

Research Article



Check for updates

OPEN ACCESS

Modulation of Apoptosis-Related Gene Expression in MCF-7 Breast Cancer Cells by *Lunasia amara*: An Integrated *In Vitro* and *In Silico* Study

Agus Saputra^{1*}, Meity Marviana Laut¹, Putri Reno Intan², Heni Nitbani¹, Ingrid T Maha¹, Yulfia Nelymalik Selan¹, Nemay Anggadewi Ndaong¹, Ietje Wientarsih³, Mohamad Rafi⁴, Lina Noviyanti Sutardi³

¹Department of Anatomy, Physiology, Pharmacology, and Biochemistry, Universitas Nusa Cendana, Kupang NTT, Indonesia

²Center for Biomedical Research, Research Organization for Health, National Research and Innovation Agency (BRIN), Bogor West Java, Indonesia

³Division of Pharmacy School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor 16680, Indonesia

⁴Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, Indonesia

ARTICLE INFO

Article history:

Received June 4, 2026

Received in revised form September 30, 2026

Accepted October 8, 2026

Available Online October 20, 2026

KEYWORDS:

Apoptosis,
Lunamarine,
Lunasia amara,
MCF7,
Molecular docking



Copyright (c) 2026 @author(s).

ABSTRACT

Empirical studies show that the *Lunasia amara* plant has been used to treat breast cancer. The previous studies on *Lunasia* extract using metabolomic and chemometric approaches obtained quinoline alkaloid compounds such as Lunamarine and Lunine which correlate highly with cytotoxic activity in the MCF7 cell line. This study aimed to determine the effect of *Lunasia amara* extract on apoptosis markers Bax and Bcl2 *in vitro* and *in silico*. Gene expression of Bax (pro apoptosis) and Bcl2 (anti-apoptosis) will be measured using the RT PCR technique, while *in silico*, it will be performed using molecular docking. Based on PCR results, ethanol extract caused an increase in Bax gene expression while Bcl2 decreased. The Bax/Bcl2 ratio increased from 1 to 4.8. This will promote cell death. The molecular interaction of quinoline compounds against Bax and Bcl2 protein receptors generally did not show significant differences based on their affinity energy values. This suggests that the ligand exerts a greater influence at the gene level than the influence exerted on the apoptotic protein. Based on our findings, ethanol extract of *Lunasia* affects the expression of apoptotic genes Bax and Bcl2.

1. Introduction

Breast cancer (BC) is the second most prevalent type of cancer globally after lung cancer and the leading cause of cancer-related deaths among women (Putra *et al.* 2020; Liu *et al.* 2024; Suo *et al.* 2024). The lack of clarity regarding the molecular heterogeneity of breast tumors has increased incidence and mortality rates (Jeibouei *et al.* 2019). Although several therapies are available, including surgery, chemotherapy, and radiotherapy, breast cancer mortality remains high, especially in cases diagnosed at an advanced stage (Al-Mahayri *et al.* 2020). There is considerable diversity among and within tumors and among cancer patients.

A comprehensive classification of breast cancer based on gene expression profiles has identified five intrinsic molecular subtypes (Luminal A, Luminal B, HER-2 loaded, Basal-like, and Claudin-low) and a normal breast-like group (Polyak 2011; Gambaro *et al.* 2023). These entities exhibit notable differences in incidence, risk factors, prognosis, and therapy sensitivity. It has been reported that breast cancer is associated with the expression of steroid hormone receptors, specifically the estrogen receptor (ER), the progesterone receptor (PR), and the amplification or overexpression of the human epidermal growth factor receptor 2 (HER2) (Chen *et al.* 2019; Permana *et al.* 2024). In recent years, several targeted treatments have been developed for ER- and HER2-positive breast cancer, including doxorubicin, tamoxifen, rapamycin, and lapatinib (Wang *et al.* 2020; Draguet *et al.* 2021; Zasheva *et al.* 2024). Research into

* Corresponding Author

E-mail Address: saputra.a@staf.undana.ac.id

the genetic and molecular underpinnings of breast cancer is essential to identify potential biomarkers that can guide patient-tailored treatment approaches. Therefore, the search for new, more effective anti-cancer drugs with minimal side effects is a priority in cancer research.

