BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 13, Number 1, April 2024 | Pages: 265-269 | DOI: 10.14421/biomedich.2024.131.265-269 ISSN 2540-9328 (online)

Potential of Soursop Leaf Extract as an Antioxidant in MCF-7 Cells

Muhammad Samsul Mustofa[1], Restu Syamsul Hadi[1], Nunung Ainur Rahmah[2,*], Pendrianto[3]
[1]Department of Biological, Faculty of Medicine, Universitas YARSI, Jakarta 10520, Indonesia.
[2]Department of Anatomical Pathology, Faculty of Medicine, Universitas YARSI, Jakarta 10520, Indonesia.
[3]Telomere Research Center, YARSI University Research Institute, Universitas YARSI, Jakarta 10520, Indonesia.

Corresponding author*
nunung.ainur@yarsi.ac.id

Manuscript received: 16 October, 2023. Revision accepted: 07 December, 2023. Published: 08 August, 2024.

Abstract

The frequency of breast cancer tends to increase. Malondialdehyde (MDA) is a marker of oxidative stress as an end product from the chain reaction of lipid peroxidation. The use of traditional medicine soursopleaf (*Annona muricata* L.) has been reported for a long time because of its bioactivity as an antioxidant. This study analyzes the relationship between MDA levels and glutathione enzymes in MCF-7 cells given the methanol extract of soursop leaves. The methanol extract of soursop leaves was carried out by infusion method. The methanol extract of soursop leaves was given to cancer cells at several doses with an incubation of 24 hours. The cytotoxic test was carried out using the MTT method. Measurement of MDA levels was carried out using the thiobarbituric acid reactive substance (TBARS/TBA) reactivity test method. GSH measurements used the colorimetric method. The results showed that the ethanol extracts of soursop leaves have cytotoxic activity in the MCF-7 breast cancer cell line with IC50 values of 23.96 ppm. Ethanol extract of soursop leaves increased levels of MDA inhibition and GSH level. Soursop leaf extract could increase MDA inhibition GSH level in human breast cancer cells MCF-7.

Keywords: Breast cancer; GSH; MCF-7; MDA; soursop leaf.

INTRODUCTION

In 2018, there were 18 million new cancer cases; by 2040, it could be 29–37 million. New cases of breast cancer reached 11.6% in 2018 (World Health Organization, 2020). Boundouki *et al.* (2021) stated there are more than 2 million new cases every year worldwide.

One of the triggers for breast cancer is oxidative stress mechanisms (Sossa *et al.*, 2013). Oxidation reactions involving free radicals can damage the surrounding normal cell membrane and damage the composition of DNA to cause a mutation. Mutations or damage to the composition of DNA can cause cancer. Free radical oxidation of proteins, nucleic acids, and lipids each produces carbonyl compounds, MDA (malondialdehyde), and deoxyguanosine P (Shaw *et al.*, 2011).

The body needs antioxidants to combat free radicals. Antioxidants are either obtained from outside the body (food) or produced from within the body (Lobo *et al.*, 2010). Examples of endogenous antioxidants include superoxide dismutase, glutathione (GSH), catalase, and glutathione peroxidase (Rizzo *et al.*, 2010). Glutathione is an enzyme with various uses, including detoxification, antioxidants, maintenance of thiol status, and modulation of cell proliferation (Lushchak, 2012).

Breast cancer treatment approaches include surgery, radiation treatment, endocrine treatment, and chemotherapy (Anjum *et al.*, 2017). However, most of the mechanisms underlying treatment involve Reactive Oxygen Species (ROS) production and result in increased oxidative damage (de Sa Junior *et al.*, 2017). One way to reduce levels of ROS by providing antioxidants. Natural antioxidants have been extensively studied to fight breast cancer and tumor development as chemopreventive agents (Griñan-Lison *et al.*, 2021).

One of the traditional food sources that contain glutathione as antioxidants are soursop (*Annona muricata* L.). Soursop is a species of the Annonaceae family, studied extensively for its therapeutic potential (Gavamukulya *et al.*, 2017). Soursop leaf extract contains phenolic and flavonoid compounds that act as antioxidants (Ovando-Domínguez *et al.*, 2019). Several studies have shown that soursop leaf extract is proven to cure disease and breast cancer cell death (Rady *et al.*, 2018; Fertilita *et al.*, 2020). However, there has been no specific research on the effect of giving soursop leaf extract on reducing oxidative stress in MCF-7 cell cultures, so this needs to be done.

