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EFFECTS OF DRYING METHODS AND SOLVENTS ON PHENOLIC AND FLAVONOIDS CONTENT, DPPH RADICAL SCAVENGING AND α-GLUCOSIDASE ACTIVITIES OF *Aquilaria* LEAVES EXTRACT

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ABSTRACT

Aquilaria is a multipurpose plant with high commercial and medicinal values. The present study aimed to evaluate the effects of drying methods and extraction solvents on the phenolic and flavonoids content in *A. malaccensis* (AMA), *A. subintegra* (ASU), and *A. sinensis* (ASI). Leaf samples were either Air-Dried (AD) or Oven-Dried (OD) and extracted using 100% Ethanol (EE100), 70% ethanol (EE70), aqueous (AE), and hexane (HE). There was a significant difference ($p<0.05$) between the drying methods and the extracting solvents. The OD in AMA leaf samples gave the highest values of total phenolic compounds (TPC) (52.98 – 85.15 mg GAE/gm) and total flavonoid compounds (TFC) (2180.97 – 3733.45 QUE ppm). The highest DPPH radical scavenging was observed in OD-EE70-AMA with the IC_{50} value of 33.60 μg/mL. Meanwhile, OD-EE100-AMA gave the lowest IC_{50} value (0.13 μg/mL) in the α-glucosidase assay, followed by OD-EE70-AMA with IC_{50} value 0.69 μg/mL with no significant differences among them. OD-EE70-AMA was found to have a higher content of TPC and TFC with the most potent DPPH scavenging and α-glucosidase inhibition activities. A strong positive correlation was reported between bioassays and TPC or TFC, hence indicating that AMA leaf used in this study might have the potential as a natural antioxidant and an antidiabetic agent.

Key words: α-glucosidase activity, *Aquilaria* sp., DPPH radical scavenging, drying method, solvent extraction

INTRODUCTION

Aquilaria is an aromatic evergreen tree, belongs to the family Thymelaeceae, which is popularly known as “Gaharu” or “Karas” in Southeast Asia. In Malaysia, native species are *A. malaccensis*, *A. hirta*, *A. microcarpa*, *A. beccariania,* and *A. rostrata* (IUCN, 2015) while *A. crasna*, *A. sinensis,* and *A. subintegra* were originated from Indochina. In this study, *A. malaccensis* (AMA), *A. subintegra* (ASU), and *A. sinensis* (ASI) were selected due to easily found in Malaysia (Forestry Department of Peninsular Malaysia, 2016).

In recent years, plants have been extensively studied for their medicinal properties and it is believed that their intake is associated with a reduced risk of degenerative diseases including diabetes (Wan-Nadilah *et al.,* 2019). Many studies proved that diabetes is associated with oxidative stress (Wan-Nadilah *et al.,* 2018) while antioxidants act by inhibiting reactive oxygen species (ROS), eliminating free radicals, or improving antioxidant defenses.

The crucial factor in retaining the biological effect of the plant extract is the drying method. The most preferred techniques were air drying and oven drying due to their easy application and low cost (Kamiloglu & Capanoglu, 2015). Furthermore, the choice of solvent is another important aspect in ensuring plant extracts of a certain quality and biological activity. Plant’s therapeutic value can be easily diminished, hence it is crucial to determine the best solvent for the optimum extraction of the

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bioactive chemical composition. With the above scenario as a background, the present study aims to evaluate the antioxidant and antidiabetic activities as well as total phenolic and flavonoid content of the highlighted plant.

MATERIALS AND METHODS

Collection of plant material

The leaf sample of AMA, ASU, and ASI were collected from Forest Research Institute Malaysia (FRIM), Merchang, Terengganu (4°58'58.4"N 103°19'19.8"E), Alor Gajah, Melaka (2°23'03.3"N 102°13'10.2"E) and Seri Kembangan, Selangor (3°00'48.5"N 101°42'12.1"E), respectively. Air-Dried (AD) samples were left at room temperature until completely dried while Oven-Dried (OD) samples dried at 40°C for 18 hr / until weight constant. The powdered samples were stored at -20°C until further use.

