

PHYTOCHEMICALS CONSTITUENT ANALYSIS AND CELL CYCLE INHIBITION EFFECT OF ETHANOL EXTRACT OF *Litsea cubeba* Lour. HEARTWOOD TOWARDS MCF-7/HER-2 CELL LINE

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ABSTRACT

Attarasa (*Litsea cubeba* Lour.) heartwood is potential anticancer especially for breast cancer which is overexpressed in HER-2. This study evaluated phytochemicals analysis of ethanol extract, cytotoxicity, cell cycle inhibition and p53 expression of ethanol extract (EE) of *Litsea cubeba* Lour. heartwood. EE was analyzed for phytochemicals content with LC-HRMS, 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 24 compounds. EE was found to have IC₅₀ 191.63 ± 1.56 µg/mL, at concentration 40 µg/mL caused accumulation in G₂-M phase (17.71% to 23.34%) and increased p53 expression (0.99% to 17.63%). The results utter that EE of *Litsea cubeba* Lour. heartwood has a cytotoxic effect through inhibition of the cell cycle. Our further study is to assess the molecular mechanism that responsible for anticancer activity.

Keywords: Breast Cancer, MCF-7/Her-2, *Litsea cubeba* Lour., Heartwood, Ethanol Extract.

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INTRODUCTION

Breast cancer is one of the most incidence rates of cancer in women after cervical cancer. Alteration in lifestyle and 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 HER2 signaling becomes an interesting field to develop a molecular targeted therapy for cancer. Several chemotherapeutic agents targeted on HER2 receptor are developed. Trastuzumab is found to be the first antibody targeting HER2. However, 70% of patients who prolonged use of trastuzumab shows a resistance phenomenon leading to a progression of metastatic cancer due to the increasing of EGFR expression.^{4,5}

Litsea cubeba (Lour.) is a Lauraceae family plant which has contains volatile oils which used as antimicrobial, anticancer on breast cancer, pesticide, antidepressants, antiinflammation, antioxidant, and neuropharmacology. It was showed to be active on HeLa cellc which causes apoptosis through the initiation of activation of caspase 3/7.^{6,7} Isoquinoline alkaloids in *Litsea* genus are active as antibacterial.⁸ 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 antioxidant effects with ABTS and DPPH methods.⁹⁻¹² The purpose of our study was to analyze phytochemicals constituent and assess the anti-breast cancer activity of ethanol extract of *Litsea cubeba* Lour. heartwood on MCF-7/Her-2 cells.

EXPERIMENTAL

Preparation of Extract

The air-dried and powdered heartwoods 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.¹³

Phytochemicals Constituent Analysis with LC-HRMS

Analysis of phytochemicals from EE was analyzed with TSQ executive (Thermo) (LSIH, Brawijaya University) with mobile phase A (0.1% formic acid in water and B (0.1% formic acid in acetonitrile) with gradient method and flow rate 40 μ L/minute for the column using Hypersil GOLD aQ 50 x 1 mm x 1.9 μ m and the time for analysis was 70 minute. The results were analyzed compound discover with mz cloud software.^{14,15}

Cytotoxicity Activity

MCF-7/Her-2 cells (1×10^4 cells) were grown in DMEM complete medium. After 24 h incubated, the medium was discharged and treated by EE. The further procedure was followed as previously described.^{16,17}

Cell Cycle Inhibition Analysis

MCF-7/Her-2 cell line (5×10^5 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.^{14,18}

p53 Expression

MCF-7/Her-2 cell line (5×10^5 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 microtube¹⁷⁻¹⁸. The cells were fixated and p53 FITC antibody was added and incubated at 37°C for 10 min and analyzed using FACScan flow cytometer.^{14,16}

RESULTS AND DISCUSSION

Phytochemicals Constituent Analysis Result of Ethanol Extract

Phytochemicals constituent analysis from EE were determined to obtain the information of compounds contain in Ee with LC-HRMS. The results were given in (Table-1).

