Cytotoxic Test of *Cassia alata* L. Leaves Ethanol Extracts, Fractions, and Main Compounds against MCF-7 Cells

Marya Salfia Khoerunisah[1], Marissa Angelina[2]*, Kasiyati[1]

[1]Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang, Central Java, 50275, Indonesia

[2]Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Inovation Agency Republic of Indonesia, South Tangerang, Banten, 15314, Indonesia

Abstract

Cancer is the primary cause of death worldwide. Conventional cancer treatment is known to be less than optimal because of its chemoresistance and toxicity to normal cells. The search for cancer drugs from natural ingredients is still being carried out as an effort to overcome these problems. *Cassia alata* L. leaf extract is known to have antibacterial and antitumor activities. The main compounds of *C. alata* L. leaves (emodin, aloe-emodin, and kaempferol) have been reported to have antiproliferative activity. This study aimed to examine the cytotoxic activity of the *C. alata* L. leaves ethanol extracts, fractions, and main compounds against breast cancer cells (MCF-7). Cytotoxic activity was carried out by the MTT method. IC_{50} was determined by linear regression analysis describing the relationship between concentration and % cell viability. The results showed that aloe-emodin, emodin, and kaempferol had better cytotoxic activity than the extract and fractions of the *C. alata* L. leaves with IC_{50} values respectively 12.7 ppm, 18.1 ppm, and 131.3 ppm.

Keywords: *Breast cancer, cytotoxic assay, Cassia alata L., ketepeng cina, MCF-7.*

INTRODUCTION

Cancer is a non-communicable disease (NCD) that is still a global health burden. Cancer disease is characterized by the presence of cancer cells, namely abnormal cells of the body that grow uncontrollably, which are invasive so that they can damage normal cells. The World Health Organization (WHO) reveals that cancer is the primary cause of death worldwide. The number of cancer cases and death rates until 2018 amounted to 18.1 and 9.6 million, respectively. The mortality rate will increase to >13.1 million in 2030. The worldwide prevalence of cancer patients is estimated at 20%, while mortality prevalence is about 12.5% for males and 9.1% for females. The prevalence of cancer in Indonesia reaches 1.49%. Breast cancer is ranked 2 of the highest cancer cases in the world (Pangribowo, 2019).

Conventional cancer treatment is known to be less than optimal due to chemoresistance and toxicity to normal cells (Zhang, *et al*., 2021).

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*Corresponding author: marissarfat@gmail.com

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Doxorubicin is a typical cancer drug (Tang, *et al*., 2021; Kullenberg, *et al*., 2021) and is recommended for breast cancer therapy (Ashariati, 2019). Doxorubicin is known to cause hair loss, heart palpitations, and a decrease in the number of leukocytes (Mulia, *et al*., 2016). The search for cancer drugs from natural ingredients continues to be carried out to overcome these problems. Natural ingredients are a valuable resource in drug discovery efforts because they support researchers in isolating new active agents for the manufacture of new efficacious drugs (Zhang, *et al*., 2021).

C. alata L. is a herbaceous, intertropical plant from the Fabaceae family (Angelina, *et al*., 2021). *C. alata* L. is native to Argentina and is known by several names worldwide like *Senna alata*, candle bush/tree, candlestick, Carion Crow Bush, etc. *C. alata* L. leaves have been used as traditional medicine in several countries in the world, namely as a remedy for several digestive problems (constipation, stomach pain, liver disease), dermatology (general skin diseases, dermatitis, skin rashes, herpes zoster, eczema, mycosis), anti-infectious (Malaria, flu), antidiabetic, and anti-inflammatory (Hennebelle, *et al*., 2009; Angelina, *et al*., 2021). In Indonesia, *C. alata* L. leaves are used traditionally as a remedy for itchy skin caused by fungus by grinding or rubbing it directly on the infected skin (Fatmawati, *et al*., 2020).