Leaf extract preparation

The powdered sample was macerated in either 100% ethanol (EE100) or 70% ethanol (EE70), distilled water/ aqueous (AE), or hexane (HE) with a ratio of 1:50 (sample: solvents) and sonicated for 20 min. The extraction was repeated three times, then filtered and concentrated in a rotary evaporator (Heidolph, Germany). The crude extracts obtained were stored at -20°C before further use.

Total phenolic content (TPC)

The TPC was determined by Nasir *et al.* (2019). The mixture consisted of 10 µL extracts or gallic acid, 10 µL distilled water, and 100 µL 10% Folin-Ciocalteu reagents (v/v) was incubated for 5 min in a 96-well plate before being added with 80 µL 7.5% Na_2CO and further incubated for 30 min under dark conditions. The 765 nm absorbance was measured and TPC was expressed as GAE/gm extract.

Total flavonoid content (TFC)

The TFC was determined by Nasir *et al.* (2019) with minor modification. Mixtures of 25 µL sample and 10 µL 5% $NaNO_3$ were incubated for 6 min in the dark. Then, 15 µL $AlCl_3$ was added and incubated for another 5 min before 50 µL of 1 M of NaOH was added. The plate was gently shaken for 1 min and read at 595 nm. TFC was expressed as mg QE/gm extract.

DPPH free radical scavenging activity

The antioxidant activity was determined according to Mahmod *et al.* (2017) with slight modification. About 50 µL extracts were mixed with 100 µL of 59 mg/L DPPH. The mixture was left for 30 min in the dark and read at 570 nm. The percentage of inhibition was then calculated using the following equation: *Inhibition (%) = [(A blank – A sample)/ (A blank)] × 100*. Where; A = Absorbance.

Alpha-glucosidase inhibition assay

This assay was followed by Wan Nadilah *et al.* (2019) with slight modification. A mixture of 10 µL sample, 50 µL of phosphate buffer, and 25 µL α-glucosidase solution (0.2 unit/mL) were incubated for 5 min. Then, 25 µL of the substrate (5 mM) was added and the reaction was incubated for 30 min. Lastly, 100 µL of 0.2 M Na_2CO_3 was added to stop the reaction and read at 410 nm. The percentage of inhibition was calculated as follows: *Inhibition (%)* $= [(A_c – A_e)/A_c] \times 100\%$. Where; A_c =difference between the control and blank and A_e =difference between a sample and blank sample.

Statistical analysis

All the experimental data were expressed as mean ± standard deviation with triplicates. Data were analyzed for one-way ANOVA while the correlation was tested by using Pearson's correlation analysis and differences at ($p<0.05$) were considered as significant.

RESULTS AND DISCUSSION

The percentage of yield ranged from 0.82 to 30.82% (Table 1) with OD had a higher yield compared to AD for all extracts. This is in agreement with Anwar *et al.* (2013) which reported that OD extracts produced a higher extraction yield than AD in cauliflower extract. Moreover, *Aquilaria* extracted with EE70 showed significantly higher yield compared to EE100, AE, and HE in both drying methods, which indicates yield is parallel with increasing polarity of solvent used. The percentage of yield by species can be reported as ASU > ASI > AMA. It is also shown that the drying method had a significant effect on the extract yield as summarized in Table 1.

Except for AD-EE100-ASI, the OD samples exhibited a higher TPC compared to AD samples. This is congruent with Nik Wil *et al.* (2014), which reported a higher TPC in OD compared to the AD method in *A. malaccensis* and tamarind leaves, respectively. Meanwhile, the HE samples exhibited a low TPC in all *Aquilaria* sp. (Table 2). This might be attributed to hexane itself which is a non-polar solvent that poorly dissolves more polar compounds such as phenolics. The TFC results showed that OD-EE70-AMA has the richest source of flavonoids with 3733.45 que ppm and a significant difference ($p<0.05$) between all samples. The previous study