The use of medicinal plants as a source of natural traditional herbal medicines such as anticancer agents has attracted much attention among the scientific community (Atanasov *et al.* 2021; Tanaka and Kashiwada 2021; Pakrashy *et al.* 2022). One plant that has been identified as a potential source of anticancer agents is *Lunasia amara*, which has a history of use in traditional medicine for the treatment of a range of diseases (Zubair *et al.* 2016; Bunga *et al.* 2024; Dhani Putri and Bakar Tawali 2024; Saputra *et al.* 2024). Previous studies have reported the presence of alkaloids in addition to flavonoids and terpenoids (Macabeo and Aguinaldo 2008). These compounds have been demonstrated to possess a range of biological activities, including anticancer properties (Prescott *et al.* 2007; Zubair and Subehan 2010; Takahashi *et al.* 2012; Adriani *et al.* 2019). However, research on the specific cytotoxic effects of *L. amara* against MCF7 breast cancer cells is still very limited. MCF7 cancer cells are a widely utilized model for the study of breast cancer due to their estrogen receptor-positive (ER+) characteristics, which are pertinent to the majority of human breast cancers (Holliday and Speirs 2011). Further research is required to elucidate the mechanisms by which these compounds exert their effects and to evaluate their potential for synergistic interactions with existing therapies.

A significant challenge in the search for anticancer drugs is the identification of signaling targets that can be inhibited to induce apoptosis (Youssif *et al.* 2024). Apoptosis is a process of programmed cell death critical for the balance and development of multicellular organisms. The process of apoptosis is regulated by many internal and external signals that activate specific molecular pathways (Pistritto *et al.* 2016; Kashyap *et al.* 2021). These include the Bcl2 family of proteins, which consists of pro-apoptotic proteins such as Bax and anti-apoptotic proteins such as Bcl2 (O'Brien and Kirby 2008). Cancer cells can evade apoptosis through the modulation of gene expression involved in apoptosis regulation. This is achieved by increasing the expression of Bcl2 and decreasing the expression of Bax, which prevents the activation of apoptotic pathways. In cancer therapy, the induction of apoptosis in cancer cells represents a principal approach, as it can result in the targeted

destruction of these cells. The ratio between these two genes is frequently a significant indicator of the efficacy of an anticancer therapeutic agent (Indriwati *et al.* 2022; Saddam *et al.* 2024; Shenoy and Abdul Salam 2024; Zhang *et al.* 2024). *In silico* approaches (molecular docking) can be used to understand the mechanism of action on apoptosis. Molecular docking is a methodology based on the interaction between bioactive compounds and proteins that serve as biomarkers of a signaling pathway (Dar and Mir 2017).

This study aims to examine the cytotoxic activity of the *L. amara* ethanol extract on the MCF7 cell line and its impact on the expression of gene markers for apoptotic pathways, specifically Bax and Bcl2. This will be achieved through a combination of *in vitro* and molecular docking approaches, to elucidate the potential mechanism of action of *L. amara* as an anticancer agent. These findings will contribute to the development of specific anticancer agents, which have the potential to enhance efficacy and reduce adverse effects in the treatment of breast cancer.

2. Materials and Methods

2.1. Materials

Samples of plant were taken from Chibal district of Manggarai regency, Flores, NTT province, Indonesia. The plant has been identified by the National Research and Innovation Agency (BRIN), and the voucher specimens (BMK0157092016) have been stored in TropBRC. The ethanol was purchased from Merck, Germany, DMSO (Sigma USA), RPMI medium (Gibco, USA), and the human breast cancer cell line MCF7 (ATCC HTB 22) was obtained from the Primate Research Centre, IPB University.

2.2. Methods

2.2.1. Sample Preparation and Extraction

Lunasia amara's stem bark was sliced and air-dried at room temperature for one week. The dried stem bark was ground into powder. Approximately 30 grams of dried powder were extracted three times with 300 millilitres of 96% ethanol. The filtrate extracts were dried in a rotary evaporator at 50°C.

