Phytochemicals Analysis and Cytotoxicity Activity of Ethanol Extract of *Litseacubeba* Lour. Heartwood

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**ABSTRACT**

Objective: The purpose of this study was to determine the chemical compounds which contain in the ethanol extract and cytotoxic activity ethanol extract of *Litseacubeba* heartwood induced in T47D cells.

Methods: The ethanol extract was extracted by maceration using ethanol 96% solvent. Cytotoxic activity was determined with MTT method and the IC50 analyzed using SPSS 23.

Results: Phytochemicals screening showed that the ethanol extract of *Litseacubeba* heartwood contained steroids/triterpenoids, glycosides, alkaloids, flavonoids, saponins and tannins. The IC50 ethanol extract of *Litseacubeba* heartwood were 349.57 ±0.35μg/ml in T47D cells.

Conclusions: Ethanol extract of *Litseacubeba* heartwood has activity as an anticancer to T47D cells breast cancer agents.

**Keywords:** heartwood, *Litseacubeba* Lour, T47D cell, cytotoxicity, phytochemicals.

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**INTRODUCTION**

Attarasa is a plant from the Lauraceae family that contains bioactive alkaloids, essential oils, flavonoids and steroids, which in this plant also contains total phenolic and flavonoids which are known to have antioxidant functions1. Traditionally, essential oils in attarasa plants are used as an antidepressant, anti-inflammatory, antioxidant, pesticide, antimicrobial, anticancer and neuro pharmacological agent2. Piyapat et al. stated that methanol extract from attarasa fruits has an activity that causes apoptosis by activating the caspase 3/7 against Hela cells. Isoquinolone alkaloids can be used as inhibitors of the cholinesterase enzyme, wherein the inhibition of the enzyme coninesterase can treat alzheimer disease, Parkinson's disease, and inhibitors of premature aging1.

Cancer is a disease that is very complex and is ranked first as the leading cause of death worldwide4. The most common type of cancer suffered by women is breast cancer (30% of all cancer cases in women), and 14% of these cases end in death5. Handling cancer with chemotherapy agents is still an option in cancer treatment. However, the presence of a multidrug resistance (MDR) mechanism results in reduced efficacy of chemotherapy drugs6. Some research began to be directed at testing the potential of natural ingredients as chemoprevention agents that have the potential as chemotherapy companion agents7. The aim is to increase the sensitivity of cancer cells and reduce the side effects caused by chemotherapy agents8. Chemoprevention agents referred to here generally have the activity of inhibiting tumor growth through the mechanism of cell cycle arrest, apoptosis tracking or inhibiting the expression of proteins that play a role in Multi Drug Resistance9. Chemopreventive agents can reduce the risk of cancer by inhibiting the initiation of preneoplastic lesions by carcinogens, or reversing cancer progression. One approach
to finding chemopreventive compounds is through exploration of natural materials, especially plants\textsuperscript{10}. The chemical composition of the heartwood in this study is intended to determine the characteristics and content of chemical compounds and to know the anticancer activity of \textit{Litsea cubeba} heartwood extract.

**EXPERIMENTAL**

**Plant and chemicals materials**

Fresh heartwood of \textit{Litsea cubeba} (Lour.) were collected from Parsoburan Village, Toba Samosir, North Sumatra, Indonesia. \textit{Litsea cubeba} Lour. was identified in Herbarium Medanese (MEDA) University of Sumatera Utara. The chemicals materials used in this study were ethanol 96%, Hepes (Sigma), dimethyl sulfoxide (DMSO) (Sigma), DMEM media, RPMI-1640 media, FBS (Gibco), penicillin-streptomycin (Gibco), Fungizon (Amphotericin B) 0.5%, trypsin-EDTA 0.25% (Gibco), Fetal Bovine Serum (Gibco), PBS, and [3-(4,5-dimethylthiazol-2-il)-2,5-difeniltetrazolium bromide] (Sigma).

**Preparation of extract**

Ethanol \textit{Litsea cubeba} Lour.

Heartwood

The air-dried and powdered heartwood of \textit{Litsea cubeba} (Lour.) (1 kg) were repeatedly macerated with ethanol 96% (3x3 d, 7.5 L), the filtrate was evaporated with a rotary evaporator at a temperature of ± 40°C to give a viscous extract\textsuperscript{11}.

**Phytochemical analysis of ethanol extract \textit{Litsea cubeba} Lour. heartwood**

Phytochemical analysis was performed on ethanol extract of heartwood \textit{Litsea cubeba} Lour. Included examination of secondary metabolites of alkaloids, flavonoids, glycosides, tannins, saponins and triterpenoids/steroids were carried out according to standard procedures\textsuperscript{12}.

**Dosage of extract**

The treatment of extract used several concentration series of 500µg /mL; 250µg /mL; 125µg /mL; 62.5µg /mL; 31.25µg /mL and 15.625µg /mL.

