ISSN 2745-455X (Online)
Indonesian Journal of Pharmacology and Therapy

Cytotoxic activity of hantap (*Sterculia oblongata* Mast) leaves extract against breast cancer cells line (MCF7/HER2): the effect on the expression of HER2 mRNA and the apoptosis

Sitti Ayu Suhartina Yahya[1]*, Mustofa[2], Indwiani Astuti[2], Woro Rukmi Pratiwi[2], Adika Suwarman[3]

[1]Master in Biomedical Sciences, Faculty of Medicine, Public Health and Nursing, Universitas Gajah Mada, [2]Department of Pharmacology and Therapy, Faculty of Medicine, Public Health and Nursing, Universitas Gajah Mada, Yogyakarta, Indonesia.

https://doi.org/10.22146/ijpther.3204

Submitted: 28/10/2021
Accepted : 12/03/2022

Keywords:
hantap (*S. oblonga* Mast.);
cytotoxic;
HER2;
apoptosis;
MCF7/HER2

ABSTRACT

Hantap leaves (*Sterculia oblongata* Mast) has been used traditionally to treat breast cancer in Palu, Central Sulawesi. However, its use is just based on empirical evidences rather than scientific evidences. The study aimed to investigate the cytotoxic activity of hantap leaves extracts against breast cancer cells line. The effect of this extract on the HER2 expression and the apoptosis was also evaluated. The hantap (*S. oblongata* Mast) leaves extracts were prepared by consecutive maceration method using n-hexane, methanol and water, respectively. The cytotoxic activity against MCF7/HER2 breast cancer cells line was evaluated using the MTT assay with doxorubicin as a positive control. The HER2 mRNA expression was examined using RT-PCR and the apoptosis after 24 h incubation was examined using a fluorescence microscope after AO-PI (acridine orange-propidium iodide) staining. Among three extracts tested, the methanolic extract exhibited the most cytotoxic against MCF7/HER2 cells with an IC_{50} of 91.25 µg/mL. Therefore, the methanolic extract was subjected to further study. The methanolic extract at concentration of $1/2IC_{50}$; $1IC_{50}$ and $2IC_{50}$ µM induced 6.8; 26.3 and 25.3% apoptosis of the MFC7/HER2 cell lines, respectively. The methanolic extract at concentration of $1/2IC_{50}$; $1IC_{50}$ and $2IC_{50}$ µM inhibited HER2 mRNA expression to be 0.6; 0.25 and 0.33 compared to control cells. In conclusion, the methanolic extract of hantap leaves (*S. oblongata* Mast) has cytotoxic activity against MCF7/HER2 breast cancer cell lines by induce cells apoptosis and inhibit HER2 mRNA expression. Further study, will be conducted to isolate active constituents as anticancer.

ABSTRAK

Daun hantap (*S. oblongata* Mast) telah digunakan secara tradisional untuk mengobati kanker payudara di Palu, Sulawesi Tengah. Namun, penggunaanya masih didasarkan bukti empiris daripada bukti ilmiah. Penelitian ini bertujuan mengkaji aktivitas sitotoksik ekstrak daun hantap (*S. oblongata* Mast) terhadap sel kanker payudara. Efek ekstrak terhadap ekspresi HER2 mRNA dan apoptosis juga dikaji. Ekstrak daun hantap (*S. oblongata* Mast) dibuat dengan maserasi bertingkat berturutan menggunakan pelarut n-heksan, methanol dan air. Aktivitas sitotoksik terhadap sel kanker payudara MCF7/HER2 dikaji menggunakan metode MMT dengan doksorubisin sebagai control positif. Ekspresi HER2 mRNA ditetapkan using RT-PCR dan apoptosis setelah inkubasi 24 jam diamati dengan mikroskop fluoresen setelah pengecatan dengan AO-API (*acridine orange-propidium iodide*). Di antara tiga ekstrak yang diuji, ekstrak methanol merupakan ekstrak yang paling toksik terhadap sel MCF7/HER2 dengan nilai IC_{50} sebesar 91.25 µg/mL. Oleh karena itu ekstrak metanol digunakan untuk uji selanjutnya. Ekstrak metanol konsentrasi $1/2IC_{50}$, IC_{50} dan $2IC_{50}$ menginduksi berturut-turut 6,8; 26,3; dan 25.3% apoptosis sel MCF7/HER2. Ekstrak metanol pada konsentrasi $1/2IC_{50}$, IC_{50} dan $2IC_{50}$ menghambat berturut-turut ekspresi HER2 mRNA sebesar 0,6; 0,25; dan 0,33 dibandingkan kontrol. Dapat disimpulkan ekstrak metanol daun hantap (*S. oblongata* Mast) mempunyai aktivitas sitotoksik terhadap sel kanker payudara MCF7/HER2 dengan menginduksi apoptosis dan menghambat ekspresi HER2 mRNA. Penelitian lanjutan akan dilakukan untuk mengisolasi kandungan aktifnya sebagai antikanker.