2.2.2. Cell Culture and Viability Test

MCF7 cells were thawed after cryopreservation, cultured in RPMI1640 media containing 10% FBS and 1% penicillin/streptomycin, and incubated at 37°C with 5% CO₂. The cells were sub cultured after they had reached confluence in the flask. After removing the

cell media, the flask was rinsed with 5 mL of sterile 1X PBS. A 5 mL solution of 0.125% trypsin was added to the flask and incubated at 37°C with 95% O₂ and 5% CO₂ for 5 minutes. The medium was added to the flask as much as 1 mL to stop the trypsin reaction. The dissociated cells were transferred to a 15 mL centrifuge tube and centrifuged at 1,500 rpm for 5 minutes. The supernatant was discarded, and 5 mL of media was used to resuspend the cells until homogenous. The cells were then counted with a hemacytometer.

2.2.3. Cytotoxic Assay

The cytotoxic test was carried out on MCF7 breast cancer cell lines using the MTT method. Cells were grown in DMEM (Dulbecco's Modified Eagle's Medium) growth medium. The cultured cells were then added with ethanol extracts with concentrations of 25, 50, 100, 200, 400, and 800 ppm and incubated for 48 hours at 37°C. Then, 10 µL/well of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, USA) MTT was added and incubated for 4 hours until purple formazan was formed. Formazan was added with HCl in isopropanol 0.1 N as much as 100 µL/well, then incubated for 10 minutes. The absorbance was read using a microplate reader at a wavelength of 595 nm. The absorption test results were converted into a percentage of viability and percent inhibition value.

2.2.4. Gene Expression (Bax and Bcl2) Analysis by RT-qPCR

MCF7 cells were cultivated at a density of up to 10⁴ cells per well in a 12-well tissue culture plate for 24 hours at 37°C with 95% O₂ and 5% CO₂. After the cells had attained approximately 50% confluence, 125 ppm of simplicia extract was applied. The cells were cultured for 48 hours. The live cells were extracted with 0.125% trypsin stained with 0.1% trypan blue, and the number of live cells was counted using a hemacytometer. Total mRNA expression of Bcl2 and Bax genes was extracted from the live cell population using an RNA extraction kit, Quick-RNA™ Miniprep Kit (Zymo Research). Reverse transcription and master mix of RNA was performed using the SensiFASrM cDNA Synthesis Kit (Bioline) and SensiFASrM SYBR® No-ROX Kit (Bioline) according to the manufacturer's instructions. Real-time PCR was generated on a CFX Opus 96 Real-Time PCR System. GAPDH was used as a control. Relative expressions were calculated using the comparative threshold cycle (2-ΔΔCt) method. The primers were as follows: GAPDH forward primer: CGGATITGGTCGTATTGG, reverse primer:

TCAAGGTGTGAGGACTGG; BAX forward primer: CCCGAGAGGTCTTTITCCGAG, reverse primer: CCAGCCCATGATGGTTCTGAT; BCL2 forward primer: GCTCTAAAATCCATCCAG, reverse primer: CCTCTCCATCATCAACTI.

2.2.5. Molecular Docking Simulation

Molecular docking studies were performed using ligands from the previous studies (data not yet published). Meanwhile, lapatinib was used as the reference ligand. The 3D structures will be downloaded from the PubChem database. Receptors used are apoptotic signaling pathway proteins such as Bcl2 and Bax. The proteins were downloaded from the protein data bank. Molecular docking was performed using PyRx software, and the results were visualized using BIOVIA Discovery Studio Visualizer.

3. Results

3.1. Identification and Extraction Yield

Based on the plant identification letter issued by the National Research and Innovation Agency (BRIN), the plant samples used in this study were identified as *Lunasia amara* Blanco and classified in the Rutaceae family. The extraction process, performed using the maceration method with 96% ethanol as the solvent, yielded 7.68% from the plant stem bark samples.

3.2. Cytotoxic Assay

The results of the cytotoxic test of ethanol extract of *Lunasia amara* with various concentrations against MCF7 cancer cells are shown in Figure 1. The higher the concentration of the extract, the significantly decreased cell viability. Changes in cell morphology are seen more clearly in the treatment with extracts with concentrations of 100 ppm to 400 ppm compared to control cell morphology. The inhibition concentration (IC₅₀) of ethanol extract of *L. amara* was obtained as 125 ppm.

