Turnitin Originality Report

Processed on: 28-Aug-2024 9:43 PM WI
ID: 2439776049
Word Count: 2452
Submitted: 1

B.2.pdf By syakira mumtaz

Similarity Index	Similarity by Source	
16%	Internet Sources:	11%
	Publications:	13%
	Student Papers:	2%

1% match (Internet from 18-May-2024)
https://d.docksci.com/the-antibacterial-activity-of-extracts-of-nine-plant-species-with-good-activity-_59f626c7d64ab20e98ba49c4.html

1% match (publications)
Jun-Ping Xu. "Cancer Inhibitors from Chinese Natural Medicines", CRC Press, 2016

1% match (Internet from 15-Nov-2021)
https://academic.oup.com/jn/article/136/11/2715/4664165

1% match (Internet from 11-Jan-2024)
https://www2.mdpi.com/2072-6651/8/5/151

1% match (Thea S. B. Møller, Joanna Hay, Malcolm J. Saxton, Karen Bunting et al. "Human β-defensin-2 production from S. cerevisiae using the repressible MET17 promoter", Microbial Cell Factories, 2017)
Thea S. B. Møller, Joanna Hay, Malcolm J. Saxton, Karen Bunting et al. "Human β-defensin-2 production from S. cerevisiae using the repressible MET17 promoter", Microbial Cell Factories, 2017

1% match (student papers from 06-Jun-2024)
Submitted to The Scientific & Technological Research Council of Turkey (TUBITAK) on 2024-06-06

1% match (student papers from 12-Aug-2023)
Submitted to University of Hull on 2023-08-12

1% match (Internet from 02-Sep-2020)
https://www.hindawi.com/journals/ecam/2011/541209/

1% match (Kabi Raj Chaudhary, Puja Banik, Karanvir Singh. "Recent trends in the delivery of plant-derived phytochemicals against various cancers using Nanotechnological approach: A comprehensive review", Journal of Drug Delivery Science and Technology, 2023)
Kabi Raj Chaudhary, Puja Banik, Karanvir Singh. "Recent trends in the delivery of plant-derived phytochemicals against various cancers using Nanotechnological approach: A comprehensive review", Journal of Drug Delivery Science and Technology, 2023

1% match (Internet from 18-Oct-2022)

https://oak.kribb.re.kr/bitstream/201005/18260/1/16294.pdf

1% match (Lili Nurmawati, Mohammad Sulchan, Siti Fatimah-Muis, Hery Djagat Purnomo, Kis Djamiatun, Martha Ardiaria, Vega Karlowee. "The effect of single clove Black garlic on the hemostasis status and lipid profile in male Sprague Dawley rats with non-alcoholic fatty liver disease", Potravinarstvo Slovak Journal of Food Sciences, 2021)
Lili Nurmawati, Mohammad Sulchan, Siti Fatimah-Muis, Hery Djagat Purnomo, Kis Djamiatun, Martha Ardiaria, Vega Karlowee. "The effect of single clove Black garlic on the hemostasis status and lipid profile in male Sprague Dawley rats with non-alcoholic fatty liver disease", Potravinarstvo Slovak Journal of Food Sciences, 2021

1% match (Internet from 31-May-2023)
https://repository.unar.ac.id/jspui/handle/123456789/1408?mode=full

1% match (Internet from 30-Apr-2024)
https://www.researchsquare.com/article/rs-180964/v1

< 1% match (Hebatollah E. Eitah, Hanan Naeim Attia, Ahmed A.F. Soliman, Amina A. Gamal el Din et al. "Vitamin D ameliorates diethylnitrosamine-induced liver preneoplasia: A pivotal role of CYP3A4/CYP2E1 via DPP-4 enzyme inhibition", Toxicology and Applied Pharmacology, 2022)
Hebatollah E. Eitah, Hanan Naeim Attia, Ahmed A.F. Soliman, Amina A. Gamal el Din et al. "Vitamin D ameliorates diethylnitrosamine-induced liver preneoplasia: A pivotal role of CYP3A4/CYP2E1 via DPP-4 enzyme inhibition", Toxicology and Applied Pharmacology, 2022