*corresponding author: sittiayusuhartina.yahya8596@gmail.com

INTRODUCTION

Cancer is a disease associated with abnormality and an uncontrolled growth of cells.[1] One of the most common types of cancer in the world is breast cancer. In 2020, it was reported 2.3 million women diagnosed with breast cancer and 685,000 deaths globally. Breast cancer is the most prevalent cancer in the world in the past 5 years with 7.8 million cases.[2] In Indonesia in 2020, 396,914 new cases with 234,511 were reported in 2020. The prevalent cases in the past 5 years was 946,088.[3,4]

Based on the expression pattern of certain gene, breast cancers are usually divided into five intrinsic or molecular subtypes.[5] HER2+ breast cancer is one of subtype of breast cancers which characterized by the high expression of the HER2 receptor and make up 10-15% of breast cancer. In contrast to normal breast cancer cells which express approximately 20,000 HER2 receptors on the cell membrane, the HER2+ breast cancer cells, express more than 2 million HER2 receptors.[6] This high expression of HER2 receptor is associated malignancy, poor prognosis and high mortality.[7,8]

Genetic and lifestyle/environmental factors are associated in the aetiology of breast cancer. Genetic mutations in the cancer cells lead to defects in cell-signaling systems that initiate apoptosis.[9,10] Apoptosis is the process programmed cell death that occur to eliminate unwanted cells. It is mediated by several intrinsic and extrinsic signalling pathways triggered by multiple factors, including cellular stress, DNA damage and immune surveillance. For last decades, apoptosis has been investigated as novel target for cancer theatment.[11,12]

Medicinal plants have been used traditionally to treat cancer in some regions in the world. Among the medicinal plants have been evaluated their *in vitro* and *in vivo* anticancer activity. Hantap leaves (*Sterculia oblonga*, Mast) has been used traditionally as an alternative therapy for breast cancer in Palu, Central Sulawesi. However, its used as anticancer just based on empirical evidences rather than scientific evidences. A biological activity study reported, hantap leaves extracts have antioxidant activity.[13] Furthermore, phytochemical analysis reported, hantap leaves contained biological active compounds such as tannins (1.24%), alkaloids (10%), flavonoids (3.4%), saponin (4%), oxalate (1.31%), cyanogenic glycoside (0.6%) and phenol (0.0067%).[14]

This study aimed to evaluate the cytotoxic activity of the leaves extract of hantap (*S. oblonga* Mast) against breast cancer cells line (MCF7/HER2). Furthermore, the effect of this extract on apoptosis and HER2 expression were also evaluated.

MATERIALS AND METHODS

Extracts preparation

The protocol of the study was approved by the Medical and Health Research Ethic Committee, Faculty of Medicine, Public Health and Nursing/Dr. Sardjito General Hospital, Yogyakarta (reff. KE/FK/1213/EC on November 5th, 2020). The plant leaves were collected from their natural habitat in Palu, Central Sulawesi and were identified and authenticated in Universitas Tadulako, Palu, Central Sulawesi. The plant leaves were air-dried at room temperature and grounded into powder. Extracts were prepared by consecutive maceration method using n-hexane, methanol and water, respectively. One hundred fifty g of the leaves powder was macerated in n-hexane, with intermittent stirring, for 24 h at room temperature. The extracts of n-hexane were then filtered and concentrated to dryness using a rotary evaporator. Dry n-hexane extracts were stored at 4 ^{o}C until further analysis. The residue obtained after maceration in n-hexane was further macerated consecutively with methanol and water. The methanol and water extracts obtained in each maceration step were

collected, dried and stored at 4 °C until further analysis.