The present study aims to investigate the cytotoxic effect and relationship between levels of MDA inhibition

and glutathione enzymes in MCF-7 cells given the ethanol extract of soursop leaves.

MATERIALS AND METHODS

Materials

The MCF-7 breast cancer cell lines were purchased from ATCC (Manassas, VA, USA). Dulbecco's minimum essential medium (DMEM) (Gibco, New York, USA) supplemented with 10% fetal bovine serum (Gibco, New York, USA), with 5% of the antibiotic-antimycotic (Corning, USA). *Annona muricata* L. powder extraction was carried out using the infusion method. The MTT kit was obtained from Abnova (Taiwan). The MDA and GSH kit were obtained from Elabscience (USA).

Soursop leaf extraction

Soursop leaf extract was obtained by maceration. The extraction was carried out at the YARSI University Herbal Research Center. A total of 100 grams of soursop leaves were blended into flour and then macerated in 250 ml of 70% ethanol for 24 hours. The sample was filtered, and the filtrate was collected. The remaining sample was added with 100 ml of 70% ethanol and macerated for 24 hours. Then, the sample was filtered again, and the filtrate was collected. The second residual sample was added to 100 ml of 70% ethanol. The filtrate was then concentrated using a rotary evaporator to produce a thick extract.

Cell culture

MCF-7 cells were routinely cultured in a tissue flask containing DMEM with 10% FBS and 5% antibiotic-antimycotic at 37°C in a humidified atmosphere with 5% CO_2. Cells from stock plates were suspended by treatment with 0.25% trypsin, buffered with 0.2% EDTA (pH 7.3), and counted using a hemocytometer to determine the effects of soursop leaf extract on cell proliferation. Cells were adjusted to a density of $1x10^6$ cells/well to the required plating medium volume supplemented with 10% PBS and soursop leaf extract dissolved in DMSO.

Cytotoxicity assay

The breast cancer cells were seeded into 96-well plates at a density of 5,000 cells per well in triplicates and were treated with 0, 10, 20, and 30 ppm concentrations of soursop leaf extract for 48 h (according to the MTT protocol datasheet). DMSO (0.1%) was added to the control wells followed by incubation at 37°C for 2 h after addition of 20 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to each well. Absorbance was measured on optical density at 490 nm. The IC_{50} was developed by an inhibition curve of three independent experiments.

Evaluation of MDA inhibition

MDA is the last marker of the lipid peroxidation pathway. This assay is according to the repercussion of MDA with thiobarbituric acid (TBA) that forms the MDATBA adduct that can be quantified calorimetrically. The sample was reacted with 200 µL of trichloroacetic acid (TCA) 20% for deproteination. Then the cortex and centrifuge at a speed of 5000 rpm for 10 minutes. The supernatant formed was taken, and 400 µl of TBA 0.67% reagent was added. Then the sample was vortexed and incubated in a water heater at 96°C, 10 minutes to produce pink color, then lift and cool at room temperature. Then read the absorption at a wavelength of 532 nm.

Calculation of results

$$\underset{(\text{nmol/mgprot})}{MMA} = \frac{\Delta A_1}{\Delta A_2} \times C \times f \div C_{pr}$$

Note:

ΔA_1 : OD_{Sample} - OD_{Blank}
ΔA_2 : $OD_{Standard}$ - OD_{Blank}
C : The concentration of standard, 10 nmol/mL
f : Dilution factor of sample before test
C_{pr} : Concentration of protein in sample, mgprot/mL

Evaluation of GSH level

The reduction of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) by GSH produced a yellow complex used to indicate the level of thiol protein; the color intensity of 412 nm was proportional to the level of GSH. Measurement of glutathione (GSH) levels by taking as much as 0.5 ml of supernatant added 0.5 ml of DNTB and 3 ml of phosphate buffer (0.2 M, pH 8). This reagent reacts with the SH group to give a yellow complex. The absorbance was read with a spectrophotometer at a wavelength of 412 nm.