Table 1. Percentage of yield for each drying method and different solvents

Sample using different solvents	Drying Method	Yield (%) (w/v)		
		AMA	ASU	ASI
EE100	AD	8.59	18.53	5.29
	OD	14.66	18.54	13.00
EE70	AD	16.93	14.70	10.51
	OD	21.32	30.82	22.35
AE	AD	11.24	12.71	13.83
	OD	13.50	16.21	17.72
HE	AD	1.18	0.82	1.04
	OD	1.30	1.42	2.39

EE100 is 100% Ethanol extract, EE70 is 70% Ethanol extract, AE is Aqueous extract and HE is Hexane extract. For the drying method, AD is air drying and OD is oven drying. Meanwhile, the AMA is *Aquilaria malaccensis*, ASU is *Aquilaria subintegra* and ASI is *Aquilaria sinensis*.

by Fu *et al.* (2017) towards walnut leaves observed that the optimum yield of flavonoids was obtained from 70% ethanol and suggested that the flavonoids were highly soluble in ethanol: water mixture. Overall, the effect of drying techniques on TFC showed a similar trend with TPC mainly because flavonoids are also a phenolic compound.

DPPH activity revealed that EE100 and EE70 significantly exhibited lower IC_{50} values compared to AE and HE in both drying methods. The lowest IC_{50} of 33.60 µg/mL belonged to OD-AMA-EE70. Nopi *et al.* (2018) stated that the combined use of water and organic solvent might facilitate the extraction of chemicals that are soluble in water or organic solvent. For the OD method, Roshanak *et al.* (2016) also showed that the DPPH scavenging activity of tea leaves was the highest followed by sun-, fresh-, freeze- and microwave-drying. It is believed that the high TPC might contribute to the high antioxidant activity by high temperature during drying (Lou *et al.,* 2015). Interestingly, the IC_{50} values for OD-EE70 (33.60 µg/mL), were slightly higher but not significantly different ($p<0.05$) compared to quercetin (20.75 µg/mL). This clearly shows that the OD-EE70-AMA might be one of the potential resources as a natural antioxidant agent.

Furthermore, all AMA extracts showed α-glucosidase inhibition activity with more than 50% inhibition. The OD-EE100-AMA extract gave the lowest IC_{50} value of 0.13 µg/mL (Table 2). The study indicated that the more organic solvent system is, particularly the EE100 sample, the more efficient it is in extracting the α-glucosidase inhibitors from AMA extract. However, there were no significant differences ($p>0.05$) detected between the IC_{50} value of EE100 and EE70. Hence, EE70 was found to be the most preferred extract instead of EE100 because the mixture of ethanol and water has been known as a good solvent for polyphenol extraction due to the different polarity of both solvents, possibility of mixing them in any proportion, and is safe for human consumption (Waszkowiak and Gliszczynska-Swiglo, 2016). Furthermore, the IC_{50} values of the two extracts were also not significantly different ($p>0.05$) from that of quercetin and acarbose. Based on these findings, the OD-EE70-AMA was found to be the most promising extraction for study in diabetic animal models which is a huge step in finding alternatives for synthetic drugs that were known for their negative effects.

The analysis showed a positive correlation between the TPC and DPPH scavenging activities (r = 0.796, $p<0.05$). This indicates that the antioxidant activity of *Aquilaria* sp. extracts could be explained by its phenolic constituents. A similar result was reported by Reihani and Azhar, (2012) suggesting that that the presence of phenolic compounds in *Aquilaria* sp. leaves extract might be responsible for its antioxidant activity. There is also a positive relationship between TFC and DPPH radical scavenging activities (r = 0.687, $p<0.05$). This is consistent with the previous study by Sariburun *et al.* (2010). Meanwhile, the relationship between TPC and α-glucosidase inhibition property is evident (r = 0.712, $p<0.05$). Meanwhile, r = 0.366 with $p<0.05$ was recorded for correlation analysis between TFC and α-glucosidase activity. The lower correlation could be explained by the fact that there were other non-polyphenolic active compounds such as polysaccharides, which could be contributing to the strong α-glucosidase inhibitory activity (Chen *et al.,* 2010). This result indicates that the phenolic content of *Aquilaria* sp. extracts was responsible for the antioxidant and anti-hyperglycemic activities measured.

