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Antioxidant activity of *aquilaria malaccensis* (thymelaeaceae) leaves

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ABSTRACT

The phytochemical and antioxidant activity of *Aquilaria malaccensis* leaves were investigated. The sequential maceration extraction methods utilizing solvents with different polarities namely hexane, ethyl acetate and methanol yielded the corresponding crude extract. The extracts were subjected to preliminary phytochemical screening and revealed the presence of alkaloids, flavanoids, triterpenoids, steroids and saponins. The phytochemical screening suggests that flavanoids present in this species might provide a great value of antioxidant activity. Preliminary screenings of the free radical scavenging activity on the extracts of the plants with 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) were tested and showed positive result. Quarcetine was used as reference standard. The extracts exhibited strong antioxidant activity radical scavenging activity with IC_{50} value of 8.0×10^2 µg/ml, 1.6×10^2 µg/ml, 1.4×10^2 µg/ml, 30.0 µg/ml and 3.33 µg/ ml for hexane, DCM, ethyl acetate, methanol and quarcetine respectively.

Keywords: *Aquilaria malaccensis*, IC_{50} value, antioxidant activity

INTRODUCTION

Antioxidants play a major role in helping to protect our body from the formation of free radicals and prevent or delay the occurrence of lipid peroxidation. In our body, oxidants and free radicals, which are formed from triplet oxygen, water and unsaturated lipid molecules, can caused oxidative stress in tissue of lungs, heart and cardiovascular system, kidneys, liver, gastrointestinal tract, blood, eye, skin, muscle and brain. Thus, free radical is to be major precursor for the development of various degenerative diseases, such as cancer, atheroschlerosis, gastric ulser, diabetic, diabetic and others. An extensive study has been carried out to evaluate antioxidant property of compounds originated from terrestrial plant sources (Habsah et al., 2006). Phenolics plant, in particular phenolic acids, tannins and flavanoids are known to be as potent antioxidants (Pratt & Hudson, 1990). In this context, one such plant is *Aquilaria Malaccensis*. *Aquilaria Malaccensis* commonly known as gaharu is an aromatic plants.

Aquilaria malaccensis is widely distributed in south and south-east Asia. There are differing accounts of the countries in which it occurs. According to Oldfield et al. (1998), *A. malaccensis* is found in 10 countries which are Bangladesh, Bhutan, India, Indonesia, Iran, Malaysia, Myanmar, Philippines, Singapore and Thailand. *Aquilaria malaccensis* is prescribed in traditional East Asian medicine to relieve pain, arrest vomiting by warming the stomach, and to relieve asthma (Anon, 2003). High-grade agarwood powder is prescribed in Chinese medicine and is also used in the production of pharmaceutical tinctures (Van Beek & Phillips, 1999). The phytoconstituents isolated so far from this leaves are kusunol, jinkoh-eremol, jinkohol II, α-agarofuran, (-)-10-epi-γ-eudesmol and oxo-agarospirol, 10-epi-γ-eudesmol (Yoneda et al. 1984) and six new 2-(2-Phenylethyl) chromone compounds (Konishi et al.

2002). According to Karim et al. (2000), *A. malaccensis leaves* contained sesquiterpene alcohols, which was 10-epi-γ-eudesmol. There are few studies have been done previously on phytochemical screening and antioxidant activity using *A. malaccensis* extracts. Therefore, this study was focused on the antioxidant activity of leaves extract. Different approached had been use to enhance the research.

CHEMICALS

2, 2-diphenyl-1-picrylhydrazyl (DPPH) and quercetin were obtained from Organic Laboratory, Department of Chemical Sciences. All the chemicals and reagents used were of analytical grade.

PLANT MATERIALS

The fresh leaves of *Aquilaria malaccensis* were collected from Malaysian Nuclear Agency (MNA), Kajang, Selangor. The sample was then identified by one of the research officer of Malaysian Nuclear Agency.

EXTRACTION

Air dried leaves (977 g) was ground into fine powder and extracted by continuously soaking with hexane, followed by dichloromethane, ethyl acetate and methanol solvent. The crude extracts were concentrated under reduce pressure to give solid residue using rotary evaporator. The yield was found to be 1.78, 2.34, 5.26 and 7.28% w/w with reference to the air dried plant.

PRELIMINARY PHYTOCHEMICAL SCREENING

Phytochemical screening test was a qualitative method which was conducted to ensure the presence of alkaloids, steroids, triterpenoids and flavonoids in each crude extract. There were four main test which are Alkaloid test (Mayer), Triterpenoid / Steroid test (Liebermann-Burchard), Saponin test and Flavonoid test. The crude of hexane, dichloromethane, ethyl acetate and methanol was subjected into four tests.