Cells and tissue sample:

$$\begin{aligned} &Reduced\ GSH\ content\ in\ cells\ and\ tissue\ (\mu mol/gprot) \\ &= \frac{\text{ODSample} - \text{ODBlank}}{\text{ODStandard} - \text{ODBlank}} \times Concentration\ of\ standard\ (40\,\mu mol/L) \\ &\times Dilution\ factor\ of\ sample\ before\ tested \div Protein\ concentration\ of\ sample(gprot/L) \end{aligned}$$

Statistical analysis

Data were processed statistically using the Statistical Program for Social Science (SPSS) software for Windows version 20.0. Hypothesis testing to analyze GSH levels used the Kruskal–Wallis test and analyzed MDA levels using the one-way ANOVA test. The Spearman nonparametric test used the correlation test between the two variables.

RESULTS AND DISCUSSION

Cytotoxicity examination

Cytotoxic test results showed the percentage of living cells is inversely proportional to the concentration increases. The higher the concentration of the test material, the lower the percent of the mean number of living cells MCF-7 cells that life is getting a little (Bahuguna *et al.*, 2017). IC_{50} values of ethanol extract of soursop leaf on MCF-7 cells were 23.96 ± 0.006 ppm (Figure 1).

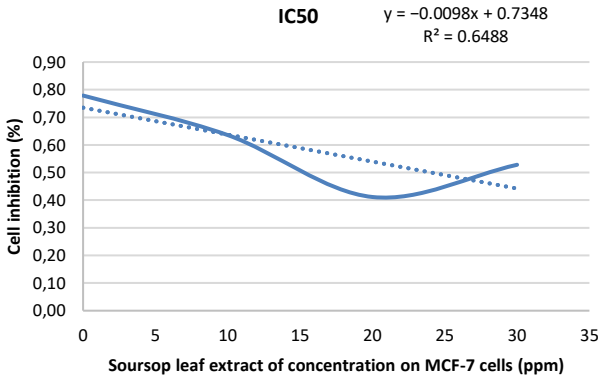

Figure 1. The cell inhibition of soursop leaf extract on MCF-7 cells.

MDA inhibition and GSH level

In this study, soursop leaf extract was used at concentrations around IC_{50}, namely 0, 10, 20, 30 ppm, to determine the state of free radical production and antioxidant levels. As shown in Figure 2, the percentage of living cells is inversely related to the increase in concentration. The higher the concentration of the test material, the lower the percentage of the average number of MCF-7 living cells.

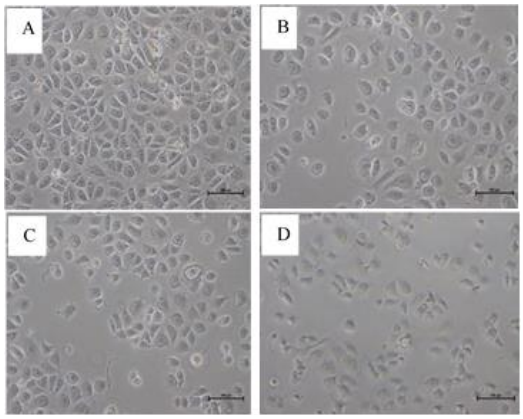

Figure 2. Microscopic photo of MCF-7 cell culture treated with soursop leaf extract (400x). (A) treatment with 0 ppm; (B) treatment with 10 ppm; (C) treatment with 20 ppm; (D) treatment with 30 ppm.

Furthermore, Figure 3 showed that at the concentration 0 ppm of soursop leaf extract the MDA inhibition level was 0.57 nmol/mgprot while GSH level was 721.05 µmol/gprot. The soursop leaf extract was increased the MDA inhibition and GSH level. The highest increase in MDA inhibition levels occurred when the soursop leaf extract was given 30 ppm, namely 388.6 nmol/mgprot, while the highest increase in GSH occurred when the soursop leaf extract was given 20 ppm, namely 1158.63 µmol/gprot.

