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Evaluation of anti-lipase activity of leaf and bark extracts from *Aquilaria subintegra* and *A. malaccensis*

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ABSTRACT: Obesity is one of the serious health condition in the world and contributes to many chronic illnesses augmenting to high risk of death rate. With the purpose of obesity treatment, natural sources come to be significant to replace commercial drugs due to their harmful effect to the body. Therefore, a lot of studies were conducted on natural sources due to their potential as medicinal herbs. *Aquilaria sp.* plants are well known in traditional medicine as a sedative, analgesic and digestive. Even though there were no reports on applications of *Aquilaria sp.* towards obesity treatment, this plant has traditionally been used as laxative for weight reducing. In this study, the anti-lipase activity of an extract of barks and leaves from *A. subintegra* and *A. malaccensis* were investigated. The anti-lipase activity was measured by colorimetric assay on pancreatic lipase activity using porcine pancreatic lipase (PPL; triacylglycerol lipase, EC 3.1.1.3). The result indicated that among these two species of *Aquilaria, A. malaccensis* bark in dichloromethane crude showed high anti-lipase activity. Thus, these results suggest that *Aquilaria sp.* plant extracts might be of therapeutic interest with respect to the treatment.

KEYWORDS: *A. subintegra* ; *A. malaccensis* ; anti-lipase ; pancreatic lipase

1. INTRODUCTION

Obesity is a severe health condition that is encountered by a lot of people today. It is an epidemic problem and contributes to many chronic illnesses augmenting to the high death rate. In order of obesity treatment, dietary fat stimulates body fat storage more operative than dietary carbohydrate [1-2]. The key of dietary fat inhibits pancreatic lipase, the main enzyme which is hydrolysed 50-70% of dietary triacylglycerols into monoacylglycerols and free fatty acid (FFA); hence, the result could decrease absorption of fat, and thus energy uptake, which plays an important role in mediating obesity problem [3-4]. Therefore, in this study porcine pancreatic lipase (PPL; triacylglycerol lipase, EC 3.1.1.3) has been used as an enzyme. There are two common anti-obesity drugs available in the market, orlistat and sibutramine; orlistat blocks the producing of FFA and glycerides in gastrointestinal enzyme lipase and sibutramine acted as a monamine-reuptake inhibitor to surge satiety [5-6]. Unfortunately, the synthetic drugs caused the harmful effect for the long term [7]. To date, herbal products and dietary supplements are widely available in the market as an alternative way to replace the use of synthetic drugs. Many researchers focused on natural sources, including plants, fruits and herbs as biological inhibitors. There are numerous studies of pharmacological properties of *Aquilaria sp.* which are currently explored for health therapy. Hence, this study will explore *Aquilaria sp.* to reveal the possible natural inhibitors.

A. subintegra and *A. malaccensis* belongs to the genus *Aquilaria* and in the family Thymelaeaceae. They are widely distributed especially in Asia due to its highest value of resin-impregnated heartwood. *Aquilaria sp.* also has been used traditionally as sedative, analgesic and digestive treatment [8]. Previous phytochemical studies of this genus showed the presence of terpenoids, phenolic compounds, flavonoids and sterols that contribute to its biological activities [9]. The leaves of *Aquilaria sp.* are believed to have major polyphenolic compounds which can be used as herbal tea and showed a laxative [10-12], anti-bacterial [13], and anti-diabetic properties [7]. In addition, stem of *A. subintegra* has been reported as promising in Alzheimer therapy [14]. As far as authors are concern, no publication was found on *Aquilaria sp.* as an anti-lipase agent. Therefore, through this publication it is hopeful that we reveal anti-lipase potential of *Aquilaria sp.* and subsequently isolate

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chemical compounds especially polyphenolic groups and understand inhibitory mechanism towards lipase, an enzyme responsible for lipid breakdown.

2. RESULTS AND DISCUSSION

2.1 Phytochemical screening of leaf and bark extracts

Preliminary phytochemical screening of all crude extracts of these two species of *Aquilaria* barks and leaves showed the presence of flavonoid, steroid and terpenoid. The biological properties of plants extract are multiplex. Thus, the anti-lipase effect may be credited to the complex pharmacological action of phytoconstituents existing in the crude extract. Adnyana and colleagues (2014) suggested that the chemical components such as flavonoids, saponin, tannin, steroid and triterpenoid able to inhibit pancreatic lipase activity.