Table-1: Phytochemicals Constituent Analysis of EE with LC-MS/MS

No	Name	Formula	Molecular Weight	Retention Time (Min)
1	N-methylhernagine	C ₂₀ H ₂₃ NO ₄	341.1623	0.835
2	Salsolinol	C ₁₀ H ₁₃ NO ₂	179.0945	1.198
3	Pipecolic acid	C ₆ H ₁₁ NO ₂	129.0789	1.228
4	2-methoxyresorcinol	C ₇ H ₈ O ₃	108.0210	1.786
5	Citral	C ₁₀ H ₁₆ O	152.1201	2.607
6	Ethyl cinnamate	C ₁₁ H ₁₂ O ₂	176.0840	3.925
7	Jasmonic acid	C ₁₂ H ₁₈ O ₃	210.1229	4.19
8	Senkyunolide H	C ₁₂ H ₁₆ O ₄	224.1024	4.293
9	Sinomenine	C ₁₉ H ₂₃ NO ₄	329.1627	4.397
10	Methyl jasmonate	C ₁₃ H ₂₀ O ₃	224.1410	4.443
11	Quercetin	C ₁₅ H ₁₀ O ₇	302.042	4.492
12	Meperidine	C ₁₅ H ₂₁ NO ₂	247.1571	5.234
13	Kaemferol-7-O-glucoside	C ₂₁ H ₂₀ O ₁₁	448.1004	5.36
14	p-cymene	C ₁₀ H ₁₄	134.1100	5.735
15	Alphaprodine	C ₁₆ H ₂₃ NO ₂	261.1727	5.803
16	Kaemferol	C ₁₅ H ₁₀ O ₆	286.0476	5.963
17	Carvone	C ₁₀ H ₁₄ O	168.1150	6.002
18	(-)-caryophyllene oxide	C ₁₅ H ₂₄ O	220.1827	7.136
19	Drofenine	C ₂₀ H ₃₁ NO ₂	317.2353	7.456
20	Samandarone	C ₁₉ H ₂₉ NO ₂	303.2200	7.647

21	2-Hydroxycinnamic acid	C ₉ H ₈ O ₃	146.0370	8.027
22	Maraniol	C ₁₂ H ₁₂ O ₃	204.0790	13.082
23	Jasmonal	C ₁₄ H ₁₈ O	202.1360	21.305
24	Erucamide	C ₂₂ H ₄₃ NO	337.3340	21.719

The results were showed that ethanol extract of *Litsea cubeba* Lour. heartwood contains alkaloids, phenolics, flavonoids, volatile oils and coumarins.

Cytotoxic Activity

In treatment, EE was shown to exhibit cell growth. The IC₅₀ value of EE was 191.63 ± 1.56 µg/mL. The cytotoxicity estimate of a natural product is related to the content of active compounds in these plants. Alkaloids, phenolics, flavonoids, volatile oils and coumarins are estimated as active compounds.^{19,20}

Analysis of Cell Cycle

The effect of EE is given in Fig.-1. Treatment with EE in 40 µg/mL caused cell accumulation at G₂-M phase (23.34%) and for the control cell (17.71%). This fact was to indicate that EE can inhibit cell growth at G₂-M phase. Flavonoids induce apoptosis and inhibition cell cycle through inhibition of survival signaling proteins such as protein kinase c (PKC-α) and the activation of death signals (PKC-δ).²¹ Evaluation of cell cycle inhibition was analyzed with flow cytometry using propidium iodide as shown in Fig.-1.

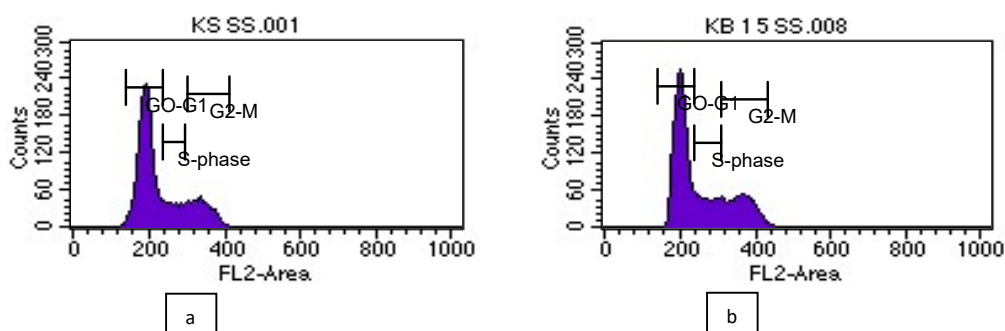


Fig.-2: Cell Cycle Analysis using Flow Cytometry:(a) Control Cells; (b) EE 40 µg/mL.

p53 Expression

The analysis of the impact of EE on p53 expression was conducted by the flow cytometry method, and the results is given in Fig.-2. Treatment with EE 40 µg/mL caused cell accumulation in M1 area (17.63%) and for the control cell (0.99%). Quercetin is one of the flavonoids compounds that have an anticancer activity which induced expression of p53 and modulation of ROS expression and the cells will be death cause of apoptosis process.²¹

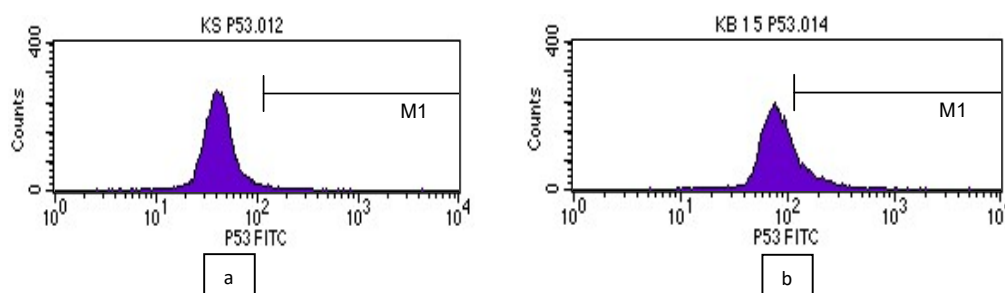


Fig.-2: p53 Expression Analysis using Flowcytometry. (a) control cells; (b) EE 40 µg/mL.