Cytotoxicity assay

The cytotoxic activity was measured by the MTT [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide assay. Briefly, each extract was pre-solubilized in dimethylsulphoxide (DMSO) at 37 °C to obtained a stock solution. Serial dilutions of the test extracts were prepared from the stock solution to obtained working concentration of 400 – 6.25 μg/mL with the final concentration of DMSO was not higher than 1%. Confluent monolayers of breast cancer cells line (MCF7/HER2) were grown in 96 well microplates for 24 h. Cells were incubated with various concentrations of the test extracts in triplicate at 37 °C in a CO_2 environment for 24 h. The negative control was growth medium cell culture alone instead of plant extract, whereas doxorubicin was used as the positive control. Following after 24 h incubation period, supernatants were removed from the wells and 25 μL of the MTT solution (2 mg/mL) in phosphate buffer saline (PBS) was added to each well and incubated at 37 °C for 4 h. The formed formazan crystals, directly correlated to the number of viable cells in the culture, were solubilized by addition of 125 μL of DMSO to each well. The plates were placed on a shaker for 15 min and the optical density was determined at λ 595 nm on a ELISA reader. The cytotoxic activity was expressed by the 50% inhibitory concentration (IC_{50}) which is defined as the extract concentration required for reduction of cell viability by half. The IC_{50} value was calculated using a non-linear regression curve and presented as mean ± standard error of the mean (SEM).

HER2 mRNA expression examination

Total RNA extraction and cDNA synthesis

The effect of the most active extract on the HER2 mRNA expression was examined using qRT-PCR.[24] After seeding and overnight incubation at 37° C, breast cancer cells line (MCF7/HER2) was treated with 3 different concentration of the test extracts (1/2 x IC_{50}; 1 x IC_{50} and 2 x IC_{50}) and incubated for 24 h. Doxorubicin in concentration of 1 x IC_{50} was used as positive control. Total RNA was extracted by using the Favorgen kit according to the manufacture's instruction. The extracted RNA was quantified using UV spectrophotometry at λ of 260 and 280 nm and then stored at -80 °C. To synthesize cDNA, 2 μg of the extracted RNA, 1 μL of oligo dT/random primer, 8 μL of DEPC-treated H_2O were mixed in a microtube. After incubation at 70 °C for 5 min ad 4 °C for 1 min, 4 μL 5X RT buffer (dNTPs), 5 μL DEPC-treated H_2O and 1 μL RNase were added for a final volume of 10 μL. The cDNA synthesis conditions were 25 °C for 10 min followed by 37 °C for 50 min and 80 °C for 5 min. The cDNA samples were then stored at –20 °C until analysis.

Real time PCR

To determine the HER2 expression, 1 μg of cDNA were reversed transcribed by using the ExelRT™ Reverse Transcription Kit II according to the manufacture's instruction. PCR reactions used PROMO ExcelTaq™ 2X Fast Q-PCR Master Mix (SYBR). PCR conditions were 2 s at 95°C, 15 s at 95°C, and 60 s at 60°C for 40 cycles. HER2 transcripts were detected using following primers. HER2-F: 5'-CCAGGACCTGCTGAACTGGT-3'; HER2-R: 5'-TGTACGAGCCGCACATCC-3'; and using glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-F:5'-GAAGATGGTGATGGGATTTC-3', GAPD-R: 5'GAAGGTGAAGGTCGGAGTC-3' as endogenous reference. The HER2 expression was quantified following the analysis of a concentration of cDNA. All samples were measured in triplicate. For each experimental sample, the amount of the HER2 and GAPDH were determined from the standard curves.

Apoptosis examination

For the most active extract, its effect on cell apoptotic cell was also examined by using acridine orange/propidium iodide (AO/PI) assay. After seeding in 24 well plate and overnight incubation at 37° C, breast cancer cells line (MCF7/HER2) was treated with 3 different concentration of the test extracts (1/2 x IC_{50}; 1 x IC_{50} and 2 x IC_{50}) and incubated for 24 h. Doxorubicin in concentration of 1 x IC_{50} was used as positive control. After being incubation, 20 µL of trypsin was added into each well. When cells had slough off, 25 µL suspensions were transferred to glass slides. Five microliter of dual fluorescent staining solution containing 100 µg/mL AO and 100 µg/mL PI was added to each suspension and then covered with a coverslip. The apoptotic cell in 100 cells was counted within 20 min by using a fluorescent microscope. Viable cells showed green fluorescent, whereas apoptotic cells showed orange fluorescent. The assay was repeated 3 time.