< 1% match (Internet from 26-May-2023)
https://docslib.org/doc/497970/download

< 1% match (Internet from 11-Jul-2024)
https://www.science.gov/topicpages/e/egfp+oligomer+mobility

< 1% match (Internet from 25-May-2019)
https://www.tandfonline.com/doi/full/10.1080/01635581.2014.864418

< 1% match (Internet from 29-Jul-2024)
https://cuir.car.chula.ac.th/dspace/bitstream/123456789/84443/1/6470072823.pdf

< 1% match (Internet from 02-Jan-2022)
http://livros01.livrosgratis.com.br/cp149654.pdf

< 1% match (Internet from 04-Dec-2023)
https://orbigen.com/elisa-kits-1/?page=11&price_max=1633&price_min=1131

< 1% match (Internet from 03-Dec-2023)
https://pdffox.com/world-journal-of-hepatology-pdf-free.html

< 1% match (Internet from 01-Nov-2022)
https://www.cusabio.com/ELISA-Kit/Rat-advanced-glycation-end-productsAGEs-ELISA-Kit-63625.html

< 1% match (Alifia Mukti Fajrani, Mohamad Sulchan, Siti Fatimah Muis, Hery Djagat Purnomo, Kis Djamiatun, Vega Karlowee, Martha Ardiaria. "Effect of black garlic on visceral fat, oxidative stress and insulin resistance in nonalcoholic fatty liver disease rats", Nutrition & Food Science, 2021)

Alifia Mukti Fajrani, Mohamad Sulchan, Siti Fatimah Muis, Hery Djagat Purnomo, Kis Djamiatun, Vega Karlowee, Martha Ardiaria. "Effect of black garlic on visceral fat, oxidative stress and insulin resistance in nonalcoholic fatty liver disease rats", Nutrition & Food Science, 2021

< 1% match (Yan Li, Robert C. G. Martin. "Herbal Medicine and Hepatocellular Carcinoma: Applications and Challenges", Evidence-Based Complementary and Alternative Medicine, 2011)
Yan Li, Robert C. G. Martin. "Herbal Medicine and Hepatocellular Carcinoma: Applications and Challenges", Evidence-Based Complementary and Alternative Medicine, 2011

< 1% match ("Polyphenols-based Nanotherapeutics for Cancer Management", Springer Science and Business Media LLC, 2021)
"Polyphenols-based Nanotherapeutics for Cancer Management", Springer Science and Business Media LLC, 2021

< 1% match (Zahra Farzaneh, Maryam Farzaneh. "Herbal Medicine-derived Compounds for the Prevention and Treatment of Hepatocellular Carcinoma", Current Cancer Therapy Reviews, 2022)
Zahra Farzaneh, Maryam Farzaneh. "Herbal Medicine-derived Compounds for the Prevention and Treatment of Hepatocellular Carcinoma", Current Cancer Therapy Reviews, 2022

