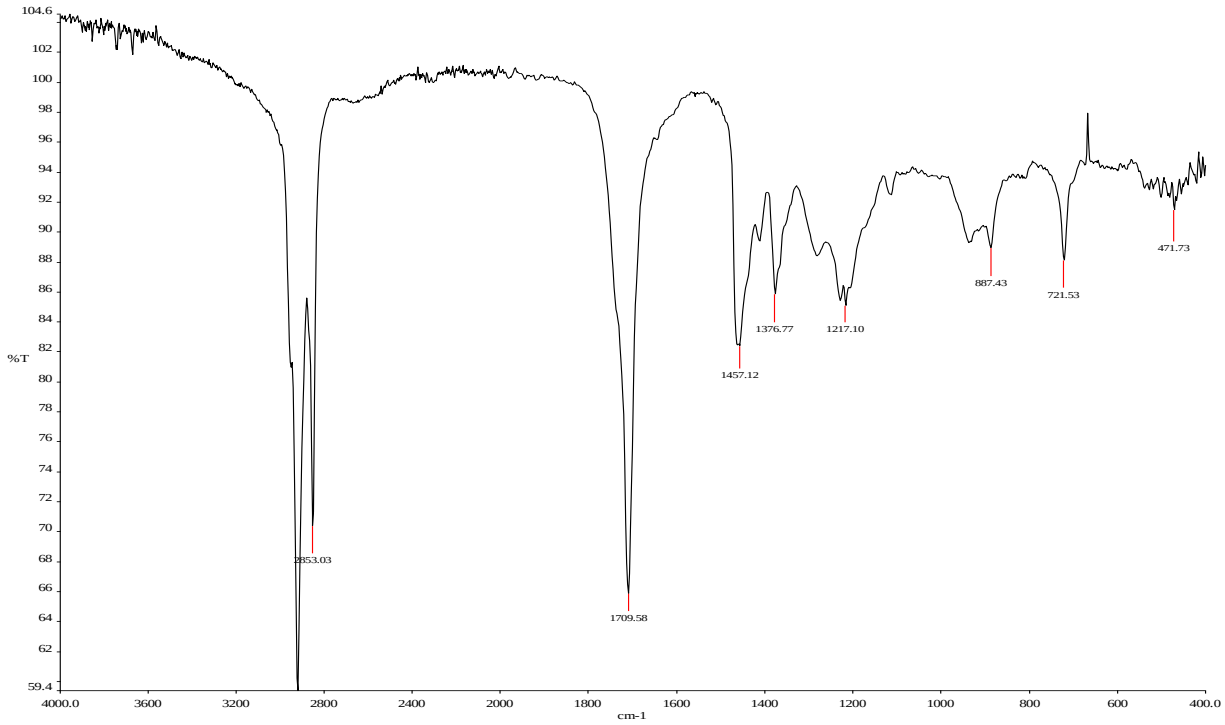

Figure 3. FTIR spectrum of inoculated *Aquilaria malaccensis* oil extracts

N-H bending of amides (1650-1560 cm^{-1}) group frequency was identified in both leaves and hydrosol extracts with frequency of 1634.85 cm^{-1} and 1638.06 cm^{-1} respectively. C-H stretching of amine (1250-1020 cm^{-1}) group frequency was also found in the hydrosol extract. C-H bond of Aromatic ring (690-900 cm^{-1}) group frequency were present in oil extract with frequency of 887.43 cm^{-1} and 721.53 cm^{-1}. Leaves and hydrosol extracts were also containing C-O stretch of ether functional group (1300-1000 cm^{-1}) with frequency of 1216.91 cm^{-1} and 1217.01 cm^{-1} respectively. The presence of ester, ether and aromatic ring in all extracts explain the phenomenon of sweet and pleasant smell being produced during extraction process. Table 1 - 3 show the summary of the functional group of leaves, hydrosol and oil extracts from inoculated *Aquilaria malaccensis* respectively.

Table 1. Functional groups of inoculated *Aquilaria malaccensis* leaves extracts [13]

Wavenumber (cm^{-1})	**Bond**	**Functional Group**	**Group band (cm^{-1})**
3323.61	H-bonded-OH	Alcohol/ Phenol	3600-3200
2160.24	C≡C-C	Alkyne	2260-2110
1634.85	N-H bending	Amide	1650-1560
1364.89	C-H bending vibration – OCOCH	Alkanes	1370-1350
1216.91	C-O stretch	Ether	1300-1000

Table 2. Functional groups of inoculated *Aquilaria malaccensis* hydrosol extracts [13]

Wavenumber (cm^{-1})	Bond	Functional Group	Group band (cm^{-1})
3750.31	Unknown	-	-
3734.77	Unknown	-	-
3312.65	H-bonded-OH	Alcohol/ Phenol	3600-3200
2970.73	C-H bonded-COCH3	Alkane	3100-2900
2154.55	C≡C-C	Alkyne	2260-2110
1737.92	C=O stretching	Ester	1750-1735
1638.06	N-H bending	Amide	1650-1560
1365.25	C-H bending vibration –OCOCH	Alkanes	1370-1350
1228.98	C-H stretching	Amine	1250-1020
1217.01	C-O stretching	Ether	1275-1200

Table 3. Functional groups of inoculated *Aquilaria malaccensis* oil extracts [13]

Wavenumber (cm^{-1})	Bond	Functional Group	Group band (cm^{-1})
2853.03	C-H bond	Alkane	2960-2850
1709.58	C=O stretching	α,β-unsaturated aldehydes,ketones	1710-1665
1457.12	C-H bend	Alkane	1470-1430
1376.77	C-H bending vibration –OCOCH	Alkanes	1370-1350
1217.70	C-O stretching	Ether	1275-1200
887.43	C-H bond	Aromatic Ring	900-690
721.53	C-H bond	Aromatic Ring	900-690
471.71	S-S	Polysulfide	500-470

From all extracts that were analyzed, it was found that hydrosols and leaves consist the alcohol/phenols functional groups that are very useful to prove the presence of phenolic compounds inside the hydrosol and leaves extract. Besides, the pattern of FTIR spectrum for both leaves and hydrosol extract were quite similar in which both spectrum did not show significance change of peaks compared to FTIR spectrum obtained from oil extract indicate their similar potential as pancreatic lipase inhibitor.

Conclusion

The present study has revealed that leaves and hydrosols extracted from inoculated *Aquilaria malaccensis* hydrosol consist of phenolic compounds. This was due to the presence of alcohol/phenols group in the FTIR analysis. The active functional groups of hydrosol and leaves extract are alcohol/phenol, alkane, alkyne, ester and amide. Meanwhile, the functional groups of oil extract are alkane, aldehydes, ketones, ether, aromatic ring and polysulfide. Finally, this analysis had achieved its objective to identify the presence of phenolic compounds from inoculated *Aquilaria malaccensis.*

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