2.2 Anti-lipase effect of Aquilaria crude extracts

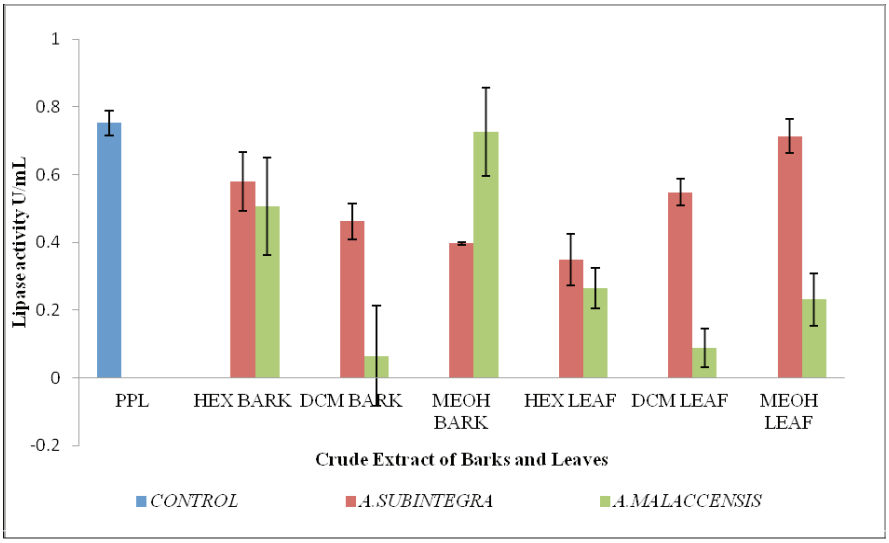
Twelve crude extracts from *A. subintegra* and *A. malaccensis* were investigated for their inhibition activities at concentration 100 µg/mL. The inhibitory activities toward porcine pancreatic lipase are shown in **Table 1**. The value of lipase activity (positive control) was 0.753 U/mL ± 0.037. The result indicated that almost all crude extracts had a potential to inhibit PPL from producing free fatty acid by comparing value of positive control. Among those examined the DCM crude from *A. malaccensis* bark (AMB) showed the highest possibility to inhibit PPL activity with 91% reduction. This is probably because the crude extracts might contain polyphenolic compounds, which may include more active compounds. It's known that polyphenolic possess several biological activities. Previous study showed that polyphenolic compounds from molokheiya leaves play a role in reducing diet-induced obesity and oxidative stress [17]. In fact, the polyphenols which comprise flavonoids group, able to prevent obesity by inhibit enzyme deal with fat absorption [18].

Table 1. Effect of *Aquilaria* crude extracts toward pancreatic lipase activity.

Plant Species	Plant Part	Crude Extract	Activity U/mL
A. subintegra	Bark	Hexane	0.580 ± 0.086
	Bark	Dichloromethane	0.462 ± 0.052
	Bark	Methanol	0.397 ± 0.004
	Leaf	Hexane	0.348 ± 0.077
	Leaf	Dichloromethane	0.548 ± 0.040
	Leaf	Methanol	0.714 ± 0.051
A. malaccensis	Bark	Hexane	0.507 ± 0.144
	Bark	Dichloromethane	0.065 ± 0.147
	Bark	Methanol	0.727 ± 0.131
	Leaf	Hexane	0.265 ± 0.061
	Leaf	Dichloromethane	0.089 ± 0.056
	Leaf	Methanol	0.231 ± 0.078

*Concentration of crude extracts = 100 µg/mL; Experiments were conducted in triplicate and values presented as average of three readings ± standard deviation.

The lipase activity was determined by observing development of color of fatty acid in solvent phase [15]; the more color develop, the activity of lipase is higher, while the activity of lipase is lower when the color is more colorless. **Figure 1** shows the mean of lipase activity (U/mL) between two species (n=3). From the graph showed that DCM crude of AMB had highest inhibition of lipase activity compared to the others. Its color also becomes colorless, suggesting that activity lipase was prevented. While looking at plant species, *A. malaccensis* was more counteract the enzyme activity than *A. subintegra*; which mean the *A. malaccensis* probably contain more active compounds compared to *A. subintegra*. This is supported with previous studies indicated that methanol extract of *A. malaccensis* leaves had highest antioxidant properties which is helpful to protect formation free radical and delay the existence of lipid peroxidation [19].



*Concentration of crude extracts = 100 µg/mL; Experiments were conducted in triplicate and values presented as average of three readings ± standard deviation.