Table 2. Value of phenolic and flavonoid content, radical scavenging, and α-glucosidase inhibitory activity of *A. malaccensis*, *A. subintegra,* and *A. sinensis* in air and oven drying extracts

Sample/ Standard	Drying Method	TPC (mg GAE/gm)			TFC (que ppm)			DPPH (IC_{50})(μg/mL)			α-glucosidase (IC_{50})(μg/mL)		
		AMA	ASU	ASI	AMA	ASU	ASI	AMA	ASU	ASI	AMA	ASU	ASI
EE100	AD	82.57 ± 0.64^{a}	18.78 ± 0.14^{b}	36.72 ± 1.63^{b}	164.77 ± 3.04^{a}	773.92 ± 4.33^{e}	nd	161.58 ± 1.16^{a}	364.60 ± 1.64^{d}	377.77 ± 4.55^{d}	0.20 ± 1.04^{a}	nd	nd
	OD	85.15 ± 1.38^{a}	20.02 ± 0.18^{b}	17.3 ± 0.28^{b}	268.92 ± 5.28^{c}	nd	nd	145.80 ± 1.44^{a}	290.41 ± 0.69^{c}	191.81 ± 3.94^{ae}	0.13 ± 0.71^{a}	nd	nd
EE70	AD	41.09 ± 0.45^{c}	39.97 ± 0.40^{c}	36.85 ± 0.60^{b}	2180.97 ± 5.48^{b}	3116.06 ± 2.14^{f}	2954.44 ± 5.77^{h}	291.68 ± 0.85^{c}	224.60 ± 0.85^{e}	315.98 ± 1.10^{cd}	3.27 ± 1.09^{b}	nd	nd
	OD	64.27 ± 0.89^{d}	42.42 ± 0.17^{b}	42.56 ± 0.86^{b}	3733.45 ± 1.77^{d}	435.68 ± 3.58^{g}	1325.52 ± 4.19^{i}	33.60 ± 4.17^{b}	189.41 ± 0.79^{ae}	257.04 ± 1.67^{ce}	0.69 ± 1.71^{aa}	nd	nd
AE	AD	36.09 ± 0.95^{c}	39.29 ± 0.11^{c}	27.05 ± 0.63^{b}	nd	nd	nd	297.37 ± 3.69^{c}	270.47 ± 0.67^{c}	nd	4.69 ± 7.09^{c}	4.71 ± 6.53^{bc}	nd
	OD	52.98 ± 0.95^{b}	48.99 ± 0.58^{b}	46.31 ± 0.89^{b}	nd	nd	nd	344.17 ± 3.35^{d}	310.28 ± 3.70^{cd}	341.10 ± 1.11^{cd}	4.21 ± 1.87^{b}	nd	nd
HE	AD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	OD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Quercetin									20.75 ± 0.20^{b}			0.44 ± 1.11^{a}	
Acarbose												0.65 ± 0.98^{a}	

EE100 is 100% Ethanol extract, EE70 is 70% Ethanol extract, AE is Aqueous extract and HE is Hexane extract. For the drying method, AD is air drying and OD is oven drying. Meanwhile, the AMA is *Aquilaria malaccensis*, ASU is *Aquilaria subintegra*, ASI is *Aquilaria sinensis*. Data are expressed as mean ± standard deviation with three replicates. The superscript ^{a,b,c,d,e,f,g,h,i} was represent the significantly of sample in each activity. The analysis was run separately according to the activity that was done (TPC, TFC, DPPH, and á-glucosidase inhibition activities). Nd means not detected due to inhibition activity was less than 50% inhibition.

CONCLUSION

The OD-EE70-AMA was found to be the most preferred extraction due to the high content of TPC and TFC with the most potent DPPH scavenging and α-glucosidase inhibition activities. A strong positive correlation was reported between bioassays and TPC or TFC, hence indicating that *A. malaccensis* leaf used in this study might have the potential as a natural antioxidant and an antidiabetic agent.

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