PHYTOCHEMICALS ANALYSIS AND CELL CYCLE ARREST ACTIVITY OF ETHANOL EXTRACT OF *Litsea cubeba* Lour. FRUITS TOWARDS MCF-7/HER-2 CELL LINE

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ABSTRACT

Attarasa (*Litsea cubeba* Lour.) fruit is a potential anticancer, especially for breast cancer. This study evaluated phytochemicals analysis of ethanol extract, cytotoxicity, cell cycle inhibition and increase p53 expression activities of ethanol extract (EE) of *Litsea cubeba* Lour. fruit. EE was analyzed for phytochemicals content based on Indonesia Herbal Pharmacopeia, cytotoxicity activity was determined by MTT method towards breast cancer cell line MCF-7/Her-2. Cell cycle inhibition and p53 expression were analyzed with the flow cytometry method. EE was found to contain alkaloids, flavonoids, steroids/triterpenoids, saponins, tannins and glycosides. EE was found to have IC\textsubscript{50} 57.92 ± 1.08 µg/mL, caused accumulation in G\textsubscript{0}-G\textsubscript{1} phase (64.52% to 70.45%) and increased p53 expression (0.66% to 61.98%). The results utter that EE of *Litsea cubeba* Lour. fruits have cytotoxic activities by inhibiting the cell cycle. Our further study is to assess the molecular mechanism of compounds responsible for anticancer activity. **Keywords:** Breast Cancer, MCF-7/Her-2, Phytochemicals Constituent, *Litsea cubeba* Lour., Ethanol Extract.

INTRODUCTION

Breast cancer is one of the most incidence rates of cancer in women after cervical cancer. Alteration in lifestyle and the daily diet is the most cause that affecting the number of breast cancer patients. The high number of incidence and the high charge treatment for cancer patients therefore is a serious problem for looking for other sources of medicine notably through traditional medicine.¹ ³ *Litsea cubeba* (Lour.) is a Lauraceae family plant that contents volatile oils which used as antimicrobial, anticancer on breast cancer, pesticide, antidepressants, antiinflammation, antioxidant, and neuropharmacology. It was showed to be active on HeLa cell which causes apoptosis through the initiation of activation of caspase 3/7.⁴ ⁵ Isoquinoline alkaloids in *Litsea* genus are active as antibacterial.⁶ The heartwoods contained a high concentration of flavonoid and phenolic and active as an antioxidant and inhibit breast cancer progression through cell cycle arrest. Alkaloid fractions of heartwoods and fruits can decrease PI3KCA, Akt-1 and Akt-2 gene expression. Alkaloid compounds from heartwood have an antioxidant effect with ABTS and DPPH methods.⁷ ¹⁰ The purpose of our study was to analysis of phytochemicals and assess anti-breast cancer activity of *Litsea cubeba* Lour. fruits ethanol extract towards MCF-7/Her-2 cells.

EXPERIMENTAL

Preparation of Extract

The air-dried and powdered fruits of *Litsea cubeba* (Lour.) (1000 g) were macerated with ethanol 96% (3x3 d, 7.5 L), The filtrate was evaporated to give a viscous extract.¹¹
Phytochemicals Constituent Analysis
Analysis of phytochemicals from EE was analyzed to determine alkaloids, flavonoids, steroids/triterpenoids, tannins, glycosides and saponins based on the previous study.  

Cytotoxicity Activity
MCF-7/Her-2 cell line (1x10^4 cells) were grown in DMEM complete medium. After 24 hours of incubation, the medium was replaced and treated by EE. The further procedure was followed as previously describe. 

Cell Cycle Inhibition Analysis
MCF-7/Her-2 cell line (5x10^5 cells) were seeded and incubated for 24 hours in incubator CO₂ 5%. After treatment and incubation, cells were harvested and analyzed with a flow cytometer were followed the procedure from the previous study. 

p53 Expression
MCF-7/Her-2 cell line (5x10^5 cells) were seeded and incubated for 24 hours in incubator CO₂ 5%. After treatment and incubation, cells were collected in a conical tube and washed thrice with cold PBS. The sediment of cells was collected in a microtube. The cells were fixated and p53 FITC antibody was added and incubated at 37°C for 10 min and analyzed using FACScan flow cytometer. 

RESULTS AND DISCUSSION
Phytochemicals Analysis Result of Ethanol Extract Litsea cubeba Lour. Heartwood
Phytochemicals constituent analysis from EE was determined to obtain the information of the group of phytochemicals contain in EE. The results were given of alkaloids, flavonoids, saponin, tanin, glycosides and steroids/triterpenoids. 

Cytotoxic Activity (IC₅₀)
The treatment of EE was shown to exhibit cell progression. The IC₅₀ score of EE was 57.92 ± 1.08 μg/mL. The cytotoxicity of a natural product is related to the content of active compounds in these plants. Flavonoids, steroids/triterpenoids and alkaloids are estimated as active compounds. 

Analysis of Cell Cycle
The effect of EE is given in Fig.-1. Treatment with EE in 25 μg/mL induced cells accumulation at G0-G1 phase (70.45%) and control cells (64.52%). This result was to show that EE inhibits cells progression at G0-G1 phase. Evaluation of cell cycle was analyzed with flow cytometry using propidium iodide as shown in Fig.-1.

Fig.-1: Cell Cycle Analysis using Flow Cytometry (a) Control Cells; (b) EE 25 μg/mL

Fig.-2: p53 Expression Analysis using Flow Cytometry. (a) Control Cells; (b) EE 25 μg/mL
Analysis of p53 Expression

To analysis, the impact of EE on p53 expression was conducted by the flow cytometry method, and the results are given in Fig.-2. Treatment with EE 25 µg/mL caused cell accumulation in M1 area (61.98%) and for the control cell (0.66%).

CONCLUSION

The results reveal that ethanol extract of *Litsea cubeba* Lour. fruits contain various active compounds and active as an anticancer against MCF-7/Her-2 cell lines by cell cycle inhibition and increase of p53 expression.

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