C. alata L. leaf extract has been reported to have antibacterial (Angelina, *et al*., 2021), antifungal (Timothy, *et al*., 2012; Triana, *et al*., 2016), antiviral (Angelina, *et al*., 2017, 2020), antitumor (Olarte, *et al*., 2013), antioxidant (Sagnia, *et al*., 2014), anti-inflammatory (Lewis & Levy, 2011), antidiabetic (Varghese, *et al*., 2013), antihepatotoxic, and hepatoprotective effects (Ali, *et al*., 2017). The chemical compounds of *C. alata* leaf extract are known to be phenolic groups (chrysophanol, kaempferol, aloe-emodin, emodin, quercetin, chrysoeriol, adenine, and glycosides), anthraquinones (alatinone and alatonal), fatty acids (oleic, palmitic, and linoleic acids), steroids, and terpenoids (sitosterol, stigmasterol, and campesterol) (Oladeji, *et al*., 2020; Angelina, *et al*., 2020). In particular, aloe-emodin has been reported to have antiproliferative and anticarcinogenic activity. Aloe-emodin can inhibit cell proliferation, cell migration, cell induction, cell death induction, cell cycle arrest, and modulation of immune signaling (Huang, *et al*., 2013; Sanders, *et al*., 2017). Emodin has been reported to have antineoplastic, anti-inflammatory, anti-angiogenesis, and antiproliferative activities (Huang, *et al*., 2013; Akkol, *et al*., 2021). Kaempferol has been reported to have anti-inflammatory, antimicrobial, antioxidant, antitumor, cardioprotective, neuroprotective, antidiabetic (Imran, *et al*., 2019), antineoplastic, and antiproliferative activities (Kim & Choi, 2013).

Phytochemical studies have been carried out on *C. alata* L. leaves extracts taken from several regions in Indonesia, namely the Bogor Botanical Gardens, South Tangerang, and South Kalimantan. It was found that the ethanol extract of *C. alata* L. leaves taken from South Kalimantan had the highest total flavonoid content (Angelina, *et al*., 2021). This study aimed to examine the cytotoxic activity of the *C. alata* L. leaves ethanol extract, fractions, and main compounds collected from South Kalimantan against breast cancer cells (MCF-7).

MATERIALS AND METHODS

Methods

The research was conducted at the Biochemistry Laboratory, National Research and Innovation Agency. The materials used were *C. alata* L. ethanol extract and fractions (hexane, ethyl acetate, water), kaempferol, aloe-emodin, emodin, doxorubicin (TCI, Japan), Dulbecco's Modified Eagles Medium (DMEM) (Gibco, USA), $NaHCO_3$ (Merck, Darmstadt, Germany), fetal bovine serum (FBS) (Sigma, USA), penstrep (penicillin-streptomycin) (Sigma, USA), aquadest, 3-(4.5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Gibco, USA), trypsin EDTA

0.25% (Gibco, USA), dimethyl sufoxice (DMSO) (Thermo scientific, France), phospat buffer saline (PBS) (Gibco, UK), MCF-7 cells were given by Mrs. Sri Ningsih (BPPT, Indonesia).

Cell Culture

The MCF-7 was cultured in complete medium growth containing high glucose DMEM, FBS 10%, and penicillin-streptomycin 1%. The cell was incubated at 37°C and 5% CO_2 content.

Sample Preparation

Dimethyl sulfoxide was used as a sample solvent. Stock solution of the ethanol extract and fractions, main compounds, and doxorubicin were made at concentration of 10000, 3000, and 5500 ppm, respectively. Each sample was diluted using the medium culture into several concentration variations, namely 400, 200, 100, and 50 ppm for extract and fractions; 100, 50, 25, 12.5, and 6.25 ppm for the main compounds and doxorubicin.