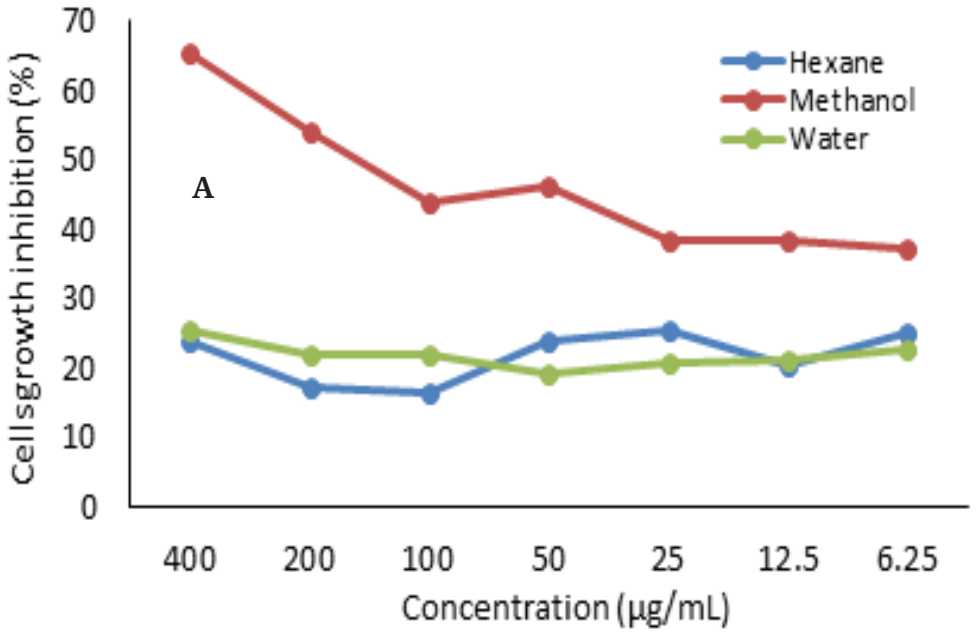
Statistical analysis

Quantitative data were presented as mean ± standard error of the mean (SEM). One way Anova was applied for parametric data and continued by Tamhane Post Hoc test to evaluate significant difference between groups.

RESULTS

Cytotoxicity assay

Curve of relationship between the concentration of hantap leaves (*S. oblonga* Mast) extracts (A) and doxorubicin (B) and the inhibition of MCF7/HER2 cells line growth after 24 h incubation is presented on FIGURE 1. Based on this curve, the IC_{50} value was calculated. Among the three extracts tested, the methanolic extract is the most active with the IC_{50} value was 91.25 µg/mL. The IC_{50} of hexanic and water extracts were > 400 µg/mL, whereas the IC_{50} of doxorubicin was 3.192 µg/mL.