3.3. Gene Expression (Bax and Bcl2)

The target genes in this study are Bax and Bcl2 genes from MCF7 cells treated with ethanol extract at a concentration of 125 ppm. The control sample refers to cells that were not exposed to the extract, and the reference gene used is GAPDH (glyceraldehyde 3-phosphate dehydrogenase). GAPDH was chosen as the reference gene because it is stably and widely expressed in various tissues and cells, making it a reliable housekeeping gene. Based on Figure 2, Bax mRNA expression in MCF7

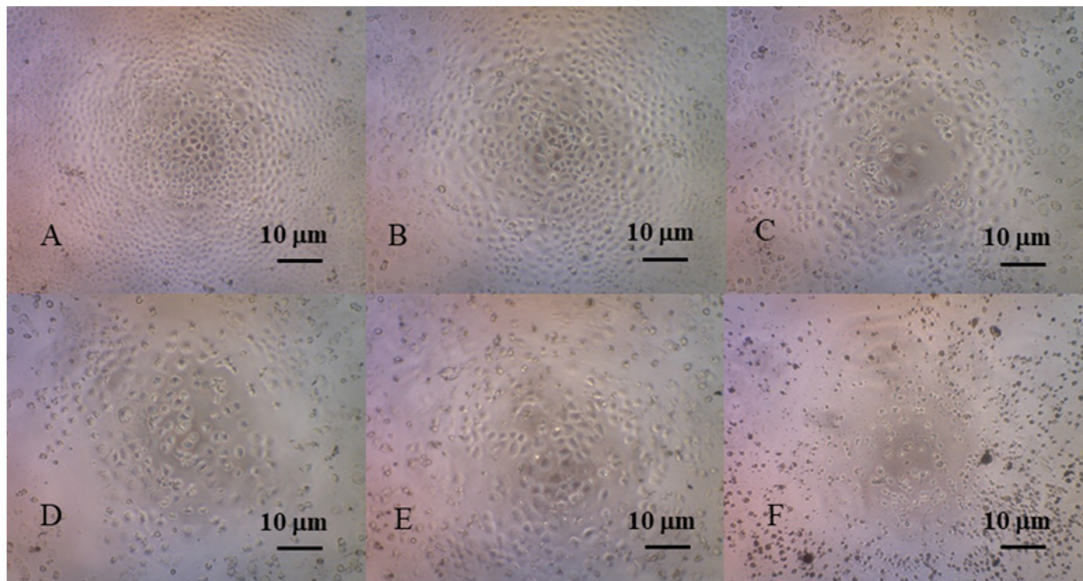


Figure 1. MCF7 breast cancer cells lines. (A) MCF7 control cells, (B, C, D, E, and F) are MCF7 cell treated with 25, 50, 100, 200, and 400 ppm ethanol extract of *Lunasia amara*