Cytotoxicity MTT Assay

Cytotoxicity MTT Assay was carried out based on the method of Sajjadi, *et al*. (2015) and Damasuri, *et al*. (2020) with some modifications. MCF-7 cells ($1.5x10^4$/well) were grown on 96 well-plates (Iwaki, Japan), incubated for 24 h. After 24 h, the culture medium was removed and replaced with the samples, incubated for 48 h. Then, samples were removed and 10% MTT solution (100% MTT = 5 mg/ml) was added, incubated for 1-4 h. After 1-4 h, the MTT was removed and replaced with DMSO. The activity of cells was analyzed using a microplate reader (Thermo scientific, Finland) at 570 nm. The absorbance data obtained were processed in Microsoft Excel, calculated the average value and % cell viability by the formula:

(sample absorbantion-medium absorbantion)/ (control absorbantion-medium absorbantionx100%

The data were analyzed by linear regression to get the IC_{50} value (Haryanti & Widiyastuti, 2017).

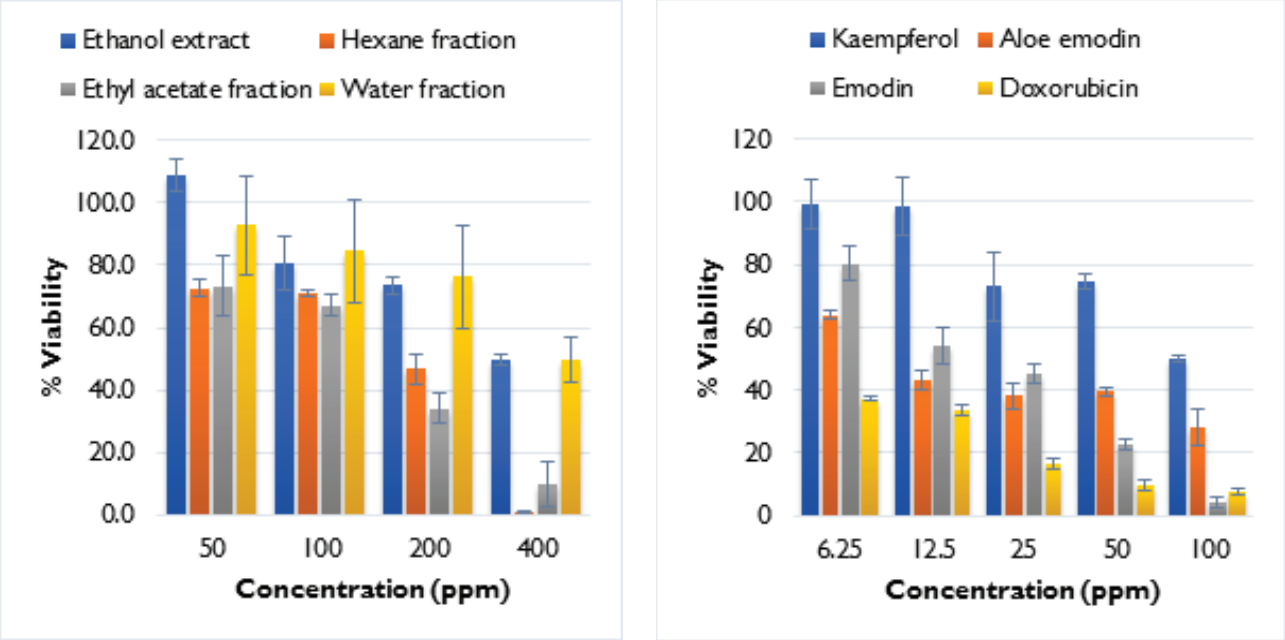

Figure 1. Graph of the relationship between compounds concentration and MCF-7 cells viability after treatment.