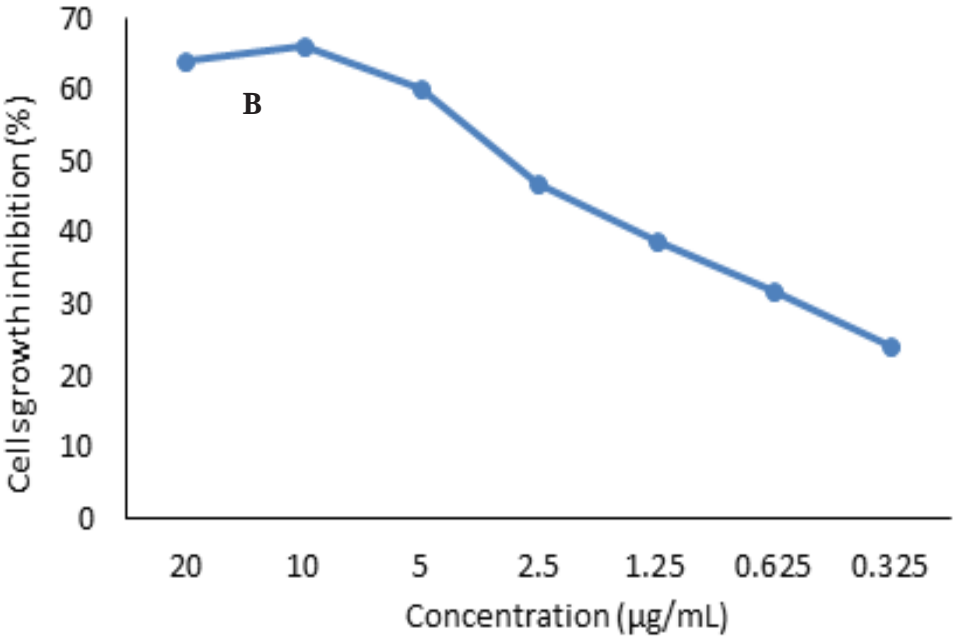


FIGURE 1. MCF7/HER2 cells growth inhibition after 24 h incubation with different concentrations of methanolic extract of hantap leaves (A) and doxorubicin (B) on inhibition of MCF7/HER2 growth after 24 h incubation.

HER2 mRNA expression examination

For the most active extract i.e. methanolic extract, its effect on HER2 mRNA cells expression was evaluated. The HER2 mRNA expression after 24 h incubation with the methanolic extract of hantap leaves (*S. oblonga* Mast) and doxorubicin is presented in FIGURE 2. In general, the methanolic extract inhibited the HER2 mRNA expression. The methanolic extract at concentration of $1/2IC_{50}$; $1IC_{50}$ and $2IC_{50}$ µM suppressed HER2 mRNA expression to be 0.6; 0.25 and 0.33 compared to control cells. Whereas doxorubicin at concentration of $1IC_{50}$ suppressed HER2 mRNA expression to 0.42.

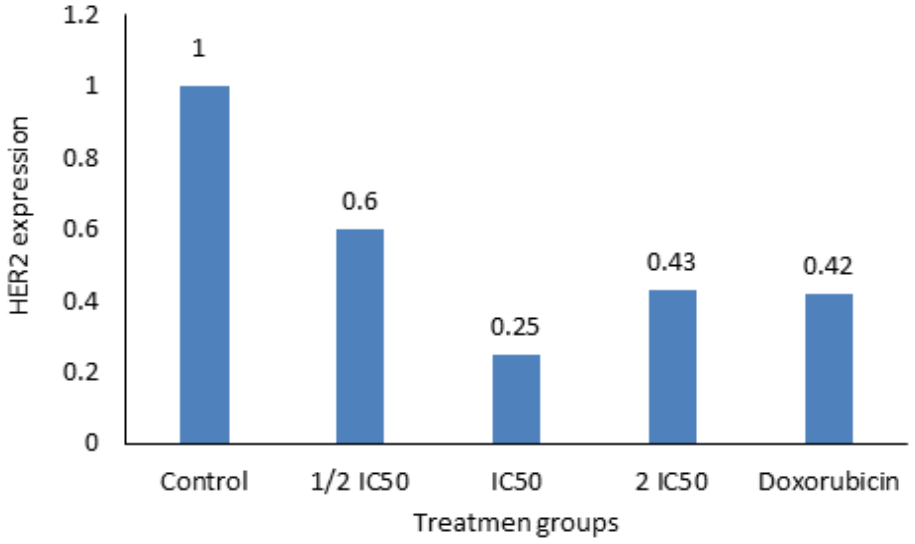


FIGURE 2. HER2 mRNA expression after 24 h incubation with the methanolic extract of hantap leaves and doxorubicin.

Apoptotic examination

The effect of the methanolic extract on cells apoptosis was also evaluated. Microscopic examination result after staining by using AO/PI is presented in FIGURE 3. Green-stained MCF7/HER2 cells represent viable cells (VL), whereas red staining represent apoptotic cells (AL). Methanolic extract at concentration of $1/2IC_{50}$; $1IC_{50}$ and $2IC_{50}$ µM induced 6.8; 26.3 and 25.3% apoptosis of the MFC7/HER2 cells, respectively. Whereas doxorubicin at concentration of $1IC_{50}$ induced 19.8% apoptosis of the cells (FIGURE 4).