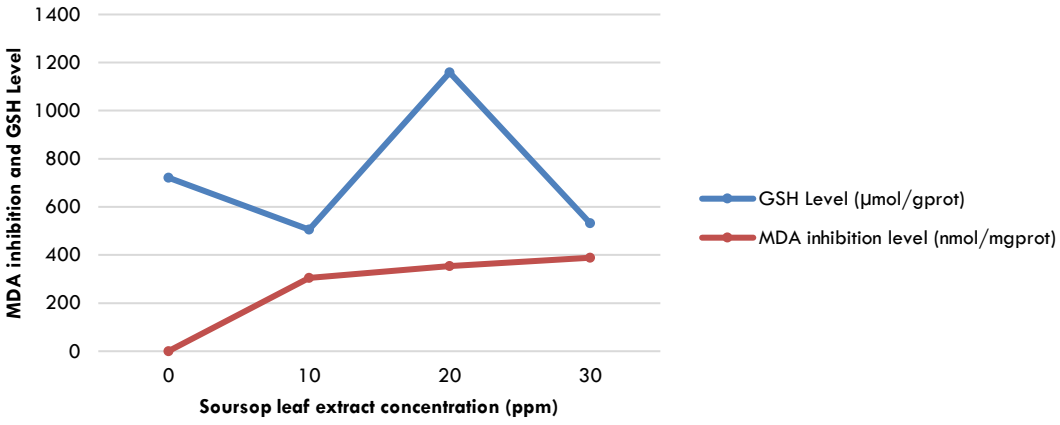

Figure 3. The growth rate of MDA inhibition and GSH level by administering soursop leaf extract to MCF-7 cell.

Oxidative stress is a condition that reflects an imbalance between ROS and antioxidant defenses. Malondialdehyde (MDA) is a marker of oxidative stress due to a chain reaction of lipid peroxidation. In this study, MDA inhibition levels in MCF-7 increased statistically significantly in the administration of soursop leaf extract. This indicates that the soursop leaf extract decreases free radical formation (Rady *et al.*, 2018; Muchtaromah *et al.*, 2015). In MCF-7 cells, soursop leaf extract can increase the MDA inhibition level, inhibit cell proliferation, stimulate cell apoptosis, inhibit metastasis, regulate the immune system, and reduce the inflammation caused by cancer (Rachmani *et al.*, 2013; Syed Najmuddin *et al.*, 2016).

Glutathione (l-J-glutamyl-cysteinyl-glycine) is a tripeptide consisting of glutamic acid, cysteine, and glycine. This compound has a sulfhydryl/thiol group (-SH) found in the amino acid cysteine. The sulfhydryl group causes GSH to act as a strong electron donor (nucleophile) in warding off free radicals. GSH can decompose H_2O_2 into H_2O with the help of the enzyme glutathione peroxidase. GSH can be synthesized in all cells, especially tissues that are highly exposed to ROS (Reactive Oxygen Species) (Wu *et al.*, 2004). In this study, GSH production levels in MCF-7 were increased in the administration of soursop leaf extract. The increased GSH levels was not statistically significant, possibly due to the large number of other antioxidants (glutathione peroxidase, SOD, and catalase) that play a role in reducing free radicals. Increased MDA inhibition levels are followed by decreased GSH levels. GSH has a role as an antioxidant by directly reducing free radicals or as a cofactor for antioxidant enzymes such as glutathione peroxidase and glutathione transhydrogenase. The main function of GSH is to detoxify drugs, xenobiotics, or pesticides catalyzed by the GSH-S-transferase enzyme. GSH also plays a role in maintaining the thiol group (-SH) in essential proteins by reducing disulfide bonds in proteins, which are catalyzed by the enzyme thiol transferase (Wu *et al.*, 2004). The administration of soursop leaf extract with a concentration of 20 ppm increased GSH production. Soursop leaf extract treatment at a concentration of 10 ppm showed a decrease in GSH levels. This may be due to other auto-oxidation when soursop leaf extract is added to a buffer solution or cell culture medium at physiological pH. The limitation of this research is that this research uses soursop leaves for the manufacture of extracts obtained from gardens that have not been certified. Ideally, the soursop leaf material used as an extract material comes from a certified soursop garden to produce good phenol and flavonoid content.

CONCLUSIONS

Soursop leaf extract was able to increase MDA inhibition and GSH level in human breast cancer cells MCF-7. Further research on the antioxidant effect of soursop leaf extract can be carried out using cancer cells other than MCF-7 cells.

Acknowledgements: The author is grateful for the internal research grant from YARSI University, Jakarta, Indonesia, 2018/2019.

Authors' Contributions: Muhammad Samsul Mustofa & Nunung Ainur Rahmah designed the study. Muhammad Samsul Mustofa carried out the laboratory work. Restu Syamsul Hadi & Pandrianto analyzed the data. Muhammad Samsul Mustofa & Nunung Ainur Rahmah wrote the manuscript. Restu Syamsul Hadi & Pandrianto revised the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

Funding: The author is grateful for the internal research grant from YARSI University, Jakarta, Indonesia, 2018/2019.