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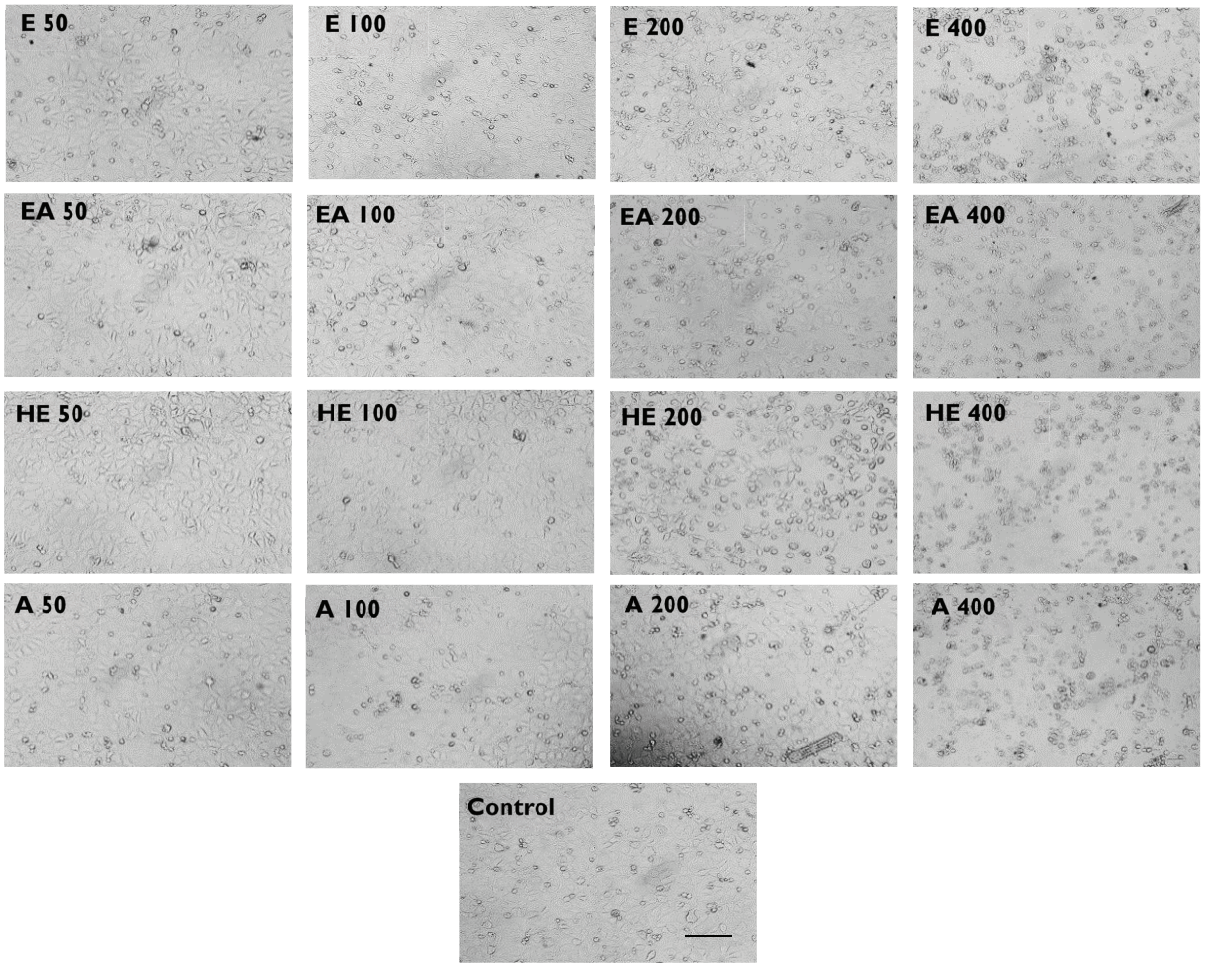

Figure 2. Morphology of the MCF-7 cells treated by ethanol extract (E), ethyl acetate fraction (AE), hexane fraction (HE), water fraction (A). INV100 microscope (200x). Scale bar 100 µm.