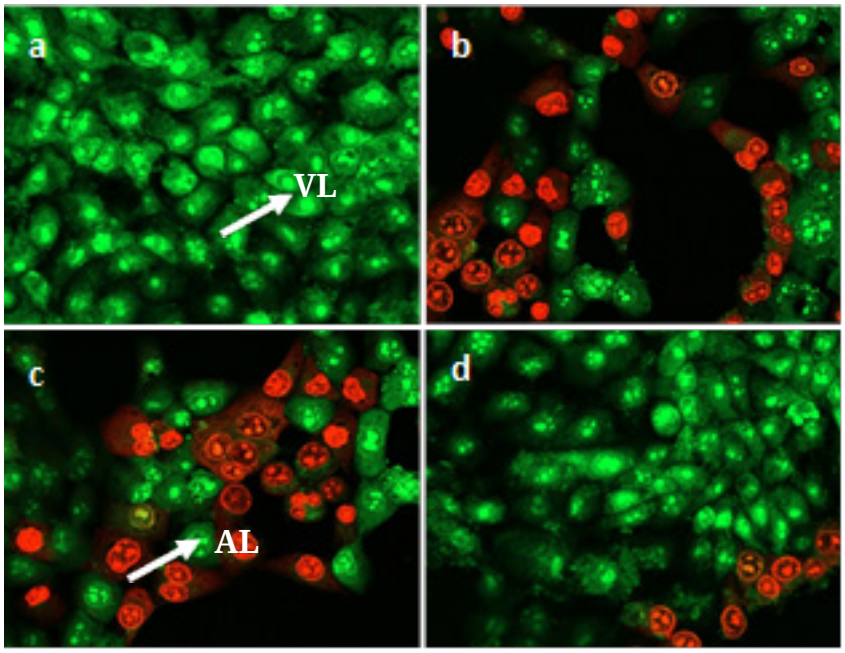


FIGURE 3. Apoptotic test results on the MCF7/HER2. breast cancer cell line after 24 hours incubation with control cells (a), treatment of S. Oblonga Mast methanol extract with 2IC50 (b), IC50 (c) and 1/2IC50 (d). viable cells (VL/Green), apoptotic cells (AL/Red)

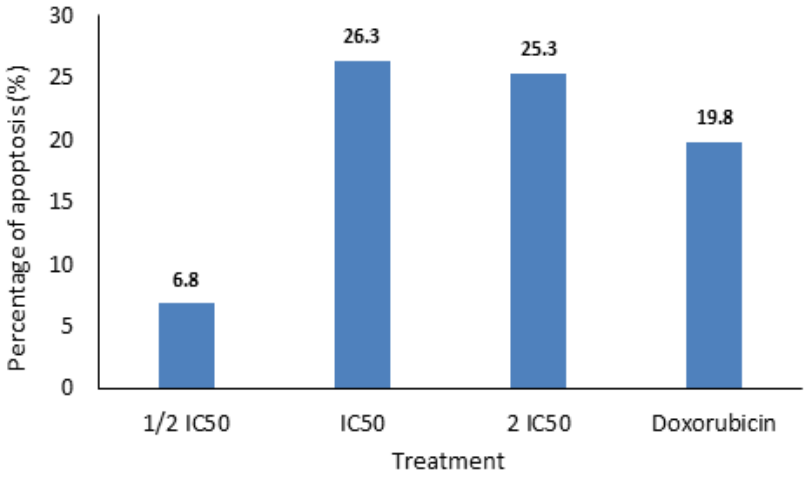


FIGURE 4. Percentage of MCF7/HER2 cells apoptotic after 24 h incubation with methanolic extract at different concentration.

DISCUSSION

Among three extracts of hantap (*S. oblonga* Mast) leaves tested, the methanolic extract exhibited the most cytotoxic against MCF7/HER2 cells line with an IC_{50} of 91.25 µg/mL. The National Cancer Institute (NCI) of America categorized the cytotoxicity of an extract into high cytotoxic activity if it has an IC_{50} value ≤ 20 µg/mL, moderate cytotoxic activity if it has an IC_{50} value ranged between 21 - 200 µg/mL, weak cytotoxic activity if it has an IC_{50} ranged between 201-500 µg/mL and no has cytotoxic activity if it has an IC_{50} > 500 µg/mL.[15] Based on this criteria, the methanolic extract of hantap (*S. oblonga* Mast) leaves could be categorized to have moderate cytotoxic activity.