ANTIOXIDANT ACTIVITY

The DPPH free radical scavenging assay was conducted on the hexane, dichloromethane, ethyl acetate and methanol crude extracts using modified method. The crude extracts (10 mg) were dissolved in 10 µl dimethylsulfoxide (DMSO). Then a clear microtiter plate was added with 5 µl DMSO solution and 5 µl of samples were added to the plate. The following concentrations of extract were prepared that is, 10, 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 mg/ml. Quarcetine was used as standard or positive control. All the solutions were prepared in the microtiter plate with DMSO. DMSO was used as the blank sample of this experiment. The experiments were carried out in replicate. The plate was left to incubate at room temperature for 30 minutes in the dark. The reduction of the DPPH free radical was measured by reading the UV absorbance at 517 nm by ELISA instrument. All tests and analyses were run in triplicate and averaged.

Percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Abs}_{\text{control (517nm)}} - \text{Abs}_{\text{sample (517nm)}}) \times 100\ \%}{\text{Abs}_{\text{control (517nm)}}}$$

Abs: absorbance

To obtain the IC_{50} value, the stocks of Quercetin (1 mg/ml) and crude extract (10 mg/ml) were diluted (two fold dilution) in 96-well micro plates to varying concentration topping from 1000 µg/ml down to the lowest of 15.6 µg/ml. The free radical-scavenging activities were determined as above (Habsah et al., 2006). A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Preliminary phytochemiacal screening in all crude showed the presence of alkaloids, steroids, tritepenoids, saponin and flavonoids. All crude except for ethyl acetate crude contains flavonoids which are generally potent inhibitors of free radicals (Havsteen, 1983). Table 1 showed the obtained result from phytochemical screening.

Antioxidant Activity-DPPH assay

This screening was done to determine whether there are any antioxidant properties in the crude. Figure 1 shows the

Table 1: Summary of the phytochemical screening tests on crude extracts of *A. malaccensis*

Crude Extract	Alkaloid Test	Triterpenoid / Steroid Test	Saponin Test	Flavonoid Test
Hexane	-	+S	-	+
Dichloromethane	-	+S	-	+
Ethyl Acetate	+	+S	+	-
Methanol	+	+T	+	+

Indicator:

-: Negative result
+: Positive result
+S: Positive results for steroid
+T: Positive results for triterpenoid

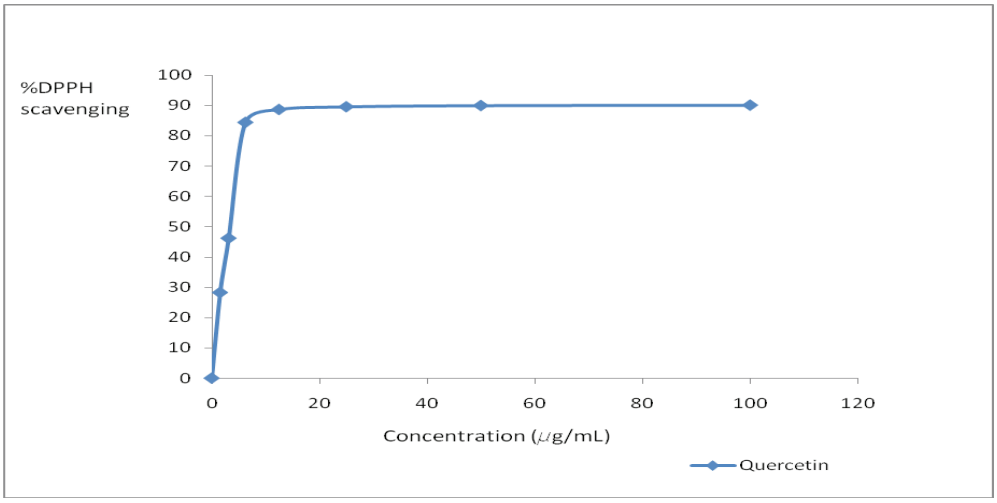

Figure 1: Percentage of inhibition H-donor activity of Quercetin as measured using DPPH assay with different concentration