RESULTS

The cytotoxic activity of the extract and the fractions from the strongest to the weakest obtained were ethyl acetate fraction, hexane fraction, ethanol extract, and water fraction with IC_{50} values of 125.0 ppm, 132.6 ppm, 388.4 ppm, and 517.7 ppm, respectively. The cytotoxic activity of the main compounds from the strongest to the weakest obtained was aloe-emodin, emodin, and kaempferol with IC_{50} values of 12.7 ppm, 18.1 ppm, and 131.3 ppm, respectively. Doxorubicin gave superior results with an IC_{50} value of 2.2 ppm. The graph showing the relationship between the concentration of the compounds and cell viability can be seen in Figure 1. The compounds treatment gave changes in the morphology of MCF-7 cells. Cell morphology changes can be seen in Figures 2 and 3.

DISCUSSION

Based on the results, ethyl acetate fraction had the highest cytotoxic activity against MCF-7 cells; followed by hexane fraction, ethanol extract, and water fraction. *C. alata* L. leaves ethanol extract is known to contain alkaloids, flavonoids, terpenoids, saponins, and tannins. Chemical compounds detected were emodin, kaempferol,

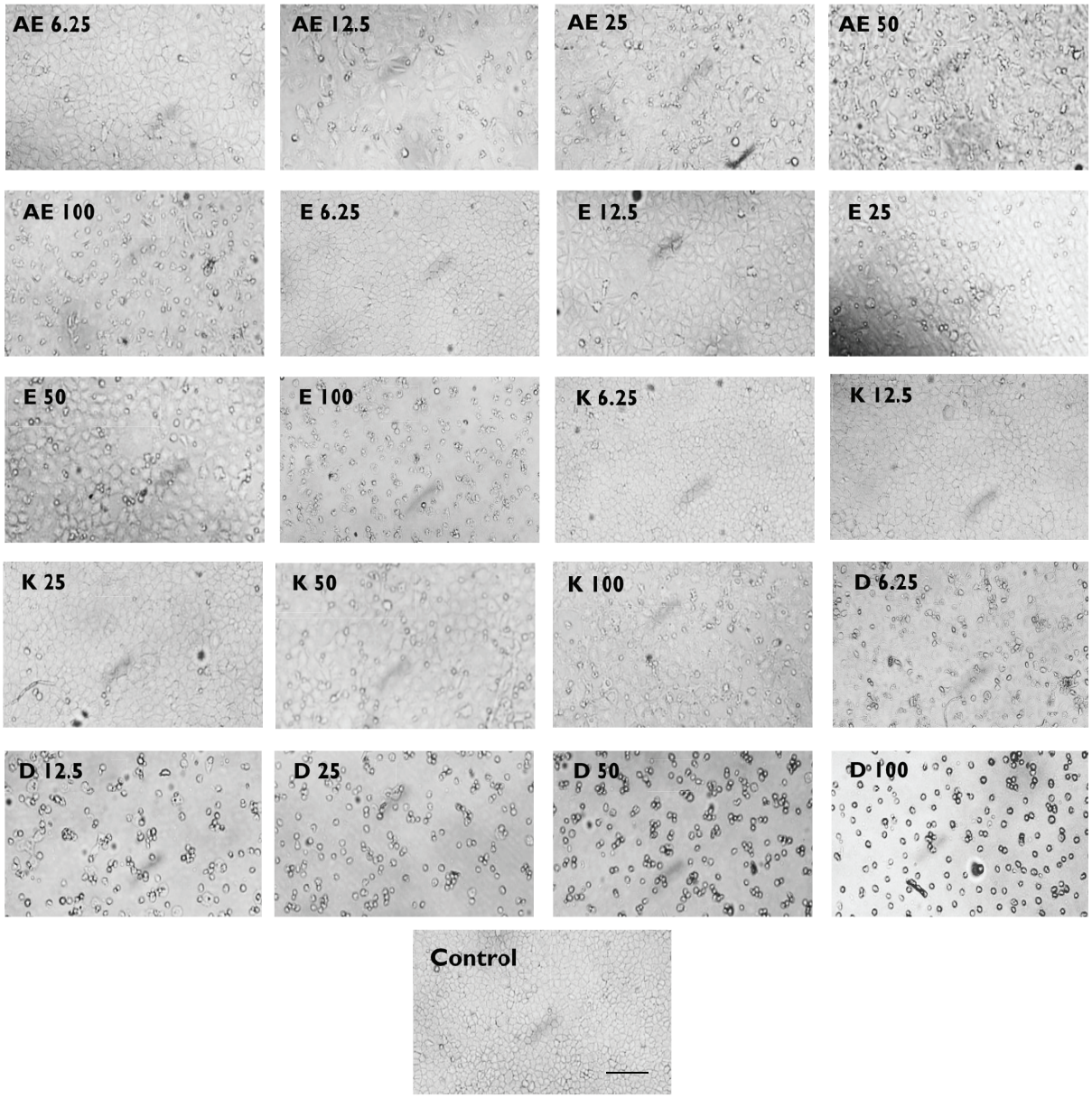

Figure 3. Morphology of the MCF-7 cells treated by aloe-emodin (AE), emodin (E), kaempferol (K), doxorubicin (D). INV100 microscope (200x). Scale bar 100 µm.

kaempferol-3,7-diglucoside, kaempferol-3-O-ß-D-glucopyranoside, aloe emodin, ω-hydroxyemodin, lunatin, physcion, ziganein, apigenin, 7,4-dihydroxy-5-methoxyflavone, diosmetin, luteolin, trans-dihydrokaempferol, trans-resveratrol, quercetin, *etc.* (Angelina, *et al*., 2021). Research conducted by Sogandi, *et al*. (2019) revealed that the screening results of licorice ethanol extract fractions contained different compounds in each fraction. The fractionation principle is the separation of compounds based on their polarity level. Compounds will dissolve in solvents of the same polarity. Butanol and water are polar solvents. Ethyl acetate is semi-polar. Hexane is non-polar.