J.Biomed.Transl.Res ISSN: 2503-2178 Available online at JBTR website: https://jbtr.fk.undip.ac.id Copyright©2023 by Faculty of Medicine Universitas Diponegoro, Indonesian Society of Human Genetics and Indonesian Society of Internal Medicine Original Research Article The Activity of Liposome-Parijoto Formula Through p53 Expression in HepG2 Cell Line Widyandani Sasikirana1*, Ragil Setia Dianingati1, Khairul Anam1, Eva Annisaa, Evieta Rohana1 1Departement of Pharmacy, Faculty of Medicine, Universitas Diponegoro, Indonesia Article Info Abstract History Parijoto, one of Melastomaceae family, has been known to have cytotoxic activity in Received: 27 Jan 2023 HepG2, a hepatocellular cancer cell line, but with low activity. However, the ethyl Accepted: 05 Jun 2023 acetate fraction of Parijoto gave the highest antioxidant and cytotoxic activity in 4T1. Available: 31 Aug 2023 Then, purification and liposome formulation need to be carried out to increase the cytotoxic activity of Parijoto extract. Objective: This research aimed to study the cytotoxic activity and p53 gene expression of LEA (Liposom-Ethyl Acetate of Parijoto Fraction) in HepG2. Method: Extraction has been done by maceration, followed by partition using n- hexane, ethyl acetate, and methanol. LEA formulation was carried out by thin-layer hydration with modification and the formula was sized using a bath sonicator. Cytotoxic activity test of LEA and extract was performed in five serial concentrations (3,9 µg/mL–250 µg/mL), while the positive control doxorubicin performed in 3,9 µg/mL – 250 µg/mL by MTT assay. P53 gene expression was analyzed by using PCR- electrophoresis. Result: Results showed that LEA increased the cytotoxic activity (IC50 = 28.40 µg/ml). Furthermore, based on the electrophoresis study, LEA induced the p53 expression while the extract only did not. Conclusion: Liposome formula from ethyl acetate fraction of Parijoto extract (LEA) was able to increase cytotoxic activity and p53 gene expression was possible through the apoptotic mechanism. This shows that this formula is a promising strategy to improve the bioavailability of herbal medicines as cytotoxic agents. Keywords: Liposome; Parijoto; p53; HepG2 Permalink/ DOI: https://doi.org/10.14710/jbtr.v9i2.17290 INTRODUCTION Hepatocellular carcinoma (HCC) is primary and mortality liver cancer that causes the

second death in worldwide1. Pathogenesis of HCC is closely associated with chronic hepatitis that is caused by several factors such as infection with a virus (Hepatitis B or C), chemical exposure (Aflatoxin), alcoholic lifestyle, and other conditions (diabetic disease, obesity). Those factors will increase the risk of liver cirrhosis which will develop to be liver cancer1. Nowadays, treatment of HCC has been done by curative methods (orthotopic liver transplantation, surgical resection, and local destruction) and palliative methods (trans arterial chemo-embolization, systemic chemotherapy, interferon, and hormonotherapy). Those treatments still have limitations such as the impact on the long-term survival of patients who need the adjuvant after/before curative treatment2. Therefore, there is a need to look for new active ingredients and strategies to improve cancer treatment with low side effects. A previous study demonstrated that Parijoto extract (Medinilla speciosa) is known to have low cytotoxic activity on the HepG2 cell line, which means it has low bioavailability3. Moreover, the ethyl acetate fraction from Parijoto extract showed the highest antioxidant activity that correlated with cytotoxic effect in 4T1 cell line4. * Corresponding author: E-mail: widyandani.sasikirana@live.undip.ac.id (Widyandani Sasikirana) Hence, the extract bioavailability will be increased by purification and lipid-based encapsulation of ethyl acetate fraction to form liposome-ethyl acetate (LEA). The liposome is a vesicle enclosed by phospholipids which is an analog to the cell membrane. This vesicle can improve the bioavailability and solubility of water- insoluble drugs which is poorly-permeable. However, liposome is more promising to deliver hydrophobic drugs than peptide and protein drugs for oral drugs, especially for phenolic compounds5. Melastomaceae family has been known has several phenolic compounds, such as ellagitannin (Medinillin A, Medinillin B6, ellagic acid7) and flavonoids which influence their activity as antioxidant and cytotoxic. Therefore, the active compounds will be able to be encapsulated into liposomes that are expected to increase their bioavailability. A molecular study of HCC pathogenesis revealed that there is a genetic change in a signaling pathway which is mediated by p53, Ras/ERK, PI3K/AKT, and wnt/B-catenin. Another study on p53 showed that its role was in the regulation of the cell cycle, apoptosis, and genomic stability. However, p53 was mutated and plays a key role in the signaling pathway of PI3K/AKT, TGF-B and B-catenin to metastasis in HCC cells8. Hence, it is necessary to investigate the cytotoxic activity of liposome-Parijoto and its molecular mechanism through the p53 gene expression MATERIALS AND METHOD Extraction and purification Extraction of Parijoto (Medinilla speciosa) fruit was carried out by three days of maceration using ethanol 70% and then evaporated using rotary evaporation. The macerate was separated using n-hexane, ethyl acetate, and methanol. Each fraction was evaporated and the ethyl acetate fraction was encapsulated to form the LEA. Figure 1. Optical microscope image of LEA formula (400x resolution) LEA formulation and characterization LEA was made using the thin-layer hydration method. Lipids were prepared by dissolving 1:1 b/b soya lecithin: cholesterol in chloroform, then evaporated using a dehydrator until the thin layer was performed. Hydration was carried out by mixing the ethyl acetate fraction solution in 1% methanol followed by water addition using a magnetic stirrer for 40 minutes. Then, sizing was done by bath sonicator for 10 minutes. The characterization of LEA was carried out by the microscopic study. Cytotoxic activity Cell lines (1x105 cells/well) were cultured into a 48- well plate and incubated overnight. The serial concentration of LEA samples was in the range of 3,9 µg/mL to 250 µg/mL and the positive control doxorubicin was 3,9 µg/mL – 250 µg/ml were put onto cells and incubated overnight. A 100 ul MTT (3-(4,5- dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide) on PBS (Phosphate Buffer Saline) 5% was put onto cell solution followed by incubation for 4 hours until the formazon formed. The reaction has been