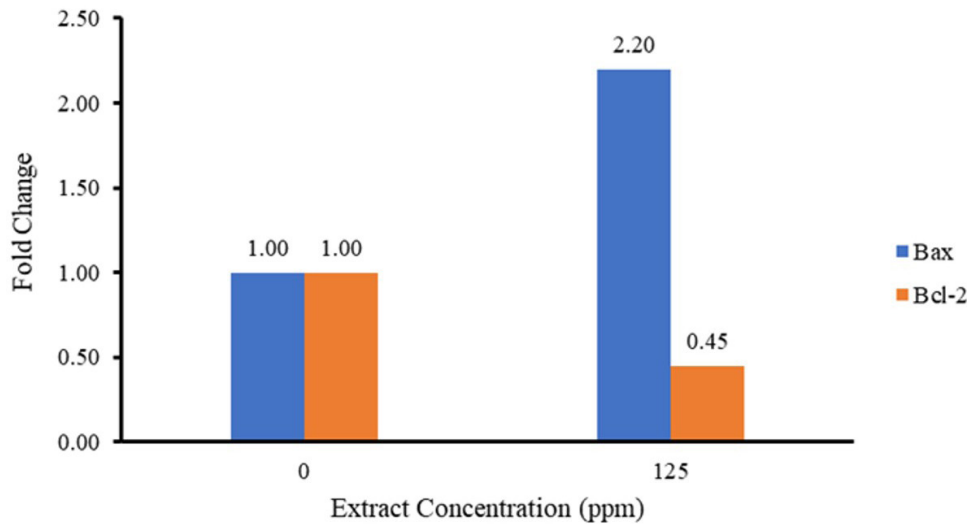


Figure 2. The effect of *Lunasia amara* stem bark ethanol extract on Bax and Bcl2 expression was assessed using RT-qPCR normalized to the GAPDH gene as a reference gene

cells increased, while Bcl2 mRNA expression decreased, showing a statistically significant difference compared to the untreated control.

3.4. Molecular Docking

Molecular docking is a computational method *in silico* tests that simulates the binding and stability of a ligand to a target protein. Table 1 presents the binding affinities of bioactive compounds from *Lunasia amara* stem bark extracts with the apoptosis-related proteins Bcl2 and Bax. Among these, Lunamarine exhibited the strongest

Table 1. The binding energy of bioactive compounds in the extracts of *L. amara* stem bark with gene marker apoptosis receptors, Bcl2 and Bax

Ligand	Binding energy (kcal/mol)	
	Bcl2	Bax
Lapatinib	-9.6	-9.0
Lunine	-8.1	-7.7
Hydroxylunine	-7.8	-8.1
Lunamarine	-8.3	-8.2
Piperlongumine	-6.8	-6.8
Lunacrine	-7.4	-7.1
Pteleprenine	-7.5	-6.6

binding to both Bcl2 (−8.3 kcal/mol) and Bax (−8.2 kcal/mol), indicating a high potential to modulate apoptosis by targeting both anti-apoptotic and pro-apoptotic pathways. Lunine and Hydroxylunine also showed favorable affinities, particularly with Bcl2, suggesting their role in inhibiting anti-apoptotic activity. While Piperlongumine displayed the weakest interactions, other compounds like Lunacrine and Pteleprenine demonstrated moderate binding energies. Compared to Lapatinib, a known anticancer agent with the highest affinities (−9.6 and −9.0 kcal/mol), the natural compounds showed slightly lower but still significant interactions, highlighting their potential as natural apoptosis modulators in cancer therapy. As illustrated in Figure 3, the molecular docking analysis revealed 3D binding interactions of lunine and lunamarine with the apoptosis-related target proteins Bax and Bcl-2.

4. Discussion

Breast cancer remains the most commonly diagnosed cancer in women and a leading cause of cancer-related mortality worldwide (Hadisaputri *et al.* 2024; Pandey *et al.* 2024; Lazovic *et al.* 2025). Its treatment is often complicated by the limited effectiveness of precision-targeted therapies. Despite the widespread use of chemotherapy, surgery, and radiotherapy, each with distinct benefits and limitations, there remains an ongoing need for more effective and less toxic alternatives (Hsu *et al.* 2024; Zasheva *et al.* 2024). One promising strategy is the use of medicinal plant-derived natural products, which serve as rich sources of bioactive metabolites with potential anticancer properties (Septaningsih *et al.* 2024).

Lunasia amara, a plant known for its quinoline alkaloid content, has attracted attention for its pharmacological