The main compound contained in *C. alata* extract having the highest cytotoxic activity against MCF-7 cells was aloe-emodin with an IC_{50} value of 12.7 ppm. Aloe-emodin was reported to induce cell apoptosis by damaging mitochondrial membrane permeability and providing an oxidative stress response (excess ROS production). Aloe-emodin can arrest the cell cycle by downregulating cyclin and cyclin-dependent kinase. Aloe-emodin can also decrease the activity of transcription factors and alter the expression of transcription that is important for cell proliferation and metabolism (Sanders, *et al*., 2017). Emodin has the second highest cytotoxic activity after aloe emodin with an IC_{50} value of 18.1 ppm. The mechanism of emodin as an anticancer was reported by increasing ROS, levels of Bax (pro-apoptotic protein), and cytosolic levels. Emodin can also reduce levels of Bcl-2 (anti-apoptotic protein) (Akkol, *et al*., 2021). Kaempferol ranks third after emodin with an IC_{50} value of 131.3 ppm. Kaempferol was reported to be able to induce cell apoptosis, disturb the cell cycle, and downregulate phosphoinositide 3-kinase (Imran, *et al*., 2019).

Doxorubicin had a more effective ability to decrease cancer cells viability with an IC_{50} value of 2.2 ppm. However, doxorubicin was reported to have many side effects. Doxorubicin is a class 1 anthracycline drug that is non-selective (toxic to normal cells) and has many side effects (Micallef & Baron, 2020; Mulia, *et al*., 2016). Doxorubicin works by blocking the topoisomerase enzyme that plays a role in the growth of cancer cells (Micallef & Baron, 2020).

CONCLUSION

The compounds contained in *C. alata* L. leaves namely aloe-emodin, emodin, and kaempferol showed better cytotoxic activity than the extract and fractions of *C. alata* L. leaves with IC_{50} values of 12.7 ppm, 18.1 ppm, and 131.3 ppm, respectively.

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REFERENCES

Akkol, E.K., Tatlı, I.I., Karatoprak, G.Ş., Ağar, O.T., Yücel, Ç., Sobarzo sánchez, E., and Capasso, R., 2021, Is emodin with anticancer effects completely innocent? Two sides of the coin, *Cancers*, **13**(11), 1–52.

