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Phenolic, flavonoid content and antioxidant aactivities of ethylacetate extract of *Litsea Cubeba* (Lour.) Pers. barks

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ABSTRACT

The oxidation induced by free radicals results in many degenerative disease. The purpose of this study to determine phenolic and flavonoid content, antioxidant activities of ethylacetate extract (EAE) of Litsea cubeba (Lour.) Pers. barks. Phenolic and total flavonoid contents in EAE was determined by colorimetric methods. EAE was found to contain high levels of phenolic (84.26 ± 0.57 mg GAE/g), total flavonoid (52.81 ± 0.03 mg QE/g). Antioxidant activity from DPPH assay measured as IC_{50} was 166.90 ± 0.10 µg/mL. The results reveal that EAE of Litsea cubeba barks has strong antioxidant potential. Our further study is to explore the mechanism action of EAE.

Keywords: Phenolic, flavonoid, Antioxidant, *Litsea cubeba*, ethylacetate extract.

INTRODUCTION

Oxidation is an important process in living organisms. Free radicals arising from metabolism or enviromental sources interact continously with biological system. Reactive species are molecules or atoms that have an electronic instability and highly reactive. Reactive oxygen species (ROS) are major sources of primary catalyst which initiate oxidation in vivo and in vitro and create oxidative stress. The uncontrolled production of oxygen free radicals and the unbalanced mechanism of antioxidant protection results in the onset of many diseases, such as cancer, diabetes, Alzheimer's, heart diseases and aging [1-6].

Attarasa (*Litsea cubeba* (Lour.) Pers.) is the plant from Lauraceae family which contain much volatile oils. Traditionally, volatile oils from attarasa was used as antideppresant, antiinflammation, antioxidant, pesticide, antimicrobial, anticancer and neuro pharmacology. Methanol extract from attarasa fruits was showed activity to HeLa cell lines which cause apoptosis with activation of caspase 3/7 [7]. The plants from *Litsea* genus contain alkaloids from isoquinolin group and were found more than 40 isoquinolin alkaloids which having antibacteria activity towards *Staphylococcus aureus* [8]. The aim of this study was to determine total phenolic and flavonoid content, antioxidant activities of ethylacetate fraction of *Litsea cubeba* Lour. Barks.

MATERIALS AND METHODS

Plant and chemicals material

Fresh barks of *Litsea cubeba* (Lour.) Pers. was collected from Balige subdistrict, Sumatera Utara province, Indonesia. *Litsea cubeba* (Lour.) Pers. was identified in Research Centre for Biology, Indonesian Institute of Science, Bogor, and the voucher specimen was deposited in herbarium. Chemicals used were $AlCl_3.6H_2O$ (Merck), distilled water, 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma), folin ciocalteu (Sigma), quercetine (Sigma), sodium acetate (Merck), sodium bicarbonate (Merck).

Preparation of Ethylacetate Extract (EAE)
The air-dried and powdered herbs of *Litsea cubeba* (Lour.) Pers. (1.5 kg) were repeatedly extracted by cold maceration with n-hexane (3x3 d, 7.5 L). The powder was dried in the air and extracted with ethylacetate (3x3 d, 7.5 L) at room temperature with occasional stirring. The filtrate was collected, and then evaporated under reduced pressure to give a viscous extract and then freeze dried to dry [9-11].

Determination of Total Phenol Concentration
The total phenol concentration (TPC) of the sample was determined using folin reagent. Briefly, 100 µL of EAE (500 µg/ml) were mixed with 7.9 mL of distilled water and 0.5 mL of folin-ciocaleu's reagent (1:10 v/v) and mixed with vortex for 1 minute. After mixing, 1.5 mL of 20% aqueous sodium bicarbonate were added, and the mixture was allowed to stand for 90 min within termittent shaking. The absorbance was measured at 775 nm using a spectrophotometer. Total phenolic concentration is expressed as gallic acid equivalentin mg per gram of extract. The methanol solution was use a blank. All assays were carried out in triplicate [5,2].

Determination of Total Flavonoid Concentration
The amount of total flavonoids in the extracts was measured spectrophotometrically as previously reported. Briefly, 2 mL of EAE in methanol was mixed with 0.10 mL of 10% aluminium chloride ($AlCl_3.6H_2O$), 0.10 mL of sodium acetate ($NaC_2H_3O_2.3H_2O$) (1 M) and 2.80 mL of distilled water. After incubation of 40 min, absorbance was measured at 432 nm using a spectrophotometer. To calculate the concentration of flavonoids, we prepared a calibration curve using quercetin as standard. The flavonoid concentrasion is expressed as quercetin equivalents in mg per gram of extract. All assays were carried out in triplicate [12,2].

Free Radical Scavenging Activity Test
The free radical scavenging activity was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH•) method of Blois (1958). 0.2mM solution of DPPH• in methanol was prepared and 100µl of this solution wasadded to various concentrations of EAF at the concentrations of 50, 100, 200 and 400 µg/ml. After 60 minutes, absorbance was measured at 516 nm. Quercetine was used as the reference material. All the tests were performed in triplicate and percentage of inhibition wascalculated by comparing the absorbance values of the control andtest samples [5,2].

Statistical analysis
Data was expressed as mean ± SD. All statistics were analyzed using the SPSS 20 software.

RESULTS AND DISCUSSION

Total Phenolic and Total Flavonoid Contents
Phenolic compounds are known as powerful chain breaking antioxidant [13]. Total phenolic content (TPC) was determined according to the Folin Ciocalteau method which is based in the reduction of phosphomolybdic-phosphotungstic acid (Folin) reagentto a blue-colored complex in an alkaline solution [14]. The EAE of *Litsea cubeba* (Lour.) Pers. barks. were found to contain high levels of phenolic content 84.26 ± 0.57 mg GAE/g. Phenolic compounds are very important plant consistuents because of their free radial scavenging ability due to their hydroxyl groups [15].

In the case of total flavonoid content (TFC), the EAE was given flavonoid content 52.81 ± 0.03 mg QE/g. Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, anti-viral and anti-cancer activities [16]. They are capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups and so they are potent antioxidant also [17].

Antiradical Activity
DPPH is one of the free radicals widely using for testing prelimenary radical scavenging activity of the plant extract [18]. Antiradical power of the plant samples was measured in term of hydrogen donating ability using DPPH which is a stable, nitrogen-centered free radical and produces deep purple colour in methanol solution. Antioxidants either transfer an electron or a hydrogen atom to DPPH, thus neutralizing its free radical character [19]. DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action [20]. The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search the in vitro general antioxidant acitivity of pure compounds as well as plant extracts [21]. It is very important to point out that a low IC_{50} value reflects a high antioxidant activity of the fraction, since the concentration necessary to inhibit the radical oxidation in 50% is low. IC_{50} for EAE and quercetin in DPPH assay were 166.90 ± 0.10 µg/mL and 4.94 ± 0.05 µg/mL respectively.

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