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Research Article

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 whichcontained 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 IC_{50}analyzed using SPSS 23.
Results: Phytochemicals screening were showed that the ethanol extract of *Litseacubeba* heartwood contained steroids/triterpenoids, glycosides, alkaloids, flavonoids, saponins and tannins. The $IC_{50}$of 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 functions[1]. Traditionally, essential oils in attarasa plants are used as an antidepressant, anti-inflammatory, antioxidant, pesticide, antimicrobial, anticancer and neuro pharmacological agent[2]. 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 alzaimer disease, Parkinson's disease, and inhibitors of premature aging[3].

Cancer is a disease that is very complex and is ranked first as the leading cause of death worldwide[4]. 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 death[5]. 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 drugs[6]. Some research began to be directed at testing the potential of natural ingredients as chemoprevention agents that have the potential as chemotherapy companion agents[7]. The aim is to increase the sensitivity of cancer cells and reduce the side effects caused by chemotherapy agents[8]. 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 Resistance[9].

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[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 Litseacubeba heartwood extract.

EXPERIMENTAL

Plant and chemicals materials

Fresh heartwood of *Litseacubeba* (Lour.) were collected from Parsoburan Village, Toba Samosir, North Sumatra, Indonesia. *Litseacubeba* Lour. was identified in Herbarium Medanense (MEDA) Universityof 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 extractethanol *Litseacubeba* Lour. Heartwood

The air-dried and powdered heartwood of *Litseacubeba* (Lour,) (1 kg) were repeatedly macerated with ethanol 96% (3x3 d, 7.5 L), the filtrate was evaporated with a rotary evaporator with a temperature of ± 40^{o}C to give a viscous extract[11].

Phytochemical analysis of ethanol extract *Litseacubeba* Lour.heartwood

Phytochemical analysis was performed on ethanol extract of heartwood *Litseacubeba* Lour. Included examination of secondary metabolites of alkaloids, flavonoids, glycosides, tannins, saponins and triterpenoids/steroids were carried out according to standard procedures[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 AssayAnd Selectivity Index

T47D cells were grown on RPMI media supplemented with 10% (Gibco) Fetal bovine, Penicillin 1% Streptomycin 1% (Gibco) and Fungizone 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[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 byusing SPSS edition 23.

RESULT AND DISCUSSION

The results of phytochemicals constituent analysis from ethanol extract of heartwood *Litseacubeba* Lour.were determined to obtain the information of the group of phytochemical which contain in *Litseacubeba* Lour. The resultscan be seen on Table 1. Alkaloids from ethanol extract[11] and Phenolic; flavonoid from ethyl acetate extract[1]were identified active as antioxidant activity, alkaloids fraction active as inhibited the development cell cancers[14].

Table 1: Phytochemicals content heartwood of *Litseacubeba* Lour.

No	Metabolite secondary	Simplicia	Extract
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Saponin	+	+
4	Tanins	+	+
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 *Litseacubeba* Lour. was carried out by MTT method [3- (4,5-dimethyl thiazol-2-il) -2,5-diphenyltetrazolium bromide] was used to determine cell viability in each observation as indicated by IC_{50} values which could inhibit cell growth after being treated and incubated for 24 hours. Inhibition of cell growth is indicated by IC_{50} values[13]. The result of Ic_{50} can be seen in the Table 2.

Table 2: IC_{50} (ug/mL) ethanol extract of *Litseacubeba*Lour.againstT47D cell

Sample	IC_{50}(ug/mL)
Heartwood of *Litseacubeba* Lour	349.57±0,35

Description: IC_{50} was measured using MTT method, ethanol extract of *Litsea cubeba* Lour.

IC_{50} values obtained from ethanol extract of heartwood *Litseacubeba* Lour.againstT47D cells were 349.57±0,35µg / mL.Dalimunthe[14] states that an extract which is declared active when giving an IC_{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_{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 (IC_{50}), 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 IC_{50} 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.

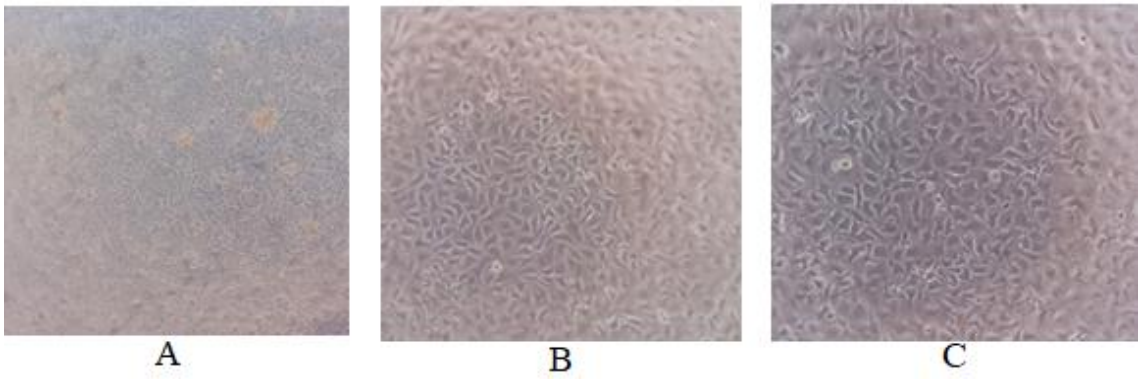

Figure 1: The cytotoxic effect of the sample on T47D cell. The observation was performed under inverted microscope with 100x magnification. A: Ethanol Extract 500 ug/mL, B: Ethanol Extract 31.25ug/mL, C: Control Cell

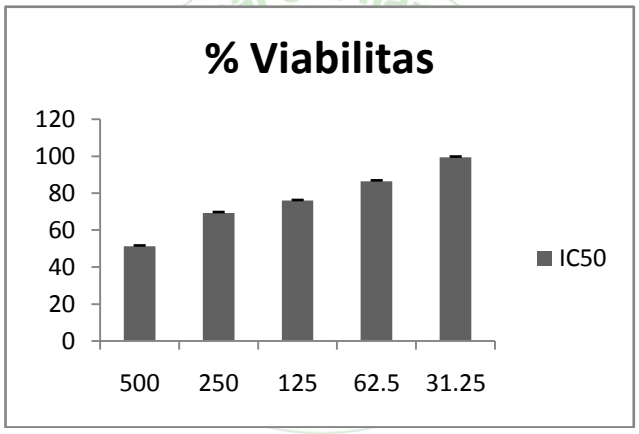

Figure 2: Percentage of viability Ethanol Extract of *Litseacubeba* (500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml and 31.25µg/ml) on T47D cell.

From figure 1, can viewed ethanol extract leaded death in T47 D 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 *Litseacubeba* Lour.hada potentially used as to co-chemotherapy agent for breast cancer therapy.

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