stopped by adding DMSO (Dimethyl sulfoxide) 10-20% in a protected light for 5 minutes and incubated. The absorbance was read using a microplate reader in 595 nm. The % of inhibition was calculated according to this formula: ((sample abs-medium abs))/(cell control abs - medium abs)) x 100% The IC50 was calculated based on linear regression of log concentration vs %inhibition. P53 gene expression study Cell lines were cultured in 48-cell wells and incubated overnight. The IC50 concentration of LEA was put into the well and incubated overnight. RNA isolation was carried out according to the Invitrogen™ TRIzol™ Plus RNA Purification Kit (Roche, Swiss) protocol for the cells. cDNA synthesis was done using Trancriptor High Fidelity cDNA Synthesis Kit (Roche, Swiss). PCR reaction was used according to the FastStart High Fidelity PCR System Kit (Roche, Swiss) condition (Pre-denaturation 95°C, 1 minute; Denaturation 95°C, 15 seconds; Annealing 58°C, 15 second; Elongation 72°C, 30 second; 35 cycles). The primer used in this research can be seen in Supplementary File 1. The visualization was done by using electrophoresis in 2% agarose RESULTS LEA characterization LEA formula showed Giant Unilamellar Vesicles (GUVs) (Figure 1) with one compartment/lamellarity after sizing with a bath sonicator. Furthermore, the average particle sizes ranged between 3,73 to 16,64 µm. Cytotoxic activity Results obtained the LEA formula has a potent cytotoxic activity when compared with the extract. Moreover, it gave a lower concentration to inhibit cell proliferation in HepG2 from IC50 value (Table 1). However, this finding suggested that the cytotoxic activity in the HepG2 cell line was greatly improved by lipid encapsulated in liposome formula p53 gene expression p53 gene expression could be seen in Figure 2. Our results showed that the LEA formula induced the p53 gene expression while the extract did not. This finding demonstrated that the LEA formula influenced cell proliferation by inducing p53 gene expression which influenced the apoptotic program. Table 1. The IC50 value of the samples in HepG2 Doxorubicin LEA Extract Doxorubicin IC50 0,23 28,4 >250* 11,12 value (µg/mL) * The extract did not show 50% inhibition of HepG2 until 250 µg/mL3. A B Figure 2. The expression of p53 in HepG2. A.GADPH (150 bp). B.p53 (300 bp). left to right: untreated cell, positive control (Doxorubicin), LEA; extract. Treated cell was induction accroding to IC50 value of LEA. There was no expression of GADH and p53 when the cell treatment to 28,4 ug/ml extract. DISCUSSION Mutations in the p53 gene cause cell malignancy and unrestricted DNA replication, resulting in uncontrolled cell proliferation and cancerous tumors. p53 activity is impaired by multiple mechanisms in HCC, hence contributing to HCC genesis. The HCC-inducing extrinsic factors which etiologically associated with p53 are AFB1 (Aflatoxin B1), vinyl Chloride, NAFLD (Non-Alcoholic Fatty Liver Disease), Iron, HBV (Hepatitis B Virus), and HCV (Hepatitis C Virus) infection9. The results of our study showed that the IC50 of the LEA formula increased sixty times higher than the extract. It indicated that the fractionation and encapsulation of the LEA formula could increase the bioavailability of the extracts. Similar results were shown in the research of Mabrok, 2002 which states that Apigenin (flavonoid from Apium graveolens) that is encapsulated into chitosan and albumin-folic acid can improve its hydrophobicity, stability, and bioavailability to target the cancer cells. The treated HePG-2 cells with Ap-CH- BSA-FANPs demonstrated the induction of apoptosis by increasing p53 gene expression, arresting the cell cycle, increasing caspase-9 levels, and decreasing both the MMP9 gene and Bcl-2 protein expression levels10. Parijoto, the Melastomaceae family, contains flavonoids11 and some ellagitannin7. Furthermore, these flavonoids and ellagitannins can exert cytotoxic activity in various cancer cell lines. Granado-Serrano et al., in 2006 reported quercetin-induced apoptosis in the HepG2 cell line and evaluated the modulation and expression of Bcl-x and Bax. Bcl-xL has been identified as a caspase substrate and the product of Bcl-xL