Several species of the *Sterculia* genus have been reported to have biological activities such as antioxidant, antiinflamatory, and cytotoxic.[13,16,17] Sterculia genus have been also reported to biological active compounds such as tannins, alkaloids, flavonoids, saponin and phenol.[13] *Sterculia oblongata* Mast rich of phenolic compounds which has strong antioxidant activity. The methanolic extract of *S. oblongata* Mast was reported to have higher antioxidant activity than that of β-carotene/linoleic acid.[16] Antioxidants are compounds that inhibit oxidation, a chemical reaction that can produce free radicals and chain reaction that may damage the cells or organisms. Antioxidants have been reported to have various biological activities including cytotoxic.[18,19] The phenolic compounds of the methanolic extract of hantap (*S. oblongata* Mast) leaves may be responsible for its cytotoxic activity.

The cytotoxic activity of the methanolic extract of hantap (*S. oblongata* Mast) leaves was supported by its effect on the expression of HER2 mRNA of MCF7/HER2 breast cancer cell lines. In this study, the methanolic extract induced inhibited mRNA expression of the cell lines with the maximal inhibition was observed at the concentration of 91.25 µg/mL (IC_{50}). Human Epidermal Growth Factor Receptor 2 (HER2) is a 185 kDa protein with intracellular tyrosine kinase domain and an extracellular ligand binding domain. It plays important roles in cell growth, survival and differentiation in a complex manner. HER2 is highly expressed in a significant proportion of breast cancer, ovarian cancer, and gastric cancer.[20] HER2 has received great attention as targeted therapy during the past two decades.[21]

The cytotoxic activity of the methanolic extract of hantap (*S. oblongata* Mast) leaves was also supported by its effect on the apoptosis of MCF7/HER2 breast cancer cell lines. In this study, the methanolic extract induced the cell cancer apoptosis with the maximal induction were observed at the concentration of 91.25 µg/mL (IC_{50}). Apoptosis is a cellular mechanism to eliminate permanent damage of the cells without causing inflammation.[22-24] It is an important homeostatic mechanism that balances cell division and cell death and maintains the appropriate cell number in the body.[25] The discovery of new anticancer targeting induction of cancer cells apoptosis has been focusing in last decade.

CONCLUSION

In conclusion, the methanolic extract of hantap (*S. oblongata* Mast) leaves has moderate cytotoxic activity against MCF7/HER2 breast cancer cell lines. Furthermore, this methanolic extract may act through induction apoptosis and inhibition HER2 mRNA expression of the cancer cell lines. Further study, will be conducted to isolate active constituents as anticancer.

ACKNOWLEDGMENT

This study is a part of a thesis research in Program of Master Biomedical Sciences, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah

Mada, Yogyakarta. This study was financial supported by Program of *Hibah Dana Masyarakat* (DAMAS) 2021, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta.