Ali, M.I., Aboul-Enein, A.M., Mohamed, S.M., Elella, F.A.M., Mohammed, M.M.D., and Hamed, A.R., 2017, Phytochemical, cytotoxicity and antioxidant investigation of *Cassia alata* leaves growing in Egypt, *Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)*, **4**(4), 97–105.

Angelina, M., Hanafi, M., Suyatna, F.D., and Dewi, B.E., 2020, Drug of action cassia alata leaves extract as antiviral to dengue virus serotype-2 *in vitro*, *Pharmacognosy Journal*, **12**(4), 864–871.

Angelina, M., Hanafi, M., Suyatna, F.D., Mirawati, S.T., Ratnasari, S., and Dewi, B.E., 2017, Antiviral Effect of Sub Fraction *Cassia alata* Leaves Extract to Dengue Virus Serotype-2 strain New Guinea C in Human Cell Line Huh-7 it-1, *IOP Conference Series: Earth and Environmental Science*, 101, 012004.

Angelina, M., Mardhiyah, A., Dewi, R.T., Fajriah, S., Muthiah, N., Ekapratiwi, Y.,

Dewijanti, I.D., Sukirno, Jamilah, and Hartati, S., 2021, Physicochemical and phytochemical standardization, and antibacterial evaluation of *Cassia alata* leaves from different locations in Indonesia, *Pharmacia*, **68**(4), 947–956.

Ashariati, A., 2019, Manajemen Kanker Payudara Komprehensif, *In Journal of Chemical Information and Modeling*, **53**(9). http://repository.unair.ac.id/96210/2/Manajemen Kanker Payudara Komprehensif.pdf.

Damasuri, A.R., Sholikhah, E.W., and Mustofa, 2020, Cytotoxicity of ((E)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one) on HeLa cell line, *Indonesian Journal of Pharmacology and Therapy*, **1**(2), 54–59.

Fatmawati, S., Yuliana, Purnomo, A.S., and Abu Bakar, M.F., 2020, Chemical constituents, usage and pharmacological activity of *Cassia alata*, *Heliyon*, **6**(7), e04396.

Haryanti, S., and Widiyastuti, Y., 2017, Aktivitas Sitotoksik pada Sel MCF-7 dari Tumbuhan Indonesia untuk Pengobatan Tradisional Kanker Payudara, *Media Penelitian dan Pengembangan Kesehatan*, 27(4), 247-254.

Hennebelle, T., Weniger, B., Joseph, H., Sahpaz, S., and Bailleul, F., 2009, *Senna alata*, *Fitoterapia*, **80**(7), 385–393.

Huang, P.H., Huang, C.Y., Chen, M.C., Lee, Y.T., Yue, C.H., Wang, H.Y., and Lin, H., 2013, Emodin and Aloe-Emodin suppress breast cancer cell proliferation through ER α inhibition, *Evidence-Based Complementary and Alternative Medicine*, **2013**, 376123.

Imran, M., Salehi, B., Sharifi-Rad, J., Gondal, T.A., Saeed, F., Imran, A., *et al.*, 2019, Kaempferol: A Key Emphasis to Its Anticancer Potential, *Molecules*, **24**(12), 2277.

Kim, S.H., and Choi, K.C., 2013, Anti-cancer effect and underlying mechanism(s) of Kaempferol, a phytoestrogen, on the regulation of apoptosis in diverse cancer cell models, *Toxicological Research*, **29**(4), 229–234.

Kullenberg, F., Degerstedt, O., Calitz, C., Pavlović, N., Balgoma, D., Gråsjö, J., *et al.*, 2021, *In vitro* cell toxicity and intracellular uptake of doxorubicin exposed as a solution or liposomes: Implications for treatment of hepatocellular carcinoma, *Cells*, **10**(7), 1717.