REFERENCES

Anjum, F., Razvi, N. and Masood, M.A. (2017). Breast cancer therapy: a mini review. *MOJ Drug Des Develop Ther*, 1(2), 35-38.

Bahuguna, A., Khan, I., Bajpai, V.K. et al. (2017). MTT assay to evaluate the cytotoxic potential of a drug. *Bangladesh J Pharmacol*, 12, 115-118.

Boundouki, G., Wilson, R., Duxbury, P. et al. (2021). Patient and public priorities for breast cancer research: a qualitative study in the UK. *BMJ Open*, 11, e036072.

de Sá Junior, P.L., Camara, D.A.D., Porcacchia, A.S. et al. (2017). The roles of ROS in cancer heterogeneity and therapy. *Oxid Med Cell Longev*, 2017, 2467940.

Fertilita, S., Sandhika, W. and Suprabawati, D.G.A. (2020). The cytotoxic activity of *Annona muricata* Linn leaves ethanolic extract (AMEE) on T47D breast cancer cell line. *Medical Laboratory Technology Journal*, 6(1), 32-39.

Gavamukulya, Y., Wamunyokoli, F. and El-Shemy, H.A. (2017). *Annona muricata* is the natural therapy to most disease conditions including cancer growing in our backyard? A systematic review of its research history and future prospects. *Asian Pac J Trop Med*, 10(9), 835-848.

Griñan-Lison, C., Blaya-Cánovas, J.L., López-Tejada, A. et al. (2021). Antioxidants for the treatment of breast cancer: are we there yet?. *Antioxidants (Basel)*, 10(2), 205.

Lobo, V., Patil, A., Phatak, A. et al. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev*, 8, 118-126.

Lushchak, V.I. (2012). Glutathione homeostasis and functions: potential targets for medical interventions. *J Amino Acids*, 2012, 1-26.

Muchtaromah, B., Romaidi, R., Pratiwi, K.H. et al. (2015). Effect of *Annona muricata* leaf extract on antioxidant activity and

histology of the mamary tissue in the breast cancer model in vivo. *Aust J Basic Appl Sci*, 9(7), 92-95.

Ovando-Domínguez, M.Y., Luján-Hidalgo, M.C., González-Mendoza, D. et al. (2019). Total phenols, flavonoids and antioxidant activity in *Annona muricata* and *Annona purpurea* callus culture. *Phyton-Int J Experimen Botany*, 88(2), 139-147.

Rachmani, E.P.N., Suhesti, T.S., Widiastuti, R. et al. (2013). Cytotoxic effects of methanol extracts of soursop leaves (*Annona muricata*) on MCF-7 cell line and its effect on expression of bcl-2. *AASIC, 2*013, 79-82.

Rady, I., Bloch, M.B., Chamcheu, R.N. et al. (2018). Anticancer properties of Graviola (*Annona muricata*): a comprehensive mechanistic review. *Oxid Med Cell Longev*, 2018, 1826170.

Rizzo, A.M., Berselli, P., Zava, S. et al. (2010). Endogenous antioxidants and radical scavengers. *Adv Exp Med Biol*, 698, 52-67.

Shaw, A.T., Winslow, M.M., Magendantz, M. et al. (2011). Selective killing of K-ras mutant cancer cells by small molecule inducers of oxidative stress. *Proc Natl Acad Sci USA*, 108(21), 8773-8778.

Sosaa, V., Molinéa, T., Somozaa, R. et al. (2013). Oxidative stress and cancer: an overview. *Ageing Res Rev*, 12(1), 376-390.

Syed Najmuddin, S.U., Romli, M.F., Hamid, M. et al. (2016). Anti-cancer effect of *Annona muricata* Linn leaves crude extract (AMCE) on breast cancer cell line. *BMC Complement Altern Med*, 16(1), 311.

World Health Organization. (2020). WHO report on cancer: setting priorities, investing wisely and providing care for all. World Health Organization. https://apps.who.int/iris/handle/10665/330745

Wu, G., Fang, Y.Z., Yang, S., et al. (2004). Glutathione metabolism and its implications for health. *J Nutr*, 134(3), 489-492

THIS PAGE INTENTIONALLY LEFT BLANK