CONCLUSION

The results reveal that ethanol extract of *Litsea cubeba* Lour. heartwood contains 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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REFERENCES

1. A. Montazeri, M. Vahdaninia and I. Harirchi, *Asia Pacific Family Medicine*, **7**, 6(2008). <https://doi.org/10.1186/1447-056X-7-6>
2. C.H. Yip, *Breast Cancer in Asia: Methods in Molecular Biology*, Human Press, Totowa, (2009).
3. P.F. Rezaei, S. Fouladdel, C. Silvia, S.M. Ghaffari, G.R. Amin and E. Azizi, *Cytotechnology*, **63**, 503(2011), <https://doi.org/10.1007/s10616-011-9373-6>
4. E. Meiyanto, D. D. P. Putri, R. A. Susudarti, R. Murwanti, Sardjiman, A. Fitriasari, *Asia Pacific Journal of Cancer Prevention*, **15**, 179(2014), <https://doi.org/10.7314/apjcp.2014.15.1.179>
5. T. Vu, F.X. Claret, *Frontiers in Oncology*, **2**, 62(2012), <https://doi.org/10.3389/fonc.2012.00062>
6. P. Trisonthi, S. Akihiko, N. Hisashi, T. Hiratoshi, *Molecules*, **19**, 6838(2014), <https://doi.org/10.3390/molecules19056838>
7. T. Piyapat, K. Miyagawa, H. Tamura, *Journal of Life Sciences*, **7(9)**, 928(2013)
8. T. Feng, Z. Rong-Ting, T. Qin-Gang, Z. Xiang-Yun, *Z Naturforsch*, **64**, 871(2009).
9. A. Dalimunthe, P.A.Z. Hasibuan, D. Satria, *Asian Journal of Pharmaceutical and Clinical Research*, **10(11)**, 404(2017), <https://doi.org/10.22159/ajpcr.2017.v1oi11.20204>
10. A. Dalimunthe, P.A.Z. Hasibuan, J. Silalahi, S.F. Sinaga, D. Satria, *Orient Journal of Chemistry*, **34(2)**, 1149(2018), <https://doi.org/10.13005/ojc/340270>
11. A. Dalimunthe, S. Achmad, D. Satria, *Der Pharma Chemica*, **8**, 466(2016)
12. A. Dalimunthe, P.A.Z. Hasibuan, D. Satria, *Open Access Macedonian Journal of Medical Sciences*, **7(9)**, 1422(2019), <https://doi.org/10.3889/oamjs.2019.317>
13. U. Harahap, P.A.Z. Hasibuan, P. Sitorus, N. Arfian, D. Satria, *Asian Pacific Journal of Cancer Prevention*, **19(2)**, 565(2018), <https://doi.org/10.22034/APJCP.2018.19.2.565>
14. P.A.Z. hasibuan, U. Harahap, P.Sitorus, D. Satria, *Heliyon*, **6**, e04449(2020), <https://doi.org/10.1016/j.heliyon.2020.e04449>
15. Y. Tine, Y. Yang, F. Renucci, J. Costa, A. Wele, J. Paolini, *Natural Product Communications*, **12(12)**, 1865(2017), <https://doi.org/10.1177/1934578X1701201213>
16. D. Satria, J. Silalahi, G. Haro, S. Ilyas, P.A.Z. Hasibuan, *Rasayan Journal of Chemistry*, **12(2)**, 803(2019), <https://doi.org/10.31788/RJC.2019.1225180>
17. A.E. Nugroho, A. Hermawan, D.D.P. Putri, A. Novika, E. Meiyanto, *Asian Pacific Journal of Tropical Biomedicine*, **3(4)**, 297(2013), [https://doi.org/10.1016/S2221-1691\(13\)60066-0](https://doi.org/10.1016/S2221-1691(13)60066-0)
18. P. Mao, Z. Ershao, C. Yang, L. Likun, R. Daqing, *Oncology Letters*, **13**, 1019(2017), <https://doi.org/10.3892/ol.2016.5509>
19. V.R. Yadav, P. Sahdeo, S. Bokyung, K. Ramaswamy, B.A. Bharat, *Toxins*, **2**, 2428(2010), <https://doi.org/10.3390/toxins2102428>
20. E. Varghese, S.M. Samuel, M. Abotaleb, S. Cheema, R. Mamtani, D. Busselberg, *Cancers*, **10**, 346 (2018), <https://doi.org/10.3390/cancers10100346>
21. M. Hashemzaei, A.D. Far, A. Yari, R.E. Heravi, K. Tabrizian, *Oncology Reports*, **38**, 819(2017), <https://doi.org/10.3892/or.2017.5766>

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