cleavage, Bcl-xS, has a pro-apoptotic function12. Moreover, hydroxygenkwanin along with kaempferol showed both cytotoxic and antioxidative potential against HepG2 cell lines13 Yohida et, al, reported that Medinilla magnifica, which has the same genus as Medinilla speciosa, was composed by medinillin A and B, the ellagitannin compounds. These compounds give cytotoxic activity through a p53-dependent pathway. Punicalagin, one of ellagitannin from Punica granatum, has been shown to impact the proapoptotic protein such as Bax, caspace 3 and 9, and the tumor suppressor p53 in human cervical cancer cell lines14. Furthermore, the other ellagitannin such as 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (PGG), showed its antiproliferative activity in humanSK- HEP-1 hepatocellular carcinoma cells which caused by the suppression of NF-kB activation and G0/G1-phase arrest via an IκB-mediated mechanism. Moreover, PGG was reported to induce atypical senescence-like S-phase arrest in HepG2 and Huh-7 human hepatocarcinoma cells. Furthermore, it also induced the senescence- associated β-galactosidase activity, inhibited proliferative capacity, and influenced the autophagy process by activating the MAPK8/9/10 on two model studies (in vitro and in vivo) of human HepG2 liver cancer15. CONCLUSION The fractionation and lipid-based encapsulated formula influenced the increasing cytotoxic activity of Parijoto extract. The induced p53 expression was a key role in the apoptotic program. This formula was a promising strategy to improve the bioavailability of herbal extracts as cytotoxic agents. ACKNOWLEDGEMENT We are grateful to the Faculty of Medicine, Universitas Diponegoro for funding our research (RDP Grant No .1198/UN7.5.4.2.1/PP/PM/2020) and Biomedic Laboratory, Universitas Sebelas Maret, Solo for the technical support. REFERENCES 1. M.L Martínez-Chantar, M.A. Avila, S.C. Lu. Hepatocellular Carcinoma: Updates in Pathogenesis, Detection and Treatment. Cancers. 2020;12:2729. https://doi.org/10.3390/cancers12102729. 2. Li Y, Martin RC 2nd. Herbal medicine and hepatocellular carcinoma: applications and challenges. Evid Based Complement Alternat Med. 2011; 2011:541209. https://doi.org/10.1093/ecam/neq044. 3. W Sasikirana, E Annisaa', N ekawati, IR Eka Dini, E Tumbilaka. Selectivity of Ethanol Extract of Parijoto (Medinilla speciosa) Fruit in HepG2, Widr, 4T1, and Vero cell lines. Jurnal Kedokteran Diponegoro (Diponegoro Medical Journal). 2021; 10:337-341. https://doi.org/10.14710/dmj.v10i5.32010 4. E Annisaa, W Sasikirana, N Ekawati, IR. Eka Dini. Correlation Between Antioxidant and Cytotoxic Activity of Parijoto (Medinilla speciosa Blume) Fractions in 4T1 Cell Line. Indones. J. Cancer Chemoprevent. 2019; 12: 21-27. https://dx.doi.org/10.14499/indonesianjcanchemopr ev12iss1pp21-27 5. Mi-Kyung Lee. Review: Liposome for Enhanced Bioavaibility of Water-Insoluble Drugs: In Vivo Evidence and Recent Approaches. Pharmaceutics. 2020; 12: 264. https://doi.org/10.3390/pharmaceutics12030264 6. Serna DM, Martínez JH. Phenolics and Polyphenolics from Melastomataceae Species. Molecules. 2015; 20:17818-47. https://doi.org/10.3390/molecules201017818 7. Takashi Yoshida , Yoshiaki Amakura, Morio Yoshimura. Review: Structural Features and Biological Properties of Ellagitannins in Some Plant Families of the Order Myrtales. Int. J. Mol. Sci. 2010; 11:79-106. https://doi.org/10.3390/ijms11010079 8. Wang Z, Jiang Y, Guan D, Li J, Yin H, et al. Critical Roles of p53 in Epithelial-Mesenchymal Transition and Metastasis of Hepatocellular Carcinoma Cells. PLOS ONE. 2013;8:e72846. https://doi.org/10.1371/journal.pone.0072846 9. Tim Link, Tomoo Iwakuma. Roles of p53 in extrinsic factor-induced liver carcinogenesis. Hepatoma Res. 2017; 3: 95–104. https://doi.org/10.20517/2394-5079.2017.07 10. Mabrouk Zayed MM, Sahyon HA, Hanafy NAN, El-Kemary MA. The Effect of Encapsulated Apigenin Nanoparticles on HePG-2 Cells through Regulation of P53. Pharmaceutics. 2022 ;14:1160.