Figure 1. The mean of lipase activity (U/mL) of twelve crude extracts from *Aquilaria* species and PPL (positive control). Results are stated as mean ± SD (n=3), *p<0.01

The statistical analysis paired t-test by SPSS was conducted towards all crude extracts of *Aquilaria* species with significant different *p<0.01. This study suggested that both *Aquilaria* sp. are worthy in their biological properties as well as DCM crude of AMB have a good promising to inhibit lipase activity. This hypothesis has been warranted with previous study indicated that *Aquilaria* species have pharmacological effect including in Alzheimer disease, diabetes mellitus and antioxidant [14]. This is followed by discovering laxative properties in *Aquilaria* leaves which believe contain active polyphenolic compound such as mangiferin and genkwanin 5-O-β-primeveroside [10,20]. Aligned with this, the anti-lipase activity of *Aquilaria* crude extracts is possibly related with existence of polyphenolic compounds.

3. CONCLUSION

In this study, we screened twelve crudes from *A. subintegra* and *A. malaccensis* and found that AMB from DCM crude have a high potential to inhibit *in-vitro* lipase activity. This findings support further research to investigate the mechanism of isolated compound from the crude for better understanding as a potential inhibitor for obesity treatment.

4. MATERIALS AND METHODS

4.1 Plant materials

The leaves and barks of *A. subintegra* and *A. malaccensis* were collected from Kajang, Selangor, Malaysia in July 2014. A voucher specimen was deposited at the herbarium of Universiti Pendidikan Sultan Idris (Voucher No: AMWNI-M6002/1).

4.2 Preparation of extract

The barks and leaves were dried at room temperature, cut into small slices and powdered using a dry grinder. Dried barks and leaves were extracted *via* maceration technique, three times with hexane, dichloromethane (DCM) and methanol, 72 h for each time, respectively. The supernatant was filtered using Whatman No. 1 filter paper. Then, the residues were concentrated at 40°C using rotary evaporator. All crude extracts were stored at 4°C until used.

4.3 Phytochemical screening

All of twelve crudes of these two *Aquilaria* species were tested with *p*-anisaldehyde to screen the presence of several active group compounds such as terpenoid, steroid and polyphenolic including flavonoid compounds.

4.4 Assay of lipase activity

In vitro pancreatic lipase activity was determined by measuring the release rate of oleic acid (free fatty acid) from the standard curve of free fatty acid. Porcine pancreatic lipase (PPL) dissolved in buffer was induced as positive control **(A)**, while crude extracts of *Aquilaria* as negative control **(B)**, were dissolved in dimethyl sulfoxide (DMSO). This method was performed colorimetrically with slightly modification from Kwon and Rhee's (1986) method. 1.0 ml of culture filtrate

was mixed with 2.5 ml of natural substrate (olive oil mixed with buffer, 1:1, v/v) and 20 µl of 20 mM $CaCl_2$. The substrate was prepared by mixing olive oil (Bertoli, Italy) and 50 mM phosphate buffer equally. The same procedure was prepared onto 1.0 ml **(A)** and 1.0 ml of **(B)** filtrate, by mixing with 2.5 ml natural substrate and 20 µl of 20 mM $CaCl_2$, respectively. The mixture was shaken 30 min in water bath shaker at an agitation rate of 200 rpm. Then 1 ml of 6N HCl was added to terminate the reaction of emulsion system and followed by adding 5 ml of isooctane and mixing for 30 sec using a vortex mixer. The upper isooctane layer containing the fatty acid was drawn off to a test tube and added 1 ml of copper reagent before analysis. Lipase activity was determined by measuring the amount of free fatty acids released from the standard curves of free fatty acid; one unit of lipase activity was defined as 1 µmole of fatty acid releasing by enzyme in 1 min [15-16].

4.5 Statistical analysis

The results obtained were analyzed using paired test in Statistical Package for the Social Sciences (SPSS) version 20. The value of $p < 0.01$ was considered to be statically significant.

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Authorship contributions

Concept – S.S.S.A.A., C.F.W.; Design – M.I., S.S.S.A.A., C.F.W., W.N.I.W.M.D.; Supervision – S.S.S.A.A., C.F.W.; Resource – S.S.S.A.A., C.F.W., W.R.W.M.; Materials – S.S.S.A.A., C.F.W.; Data Collection and/or Processing – M.I., S.S.S.A.A., C.F.W., W.N.I.W.M.D.; Analysis and/or Interpretation – M.I., S.S.S.A.A., C.F.W.; Literature Search – M.I., S.S.S.A.A., C.F.W., Y.M.B., S.A., R.Y., N.H.I., W.M.N.H.W.S.; Writing, Critical Reviews – M.I., S.S.S.A.A., C.F.W., W.N.I.W.M.D., W.R.W.M., Y.M.B., S.A., R.Y., N.H.I., W.M.N.H.W.S.

Conflict of interest

The authors declared no conflict of interest.

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