REFERENCES

1. American Cancer Society. Cancer facts and figures. American Cancer Society, Atlanta; 2017. Available from: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2017.html
2. World Health Organization. Breast cancer. WHO; 2021. Available from: https://www.who.int/news-room/fact-sheets/detail/breast-cancer
3. International Agency for Reseach on Cancer. Indonesia. World Health Organization, 2020. Available from: https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf
4. Kementiran Kesehatan RI. Info jenis kanker ini rentan menyerang manusia 2020. Kementerian Kesehatan Republik Indonesia. Jakarta, 2020. Available from: https://www.kemkes.go.id.
5. Yuan C, Zeng Z, Zhang L, Ji X, Liu W, Huang B, *et al.* Breast cancer development and progression: risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Gene Dis 2018; 5:77-106. https://doi.org/10.1016/j.gendis.2018.05.001
6. Wong DJL, Hurvitz SA. Recent advances in the development of anti-HER2 antibodies and antibody-drug conjugates. Ann Transl Med 2014; 2(12):122. https://doi.org/10.3978/j.issn.2305-5839.2014.08.13
7. Mendes D, Alves C, Afonso N, Cardoso F, Passos-Coelho JL, Costa L, *et al.* The benefit of HER2 targeted therapies on overall survival of patients with metastatic HER2-positive breast cancer: a systematic review. Breast Cancer Res 2014; 17(140):1-14. https://doi.org/10.1186/s13058-015-0648-2
8. Yokoyama Y, Matsumoto A, Hieda M, Shinchi Y, Ogihara E, Hamada M, *et al.* Loss of histone H4K20 trimethylation predicts poor prognosis in breast cancer and is associated with invasive activity. Breast Cancer Res 2014; 16(R66):1-12.
9. Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M. Genetic susceptibility to breast cancer. Mol Oncol 2010; 4(3):174-91. https://doi.org/10.1016/j.molonc.2010.04.011
10. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144(5):646-74. https://doi.org/10.1016/j.cell.2011.02.013
11. Carneiro BA, El-Deiry WS. Targeting apoptosis in cancer therapy. Nat Rev Clin Oncol 2020; 17:395–417. https://doi.org/10.1038/s41571-020-0341-y
12. Sun SY, Hail NJ, Lotan R. Apoptosis as a novel target for cancer chemoprevention. JNCI 2004; 96(5):662-72. https://doi.org/10.1093/jnci/djh123
13. Murningsih T, Praptiwi, Liana, Fathoni A. TLC profiling and antioxidant activity of phenolic compound from *Sterculia oblongata* bark extract. Nusantara Bioscience 2019; 11(1):44-8. https://doi.org/10.13057/nusbiosci/n110108
14. Ezeonu CS, Ejikeme CM. Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian Softwoods. New J Sci 2016; 5601327. https://doi.org/10.1155/2016/5601327
15. Sajjadi SE, Ghanadian M, Haghighi M, Mouhebat L. Cytotoxic effect of *Cousiniaver bascifolia* bunge against OVCAR-3 and HT-29 cancer cells. J Her Med Pharmacol 2015; 4(1):15-9.
16. Murtiningsih T, Pratiwi, Liana, Fathoni A. TLC profiling and

antioxidant activity of phenolic compound from *Sterculia oblongata* bark extract. Nusa Biosci 2019; 11(1):44-8. https://doi.org/10.13057/nusbiosci/n110108

17. El Sherei MM, Ragheb AY, Kassem M, Marzouk M, Mosarrafa S, Saleh N, Phytochemistry, biological activities and economical uses of the genus Sterculia and the related genera: A review. Asian Pacific J Trop Dis 2016; 6(6):492-501. https://doi.org/10.1016/S2222-1808(16)61075-7
18. Neha K, Haider MR, Pathak A, Yar MS. Medicinal prospects of antioxidants: A review. Eur J Med Chem 2019; 178:687-704. https://doi.org/10.1016/j.ejmech.2019.06.010
19. Athreya K, Xavier MF. Antioxidants in the treatment of cancer. Nutr Cancer 2017; 69(8):1099-1104. https://doi.org/10.1080/01635581.2017.1362445
20. Tai W, Mahato R, Cheng K. The role of HER2 in cancer therapy and targeted drug delivery. J Control Release 2010; 146(3):264-75. https://doi.org/10.1016/j.jconrel.2010.04.009
21. Lewis Phillips GD, Li G, Dugger DL, Crocker LM, Parsons KL, Mai E, *et al.* Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. Cancer Res 2008; 68(22):9280-90. https://doi.org/10.1158/0008-5472.CAN-08-1776
22. Galati G, Teng S, Moridani MY, Chan TS, O'Brien PJ. Cancer chemoprevention and apoptosis mechanisms induced by dietary polyphenolics. Drug Metabol Drug Interact 2000; 17(1-4):311-49. https://doi.org/10.1515/dmdi.2000.17.1-4.311
23. Elmore S. Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol 2007; 35(4):495-516. https://doi.org/10.1080/01926230701320337
24. Paus R, Rosenbach T, Haas N, Czarnetski BM. Patterns of cell death: the significance of apoptosis in dermatology. Exp Dermatol 1993; 2(1):3-11. https://doi.org/10.1111/j.1600-0625.1993.tb00192.x
25. Martin SJ, Green DR. Apoptosis and cancer: the failure of controls on cell death and cell survival. Crit Rev Oncol Hematol 1995; 18(2):137–53. https://doi.org/10.1016/1040-8428(94)00124-c