**Cytotoxicity Assay And Selectivity Index**

T47D cells were grown on RPMI media supplemented with 10% (Gibco) Fetal bovine, Penicillin 1% Streptomycin 1% (Gibco) and Fungizon 0.5% (Gibco) were incubated at 37°C, CO2 5%. The inoculums seeded on a 96 well plate (Iwaki), each well 1 x 104 cells/0.1 mL. Cell culture were incubated at 37°C, 5% CO2 for 24 hours. After 24 hours the media was discarded and the cell plus ethanol extract and doxorubicin were incubated for 24 hours then the medium was removed and 0.5 mg / mL of MTT was added and incubated for 4 hours at 37°C, 5% CO2. after crystal formazan was formed and 10% SDS was added to dissolve the formazan crystals, then incubated for 24 hours at room temperature and shielded from light. The absorbance was measured with microplate reader at λ 595 nm. The resulting absorbance was converted to a percentage of cell viability, then the selectivity index (IS) ethanol extract was determined against T47D cells\textsuperscript{13}.

The equation to determine the viability of cells

\[
\% \text{Viability} = \frac{\text{Absorbance of treatment} - \text{absorbance of medium}}{\text{absorbance of control cells} - \text{absorbance of medium}} \times 100\%
\]

**Statistical Analysis**

The results were presented as means ± SD. The statistical analysis was carried out by using SPSS edition 23.

**RESULT AND DISCUSSION**

The results of phytochemicals constituent analysis from ethanol extract of heartwood \textit{Litsea cubeba} Lour. were determined to obtain the information of the group of phytochemical which contain in \textit{Litsea cubeba} Lour. The results can be seen on Table 1. Alkaloids from ethanol extract\textsuperscript{11} and Phenolic; flavonoid from ethyl acetate extract were identified active as antioxidant activity, alkaloids fraction active as inhibited the development cell cancers\textsuperscript{14}.

| No | Metabolite secondary | Simplicia | Extract |
|----|----------------------|----------|---------|
| 1  | Alkaloids            | +        | +       |
| 2  | Flavonoids           | +        | +       |
| 3  | Saponin              | +        | +       |
| 4  | Tannins              | +        | +       |
| 5  | Glikosid             | +        | +       |
| 6  | Steroid/Triterpenoid | +        | +       |

Description: (+) shows that the simplicia and ethanol extract contains secondary metabolite, (-) shows that the simplicia and ethanol extract not contain secondary metabolite. Phytochemical compounds in simplicia and ethanol extract isn’t different.

Cytotoxic effect of ethanol extract \textit{Litsea cubeba} Lour. was carried out by MTT method [3-(4,5-dimethyl thiazol-2-il) - 2,5-difeniltetrazolium bromide] was used to determine cell viability in each observation as indicated by IC\textsubscript{50} values which could inhibit cell growth after being treated and incubated for 24 hours. Inhibition of cell growth is indicated by IC\textsubscript{50} value of 100 µg / ml, with the smaller IC\textsubscript{50} value means the higher the value of its cytotoxic activity. IC\textsubscript{50} values obtained from ethanol extract of heartwood \textit{Litsea cubeba} Lour. against T47D cells were 349.57±0.35µg / mL.Dalimunthe\textsuperscript{14} states that an extract which is declared active when giving an IC\textsubscript{50} value of 10-100 µg / ml, with the results obtained from the alkaloid fraction of attarasa heartwood and fruit at pH 7 and 9 were 46.60± 0.19; 123.01 ± 14.63dan 35.89 ± 1.04; 98.31 ± 2.51µg/mL. The smaller the IC\textsubscript{50} value means the higher the value of its cytotoxic activity. Cytotoxicity can be grouped into three namely: (1) cytotoxic potential if IC50 <100µg / ml, (2) moderate
cytotoxic if 100μg/ml < IC50 <1000μg/ml and non-toxic if IC50> 1000 μg / ml. Groups of compounds with potential cytotoxicity can be used as anticancer agents while moderate cytotoxicity can be used for chemoprevention that can prevent and inhibit the growth of cancer cells15. NCI (National Cancer Institute) has established anticancer activity criteria based on Inhibition Concentration 50 (IC50), which is the concentration of substances needed to inhibit cell growth by 50%. A substance is called cytotoxic (anticancer) if its activity on a test has an IC50 value <4μg / ml.16

Ethanol extract of Litsea cubeba Lour. cytotoxic activity was also showed by changes in T47D cells morphology and viability data after treatment. T47D cells morphology and viability data can be seen in figure 1 and figure 2.

**Figure 1:** The cytotoxic effect of the sample on T47D cell. The observation was performed under inverted microscope with 100x magnification. A: Ethanol Extract 500 μg/mL, B: Ethanol Extract 31.25μg/mL, C: Control Cell

**Figure 2:** Percentage of viability Ethanol Extract of Litsea cubeba (500μg/ml, 250μg/ml, 125μg/ml, 62.5μg/ml and 31.25μg/ml) on T47D cell.

From figure 1, can viewed ethanol extract leaded death in T47D Cell morphology was changed and having damaged. If concentration of ethanol extract was increased, then it will cause percentage of viability will decreased. Figure 2 showed, an increased in ethanol extract concentration caused decreased percentage of viability at 51, 25%, 69, 21%, 75, 96%, 86, 41% and 99, 32%.

**CONCLUSION**

Based on the results we obtained ethanol extract of heartwood Litsea cubeba Lour.hada potentially used as to co-chemotherapy agent for breast cancer therapy.

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