Lewis, A., and Levy, A., 2011, Anti-inflammatory activities of *Cassia alata* leaf extract in complete freund's adjuvant arthritis in rats, *West Indian Medical Journal*, **60**(6), 615–621.

Micallef, I., and Baron, B., 2020, Doxorubicin: An Overview of the Anti-Cancer and Chemoresistance Mechanisms, *Annals of Clinical Toxicology*, **3**(2), 1031.

Mulia, K., Hasan, A.E.Z., and Suryani, 2016, Total phenolic, anticancer and antioxidant activity of ethanol extract of Piper retrofractum Vahl from Pamekasan and Karang Asem, *Current Biochemistry*, **3**(2), 80–90.

Oladeji, O.S., Adelowo, F.E., Oluyori, A.P., and Bankole, D.T., 2020, Ethnobotanical Description and Biological Activities of *Senna alata*, *Evidence-Based Complementary and Alternative Medicine*, **2020**, 2580259.

Olarte, E.I., Herrera, A.A., Villaseñor, I.M., and Jacinto, S.D., 2013, *In vitro* antitumor properties of an isolate from leaves of *Cassia alata* L., *Asian Pacific Journal of Cancer Prevention*, **14**(5), 3191–3196.

Pangribowo, S., 2019, Beban Kanker di Indonesia, *Pusat Data Dan Informasi Kesehatan Kementerian Kesehatan RI*, 1–16.

Sagnia, B., Fedeli, D., Casetti, R., Montesano, C., Falcioni, G., and Colizzi, V., 2014, Antioxidant and anti-inflammatory activities of extracts from *Cassia alata*, Eleusine indica, Eremomastax speciosa, Carica papaya and Polyscias fulva medicinal plants collected in Cameroon, *PLoS ONE*, **9**(8), 1–10.

Sajjadi, S.E., Ghanadian, M., Haghighi, M., and Mouhebat, L., 2015, Cytotoxic effect of Cousinia verbascifolia Bunge against OVCAR-3 and HT-29 cancer cells, *Journal of HerbMed Pharmacology*, **4**(1), 15–19.

Sanders, B., Ray, A.M., Goldberg, S., Clark, T., McDaniel, H.R., Atlas, S.E., *et al.*, 2017, Anti-cancer effects of aloe-emodin: a systematic review, *Journal of Clinical and Translational Research*, **3**(3), 283–296.

Sogandi, G., Darma, W.S.T., and Jannah, R., 2019, Potential of Antibacterial Compounds from Sweet Root Extract (*Glycyrrhiza glabra* L.) on Bacillus cereus, *Jurnal Kimia Sains Dan Aplikasi*, **22**(4), 105–111.

Tang, Z., He, J., Zou, J., Yu, S., Sun, X., and Qin, L., 2021, Cisplatin-resistant HepG2 cell-derived exosomes transfer cisplatin resistance to cisplatin-sensitive cells in HCC, *PeerJ*, **9**, 1–13.

Timothy, S.Y., Wazis, C.H., Adati, R.G., and Maspalma, I.D., 2012, Antifungal activity of aqueous and ethanolic leaf extracts of *Cassia alata* linn, *Journal of Applied Pharmaceutical Science*, **2**(7), 182–185.

Triana, O., Prasetya, F., Kuncoro, H., and Rijai, L., 2016, Aktivitas Antijamur Ekstrak Daun Ketepeng Cina (*Cassia alata* L.), *Jurnal Sains dan Kesehatan*, **1**(6), 311–315.

Varghese, G.K., Bose, L.V., and Habtemariam, S., 2013, Antidiabetic components of *Cassia alata* leaves: Identification through α-glucosidase inhibition studies, *Pharmaceutical Biology*, **51**(3), 345–349.

Zhang, Z., Shi, J., Nice, E.C., Huang, C., and Shi, Z., 2021, The multifaceted role of flavonoids in cancer therapy: Leveraging autophagy with a double-edged sword, *Antioxidants*, **10**(7), 1–15.