

ANTIOXIDANT ACTIVITY OF ALKALOID FRACTIONS AND COMPOUNDS FROM *Litsea cubeba* Lour. Fruits

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ABSTRACT

Maceration was used to extract the fruit of *Litsea cubeba* (Lour.). To get alkaloid fractions, ethanol extract was fractionated by liquid-liquid extraction using n-hexane, and chloroform at pH 3, 7, 9, and 11. Preparative thin layer chromatography was used to isolate alkaloid compounds. The ABTS test was used to assess the antioxidant activity of fractions and isolates. Chloroform fractions at pH 7 and compound III had the highest IC₅₀. They IC₅₀ were 80.47 ± 0.19 and 17.73 ± 0.04 µg/mL. Compound III was showed maximum absorbance at (279.0; 227.0; 212.5 nm) and wave number (3394.72; 2943.37; 2839.22; 1599.34; 1462.04; 1369.46; 1319.31; 1111.00; 817.82 cm⁻¹). This indicates that it is an alkaloid compound containing hydroxyl, methyl, methylene, and methoxy groups. The findings show that the alkaloid fractions and components of *Litsea cubeba* fruit have a high antioxidant potential.

Keywords: Antioxidant Activity, Fruit, , *Litsea cubeba* (Lour.), Compounds, an Alkaloid.

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INTRODUCTION

Free radicals are produced during the process of metabolism or from other sources such as the environment, meals, and cigarettes that interact with the biological system. Reactive species are molecules that are particularly reactive due to their electronic instability. The key catalyst that initiates the entire process of oxidation is reactive oxygen species (ROS). Oxidative stress occurs when reactive forms of oxygen are created quicker than they can be safely neutralized by antioxidant systems and/or when the antioxidant defense is reduced. The unregulated creation of free radicals and the unrated antioxidant system in defense are the root causes of many diseases, including cancer, diabetes, heart disease, Alzheimer's, and aging.¹⁻⁶ Attarasa (*Litsea cubeba* (Lour.)) is a plant in the Lauraceae family that contains various essential oils that have been utilized as antidepressants, antiinflammation, antioxidants, pesticides, antimicrobials, anticancer for breast cancer, and neuropharmacology. The methanol extract from attarasa fruits was found to be active on HeLa cell lines, causing apoptosis by caspase 3/7^{7,8} activation. The *Litsea* genus contains around forty isoquinoline alkaloids that are potent as antibacterial agents against *Staphylococcus aureus*.⁹ The heartwoods of *Litsea cubeba* contained a high amount of phenolic and flavonoid content, which was found to be active as an antioxidant and to have anti-breast cancer and anti-pancreatic cancer action, causing cell cycle inhibition. The alkaloid fractions of heartwoods and fruits have the ability to reduce the expression of the PI3KCA, Akt-1, and Akt-2 genes. Alkaloids that extracted from heartwood have antioxidant activity when tested using the DPPH and ABTS methods.¹⁰⁻¹⁵ The purpose of this study was to assess the antioxidant activity of alkaloid fractions and compounds that found in the fruits of *Litsea cubeba* Lour.

EXPERIMENTAL

Plant and Chemicals

Litsea cubeba (Lour.) fresh fruits were obtained from Balige subdistrict, Sumatera Utara province, Indonesia. *Litsea cubeba* (Lour.) was identified in Herbarium Medanense, Faculty of Mathematics and Natural Products, University of Sumatera Utara, and the voucher specimen was deposited in the herbarium. Distilled water (Bratachem), chloroform (Fulltime), hydrochloric acid (Merck), sodium hydroxide (Merck),

quercetin (Sigma), sodium persulfate (Merck), 2,2'-azino-bis(3-ethylbenzothiazoline-sulphonic acid (ABTS) (Sigma), and methanol were the chemicals utilized (Merck).