https://doi.org/10.3390/pharmaceutics14061160. 11. T.Milanda, K.Lestari, Nimas TIT. Antibacterial Activtiy of Parijoto (Medinilla speciosa Blume) Fruit Against Serratia marcescens and Staphylococcus aureus. Indoensian Journal of Pharmaceutical Scinece and Technology. 2021. 8:76-85. https://doi.org/10.24198/ijpst.v8i2.32166 12. Granado-Serrano AB, Martín MA, Bravo L, Goya L, Ramos S. Quercetin induces apoptosis via caspase activation,regulation of Bcl-2, and inhibition of PI-3- kinase/Akt and ERK pathways in a human hepatoma cell line (HepG2). J. Nutr. 2006;136: 2715–2721.https://doi.org/10.1093/jn/136.11.2715 13. Li N, Liu JH, Zhang J, Yu BY. Comparative evaluation of cytotoxicity and antioxidative activity of 20 flavonoids. J. Agric.Food Chem. 2008;56:3876–3883. https://doi.org/10.1021/jf073520n 14. Izabela Berdowska, Małgorzata Matusiewicz, Izabela Fecka, Review: Punicalagin in Cancer Prevention—Via Signaling Pathways Targeting. Nutrients. 2021;13:2733. https://doi.org/10.3390/nu13082733 15. Tariq I, Cinzia C, Anna R D, Carmela F, Eleonora T, Elena C, et al. Review: Ellagitannins in Cancer Chemoprevention and Therapy. Toxins. 2016; 8:151. https://doi.org/10.3390/toxins8050151

Journal of Biomedicine and Translational Research, 9 (2) 2023, 68-71 70
Journal of Biomedicine and Translational Research, 9 (2) 2023, 68-71 71