Antioxidant and Cytotoxic Bioassay on *Blumeodendron toxbrai* (Blume.) Stem Bark Hexane, Dichloromethane, and Methanolic Extract

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**ABSTRACT**

**Introduction** *Blumeodendron toksbraii* has the potential to be anti-HIV and anti α-glucosidase. **Objective:** This research was conducted to examine the effects of antioxidant and cytotoxicity in vitro from these compounds from methanolic stem bark extract. **Method:** Stem bark to be extracted with maceration using hexane, dichloromethane, and methanol solution. Extracts were quantified with respect to in vitro antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. Anticytotoxic activity was determined by cytotoxicity assay using MCF-7 cell line with Alamar Blue method. **Results:** The observed *IC₅₀* value from hexane, dichloromethane, and methanol extract for antioxidant assay were 88.33 ± 0.19 μg/mL, 74.54 ± 0.61 μg/mL and 94.1 ± 0.19 μg/mL respectively. *IC₅₀* value of anti-cytotoxic assay from hexane extract, dichloromethane and methanol extract is 121.24 ± 0.15 μg/mL, 55 ± 0.48 μg/mL and 70.71 ± 0.15 μg/mL. Conclusion: dichloromethane extract showed good promising result for anti-oxidant and cytotoxic assay, further study needed to isolate compound from this plant. **Key Words:** *Blumeodendron toksbraii*, Cancer, Antioxidant, Anticytotoxic DPPH, MCF-7.

**INTRODUCTION**

Cancer is the most prevalence disease in the world. The number of cancer sufferers has increased worldwide. WHO data reports that there were 8.8 million of cancer sufferers in 2015. This increase is due to the widespread pollution of waste that has the potential as a carcinogen, UV intensity, and unhealthy lifestyles such as cigarettes and alcohol1.

Cancer treatments have used surgery, radiation, chemotherapy, hormonal therapy, and monoclonal antibodies. Surgery of body tissues contained in cancer cells is the first treatment for some cancer sufferers, but this is effective if done in early-stage cancer. The use of chemotherapy can also be an effective treatment option for treating cancer. So far, the chemotherapy drugs in circulation have come from synthetic drugs. Synthetic drugs are derived from natural materials such as the discovery of taxol from the Pacific Pine plant, vincristine from *Catharanthus roseus* (40 μL) was added to the sample solution, then 155 μL methanol (4 mg/mL), 5 μL sample was pipetted into a 96-well plate. DPPH free radical scavenging activity was conducted according to Yen and Che1. Sample was dissolved in methanol (4 mg/mL). 5 μL sample was pipetted into the microplate. Methanolic 1 mM DPPH solution (40 μL) was added to the sample solution, then 155 μL methanol was added to give final concentration of the sample was 100 μg/mL. The sample was measured at 515 nm.

**MATERIAL AND METHOD**

**Sample preparation**

Stem bark of *Blumeodendron toksbraii* 1000 grams were collected from Bogor Botanical Garden, Bogor. The stem bark were dried then crushed to be fine powder.

**Extraction**

The extraction process was done by maceration method. 1 kg of dried bark, the bark powder is then extracted with hexane solvent to obtain hexane extract. The remaining residue was extracted with dichloromethane (1.5 L / kg) solvent to obtain dichloromethane extract. Then the remaining residue was macerated again with methanol so as to obtain methanol extract with a vaccum rotary evaporator.

**Antioxidant assay**

DPPH free radical scavenging activity was conducted according to Yen and Che1. Sample was dissolved in methanol (4 mg/mL). 5 μL sample was pipetted into the microplate. Methanolic 1 mM DPPH solution (40 μL) was added to the sample solution, then 155 μL methanol was added to give final concentration of the sample was 100 μg/mL. The sample was measured at 515 nm.

**Cytotoxic assay**

Breast cancer cell line MCF-7 was obtained from Dr. Churiah (LAPTIAB-BPPT) and maintained in RCCChemLIPI). The cell lines were cultivated at 37°C with 5% CO₂ in RPMI60 or DMEM (GIBCO)
medium supplemented with 10% v/v Fetal Bovine Serum (Sigma), 1% antibiotic-antimycotic (GIBCO). Cell suspensions (5x104 cells/mL) were seeding to each well and the cells were incubated for at 37°C under 5% CO₂. After 24 hours, extracts were added and microscopic observation was conducted after 24 hour incubation.

RESULT AND DISCUSSION

Antioxidant assay

The observed IC₅₀ value from hexane, dichloromethane, and methanol extract were 88.33 ± 0.19 µg/mL, 74.54 ± 0.61 µg/mL, and 94.1 ± 0.19 µg/mL respectively. It shows that dichloromethane extract has better antioxidant activity than methanol extract, whereas hexane extract is inactive due to the high content of non-polar compounds such as fatty acid compounds. These results are in accordance with the antioxidant test. According to Nurul Jadid dkk. Anti-oxidant activity range between 50-100 µg/mL exhibit mild anti-oxidant activity. The possibility of these results is due to the phytochemical content that exists are alkaloids, saponins, and glycosides. Based on the literature, the most antioxidant properties found in flavonoids and some of the alkaloids. In Blumeodendron toxbraii plants there are only alkaloids, saponins, and glycosides so that the results of measurement of antioxidant activity indicate an intermediate level.

Cytotoxic assay

From hexane crude extract, dichloromethane, and methanol extract represented effects on MCF-7 with IC₅₀ values of 121.24 ± 0.15 µg/mL, 55 ± 0.48 µg/mL, and 70.71 ± 0.15 µg/mL. It showed dichloromethane extract was good cytotoxic activity against breast cancer cell line MCF-7 which better than hexane and methanolic extract, whereas Blumeodendron toxbraii showed very low cytotoxicity. The test results showed dichloromethane showed inhibitory activity of cancer cell growth because it had a value in accordance with the criteria where the IC₅₀ value was below 100 µg/mL, whereas hexane is inactive because it has an IC₅₀ value above 100 µg/mL. A compound is categorized into a potential cytotoxic compound if it has an IC₅₀ value <100 µg/mL, has moderate cytotoxic activity if the IC₅₀ value is in the range of 100-1000 µg/mL, and has no cytotoxic activity if the IC₅₀ value is > 1000 µg/mL. Hexane extract also contains non-polar compounds such as fatty acid compounds. This hexane extract does not have strong antioxidant or cytotoxic activity because the main content of hexane extract is fatty acids. Semipolar and polar extracts like dichloromethane and methanol are thought to contain more phenol compounds. Increase or decrease in antioxidant IC₅₀ values is positively correlated with increase / decrease in anticancer IC₅₀ values. The higher the IC₅₀ value of antioxidant activity, the higher the IC₅₀ value of anticancer activity. The content of phenolic compounds can increase the inhibitory effect on cancer cells. This is consistent with the results obtained in this study, in which the antioxidant extract of dichloromethane extract had IC₅₀ 74.54 µg / mL and IC₅₀ for anti-cytotoxic is 55 µg / mL.

CONCLUSION

Blumeodendron toxbraii has show good antioxidant and cytotoxicity based on bioassay result from dichloromethane extract. From dichloromethane extract, it show 74.54 ± 0.61 µg/mL for antioxidant assay and 55 ± 0.48 µg/mL for cytotoxic assay. Further attempt needed to isolate compound from dichloromethane extract which show good anti-oxidant and cytotoxic assay from this plant.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest in this study.

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