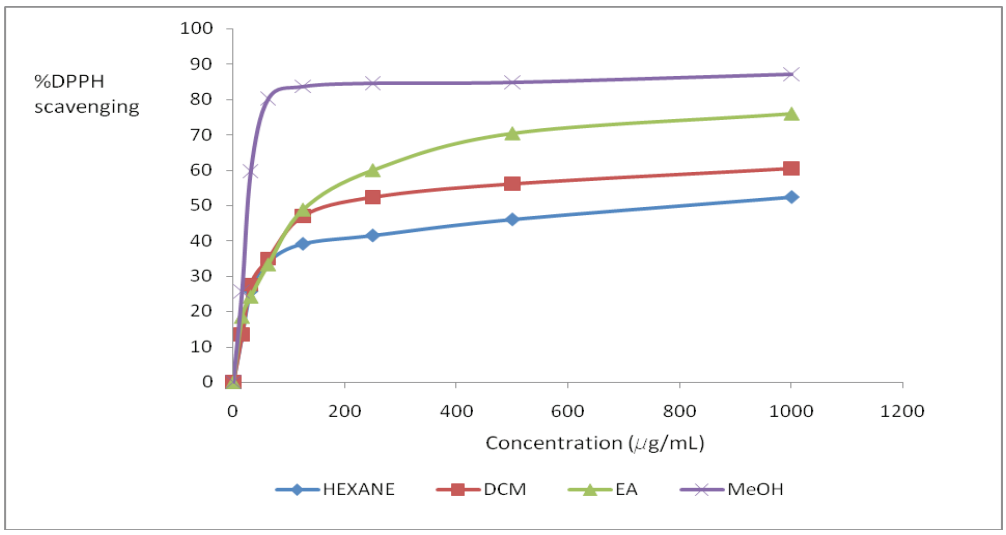

Figure 2: Percentage of inhibition H-donor activity of hexane, dichloromethane, ethyl acetate and methanol crude extract as measured using DPPH assay with different concentration

Table 2: Percentage of DPPH Free Radical Scavenging Activity of the crude extracts of *A. malaccensis*

% DPPH Free Radical Scavenging Activity				
Concentration of Crude Extract (x10² µg/ml)	**Type of Crude Extracts (%)**			
	Hexane	**DCM**	**EA**	**MeOH**
10.000	52.41 ± 1.02	60.46 ± 0.83	75.87 ± 1.64	87.21 ± 0.69
5.000	46.08 ± 1.18	56.10 ± 0.84	70.31 ± 1.47	84.91 ± 0.18
2.500	41.54 ± 1.76	52.33 ± 1.39	59.90 ± 0.31	84.65 ± 0.34
1.250	39.17 ± 1.02	47.03 ± 1.18	48.76 ± 0.76	83.72 ± 0.67
0.625	34.06 ± 0.28	34.93 ± 0.91	33.28 ± 0.98	80.23 ± 0.06
0.313	25.77 ± 4.65	27.53 ± 0.79	24.16 ± 1.45	59.75 ± 2.60
0.156	13.03 ± 2.95	13.44 ± 0.96	18.46 ± 0.63	21.11 ± 1.25

percentage of inhibition H-donor activity of quercetin as measured using DPPH assay with different concentration and Figure 2 shows the percentage of inhibition H-donor activity of hexane, dichloromethane, ethyl acetate and methanol crude extract as measured using DPPH assay with different concentration.

DPPH assay clearly shows the highest potential of antioxidant properties in each crude extracts of *A.*

Table 3: Percentage of DPPH Free Radical Scavenging Activity of the Quarcetine

Concentration of Quarcetine (x10^2 µg/ml)	% DPPH Free Radical Scavenging Activity
1.0000	90.06 ± 0.62
0.5000	89.93 ± 0.83
0.2500	89.54 ± 1.50
0.125	88.65 ± 1.30
0.063	84.38 ± 0.62
0.031	46.16 ± 0.45
0.016	28.26 ± 0.87

Table 4: IC_{50} Values of DPPH Free Radical Scavenging Activity for Crude Extracts of *A. malaccensis*

Crude Extracts	Value of IC_{50} (µg/ ml)
Hexane	800
DCM	160
EA	140
MeOH	30
Quarcetine (Std.)	3.33

malaccensis especially the methanol crude extract. Table 2 shows the percentage of DPPH Free Radical Scavenging Activity of each crude extracts while Table 3 shows the percentage of DPPH Free Radical Scavenging Activity of quarcetine as the standard used in this study.

The IC_{50} value of each crude extracts is the inhibition concentration to obtain 50% of a maximum scavenging capacity. The DPPH free radical scavenging activity for each crude extract can be simplifies as shown in Table 4, respectively. It could be observed that all the crude extracts exhibited a positive DPPH free radical scavenging activities. The IC_{50} values of DPPH free radical scavenging activity was in decreasing order:

MeOH > DCM > EA > Hexane

The methanol crude possessed the highest antioxidant activity than DCM, EA and Hexane crude extract. Methanol extracts were the most effective DPPH scavengers. Quarcetine is a potent free radical scavenging. So when compared to such pure compounds, IC_{50} value of the different crude extract is quite good proving that they are potent DPPH free radical scavenger. This can be attributed to the presence of flavonoids contains in the extract. Therefore, the extended researches are needed for the isolation and identification of the antioxidant compounds.

CONCLUSION

In conclusion, the preliminary phytochemical screening of the fruits of *A. malaccensis* indicates the presence of secondary metabolities, having an essential role in medicine. Overall, this study indicates the antioxidant activity of *A. malaccensis* and provides some idea about phytochemical investigation on *A. malaccensis*.

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