Preparation of Extract, Fractions, and Isolates

The air-dried and powdered fruits of *Litsea cubeba* (Lour) (1 kg) were repeatedly macerated with 96 percent ethanol (3x3 d, 7.5 L), and the filtrate was evaporated to get a viscous extract. At pH 3, 7, 9 and 11, the viscous extract was separated with n-hexane and then with chloroform. Preparative thin-layer chromatography (P-TLC) (Merck) was used to fractionate chloroform fraction at pH 7 using mobile phase dichloromethane: methanol: ammonia (85:15:1) and sprayed with Bouchard at reagent. The alkaloid compounds were examined using a UV-Vis spectrophotometer (Shimadzu) and FTIR (Shimadzu).^{11,16,17}

ABTS Radical Anion Scavenging Activity

The ABTS radical was created by reacting 5 mL of 14 mM ABTS solution with 5 mL of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution in the dark for 16 hours at room temperature. Prior to use, the solution was diluted with phosphate buffer saline (PBS) to achieve an absorbance of 0.700 ± 0.020 at 734 nm. The fractions and isolates were homogenized with 1 mL of ABTS solution and their absorbance was measured at 734 nm, PBS blanks were run in each test, and all measurements were completed after at least 6 minutes. Similarly, the standard group reaction mixture was prepared using quercetin, and the ABTS scavenging ability was quantified as IC_{50} (g/mL).^{5,11}

Statistical Analysis

Data was interpreted as mean $\pm$ SD which were analyzed using the SPSS 21 software.

RESULTS AND DISCUSSION

Antiradical Activity

The antioxidant assay with ABTS, which is based on the ability of ABTS, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action¹⁸ and has been widely used as a quick, reliable, and reproducible in vitro antioxidant activity assay.¹⁹ Compounds' reducing capacity might be used to predict prospective antioxidant activity.^{12,20,21} Alkaloids are compounds with OH and NH functional groups that may donate hydrogen to the radical ABTS.²² The IC_{50} for each percentage and molecule is listed in Table-1.

Table-1: IC_{50} Value of Alkaloid Fractions and Compounds of *Litsea cubeba* Fruit with ABTS Assay (Mean $\pm$ SD, 3 times of replication)

Treatment	IC_{50} (μ g/mL)
Ethanol extract	492.29 ± 10.68
n-hexane Fraction	549.17 ± 14.10
Chloroform Fraction pH 3	290.40 ± 10.46
Chloroform Fraction pH 7	80.47 ± 2.29
Chloroform Fraction pH 9	88.94 ± 5.92
Chloroform Fraction pH 11	153.75 ± 7.83
Water Fraction	408.79 ± 13.17
Compound I	151.47 ± 2.50
Compound II	70.90 ± 0.88
Compound III	17.73 ± 0.15
Quercetin	13.88 ± 0.10

Analysis of Compounds

Compounds were extracted from a chloroform fraction at pH 7 and identified using spectroscopic techniques such as UV-Vis and FTIR spectrophotometers. Compound I's UV-Vis spectra was shown. (403.5; 277.0 and 204.5 nm), compound II (276.0 and 213.0 nm), compound III (279.0; 227.0 and 212.5 nm). The FTIR spectrum were showed compound I (3379.29; 2927.94; 2862.36; 1724.36; 1662.64; 1454.33; 1377.17; 1161.15; 1072.42 cm^{-1}), compound II (3406.29; 2924.09; 2858.51; 1728.22; 1624.06; 1454.33; 1377.17; 1246.02; 1114.86; 883.40 cm^{-1}), compound III (3394.72; 2943.37; 2839.22; 1599.34; 1462.04; 1369.46; 1319.31; 1111.00; 817.82 cm^{-1}). The O-H groups were classified based on the

wavenumber between 3000 – 3500 cm^{-1} (3394.72 and 3286.70 cm^{-1}). The N-H group was shown at 3109.25 cm^{-1} . The C-H stretching groups were shown at (2800 – 2950 cm^{-1}) and C-H bending groups were shown at (2800 – 2950 cm^{-1}). Many researchers have found that alkaloid compounds include significant functional groups with stretching and bending groups O-H, N-H, and C-H.^{23,24}

CONCLUSION

This research found that the alkaloid fractions and chemicals in *Litsea cubeba* fruit had antioxidant properties.

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