CHEMICAL COMPOSITION AND ANTIOXIDANT ACTICITY OF AQUILARIA MALACCENSIS LEAF EXTRACT

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ABSTRACT - In this study, water extraction (WE) and methanol extraction (ME) of *A. malaccensis* dried leaves (DL) and fresh leaves (FL) were investigated for its in vitro antioxidant activity that may contribute to their pharmacological effects. Total phenolic content (TPC) of this plants were determined by Folin-Ciaocalteu assay while antioxidant potential were evaluated by CUPRAC and DPPH method. WEDL showed the highest inhibition of the DPPH radical (48.07 ± 0.68 %) at concentration 1000 ug/ml and IC_{50} value was found to be 1.091mg/ml, relative to ascorbic acid, having an IC_{50} of 0.219 mg/ml. It also showed the highest CUPRAC value (3.32 ± 0.01µg/ml) as well as the highest TPC (181.11 ± 0.61gallic acid equivalent (GAE) mg/g) at a concentration of 1000 µg/ml) as compared to the other studied extracts. In conclusion, the results of this study clearly indicated that the extracts of *A. malaccensis* possess significant antioxidant activities and could be used as a potential source of natural antioxidant agents that may be due to the presence of phytochemicals.

Keywords: *Aquilaria malaccensis,* antioxidant activity, phytochemicals

INTRODUCTION

Aquilaria spp. are the most valuable and highly fragrant forest products locally known as agarwood, aloeswood, eaglewood, gaharu, kalamabak or oudh depending on the region[1]. Several genera that might be source of agarwood production from the family Thymelaeaceae might be endangered due to the deterioration of their natural resources include *Aquilaria, Gonystulus*, and *Gyrinops* having been listed in Appendix II of the Convention on Internal Trade in Endangered Species of Wild Fauna and Flora[2]. The most countries producing agarwood are Australia, India, China, Indonesia, Myanmar, Singapore, Laos, Thailand, Vietnam and Malaysia[3]. The main markets for these products are in South and East Asia and the Middle East. The identification of the botanical and ecological aspects of this species has been widely identified from a chemical constituent point of view that can be explain its uses as an important and excellent source of pharmaceutical products[4]. Many parts of this plants including the leaves, skin, seeds, wood and roots are valuable in medicinal properties. It is highly sought after for its resin and essential oils while less has been focused on the health beneficial effects of other parts of the plant despite the various ethnopharmacological evidences. These include antioxidant activities, analgesic, antipyretic, anti-inflammatory [5,6], antihyperglycemic [7], and antimicrobial [8] for various medicinal purposes.

Antioxidant has been ability to destroy single oxygen molecules and neutralize chemically active products of metabolism in order to protecting oxidative damage to cells which cause several diseases such as cancer, ageing [9] and diabetes [10]. Most researchers commonly use different methods for measuring antioxidant capacity of the compound extract. The antioxidant capacity and flavouring properties of many plant extracts is related to the presence of phenolic compounds such as phenolic acids, polyphenols and flavonoids [11]. However there is a little known about the potential phenolic profiles and antioxidant activity of herbs on human health [12].

Even though more research works has been carried out on this plant including woods and leaves but there is no enough scientific data available on the antioxidant activities of various extracts of the dried and fresh leaves. Thus, we have carried out the research work on antioxidant activities by using radical scavenging assays such as 2,2-diphenyl-1-picrylhydrazy (DPPH) and Cupric Reducing Antioxidant Capacity (CUPRAC) assay. The present study was also investigate the total phenolic content and phytochemical screening by using standard methods.

MATERIAL AND METHOD

Plant Collection and identification: The *A. malacenssis* dried leaves were purchased from Selangor and fresh leaves were collected at Ladang Karas, Merchang in Terengganu. Plants were identified with herbarium of Institute of Bioscience UPM, voucher specimen No. SK 2422/14 and 2423/14. The leaves were cut into small slices (~ 0.5-1.5 cm) and powdered using dry grinder.

Sample Preparation: Dried leaves were ground into fine powder and extracted by distilled water and methanol solvent. The supernatant were filtered using Whatman No. 2 filter paper. The filtrates from water were freeze dried using a vacuum freeze dryer for 42 hr at -62 °C. Meanwhile, the solvents (methanol) in the extract were removed under reduced pressure at 40 °C using rotary evaporator. The extracts were labeled as WEDL, WEFL, MEDL and MEFL. The process was repeated triplicates for each different sample. The extracts were placed in a glass bottle and then stored at −4 °C prior analysis.

Determination of total phenol content: Samples were measured for total phenolic content according to the previuous method [13]. Briefly, an aliquot of 12.5 µl of each plant was mixed with 250 µl of 2% sodium carbonate solution in 96-well microplate and allowed to react for 5 min at room temperature. Then 12.5 µl of diluted Folin-Ciocalteu phenol reagent (1:1 with water) and allowed to stand for 30 min at room temperature before the absorbance of the reaction mixture was read at 650 nm using a spectrophotometer. Calibration was achieved with an aqueous garlic acid solution. The TPC of the extracts was expressed as mg gallic acid equivalent (GAE) per gram of each plant on dry basis and all determinations were performed in triplicates.

Antioxidant assay

DPPH radical-scavenging activity: The scavenging activity of the extracts was determined using DPPH-scavenging assay. Briefly, 1 ml of each extract was allowed to react with 1 ml of 0.2 mM DPPH in 95% methanol. The solution was then incubated in dark at room temperature for

30 min and the absorbance (A) was measured at 517 nm using spectrophotometer. All samples were performed in triplicates. Ascorbic acid was used as a positive control. The scavenging activity was calculated as a percentage of DPPH decolouration relative to a negative control using the following equation:

Free-radical scavenging activity (%) = A (blank) - A (extract) / A (blank) x 100

CUPRAC Test: The capacity to reduce cupric ions was determined using the CUPRAC assay. The extracts was mixed with 50 µl samples extracts, 50 µl of $CuCl_2$ solution (1.0×10^{-2}M), 50 µl of neocuproine alcoholic solution (7.5×10^{-3}M) and 50 µl NH_4Ac buffer solution. Absorbance against a reagent blank was measured at 450 nm after 30 minutes.

Statistical Analyses: The data obtained in this study are means ± confidence interval of three replicate determinations. Statistical comparisons employed Tukey's test, with $P<0.05$ considered statistically significant. Concentrations yielding 50% inhibition (IC_{50}) value were calculated by interpolation from linear regression analysis. All statistical analyses used SPSS software.

RESULT AND DISCUSSION

The preliminary phytochemical screening tests for the crude extracts of A.M leaves revealed the presence of alkaloids, terpenoids, flavonoids, steroids, saponins and tannins (Table 2). Some of the secondary metabolites from a single or combination with others in plant extracts are liable for the antioxidant activity [14]. Some phytochemicals have antioxidant activity where it provides protection against damage and risk of developing chronic disease can be substantially reduced [15].

The total phenol content is reported as gallic acid equivalents by reference to standard curve ($Y= 0.002x - 0.045$ and $r^2=0.996$). Among the samples studied at 100 to 1000 µg/ml, WEDL had the highest total phenolic content (181.11 ± 0.61 mg GAE/g) followed by MEDL<MEFL<WEFL (Table 1). The phenolic contents in these extracts showing the leaf extracts contains the main active compound groups and acts as a type of antioxidants. In previous studies of *Aquilaria* spp., the presence of various polyphenol compounds was reported [16, 17, 18]. Boiling water extracted more polyphenols from dried plant that led to higher total phenols being extracted. However, methanols being organic and volatile solvent which able to denature polyphenol oxidases and extract a greater amount of endocellular material than water extract for fresh leaf extract [19].

Antioxidant activity was assessed by determining percentage of inhibition of DPPH. The quality of the antioxidants in the extracts was determined by the IC50 values which represent the concentration of the sample need to scavenge 50% of the DPPH free radicals. Ascorbic acid was used as standard for present investigation. The water and the methanol of dried leaves showed very similar values percentage inhibition of DPPH at a concentration of 1000 µg/ml which is 48.07 ± 0.68 % and 40.76 ± 0.55 %. The IC_{50} of extracts are presented as in Table 2. It showed that the lower the IC_{50} value indicated the higher scavenging potential. Their antioxidant activity are affected by phenolic compound on DPPH which able to donate hydrogen atom to form stable [20].

The chromogenic oxidizing reagent of the developed CUPRAC, i.e., bis-(neocuproine) copper (II) chloride (Cu (II)-Nc) reacts with polyphenolic antioxidants and oxidized them into the corresponding quinones and Cu(II)-Nc. Results showed reducing powers of water and methanolic extracts from fresh and dried samples increased rapidly at low concentrations from 100 to 1000 µg ml. Table 3 shows the dose-response curves for the reducing powers of different solvent extracts comparable with that of ascorbic acid (the positive control). It was found that the reducing power of the leaf extract also increased with increasing the concentrations by different system.

CONCLUSION

Water and methanol extract of dried leaves showed high extraction efficiency in antioxidant activity, followed by fresh leaves. However, dried and fresh leaves for various extract had significally higher TPC, DPPH, and CUPRAC than commercial teas. This study on in vitro antioxidant activities on agarwood from various A.M varieties has beneficial input to the agarwood leaves industry as well as food industry to develop a new food product and also stimulates the use of agarwood leaves as a new value added food ingredients. The screening of phenolic compound and the antioxidant activities were found to be very useful tools to provide in depth the characteristic of phenolics that are present agarwood leaves.

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