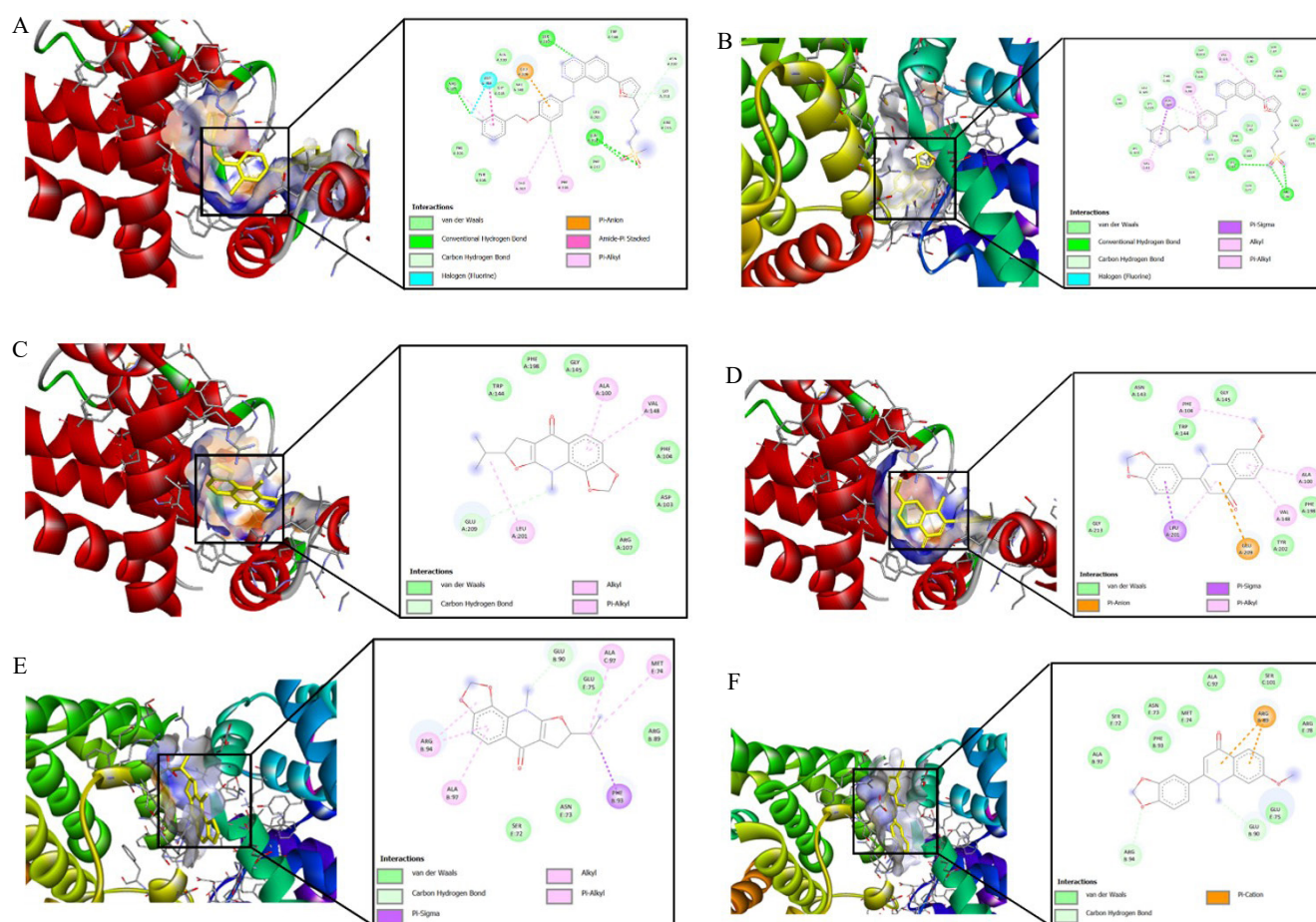


Figure 3. Protein–ligand interaction: (A) Bcl-2 and Lapatinib, (B) Bax and Lapatinib, (C) Bcl-2 and Lunine, (D) Bcl-2 and Lunamarine, (E) Bax and Lunine, and (F) Bax and Lunamarine

properties, including cytotoxic and antitumor activities (Macabeo and Aguinaldo 2008). In the present study, ethanol extracts of *L. amara* stem bark were tested against MCF7 breast cancer cells, demonstrating a concentration-dependent decrease in cell viability, with an IC_{50} value of 125 ppm. According to the U.S. National Cancer Institute (NCI) guidelines, crude extracts with IC_{50} values below 200 ppm are considered to have promising cytotoxic potential. Morphological changes observed in treated MCF7 cells, including membrane blebbing, shrinkage, and reduced cell density, are characteristic of apoptosis, indicating that the extract may induce cell death through apoptotic mechanisms.

The MCF7 cell line lacks caspase-3, a key effector in the apoptotic pathway, and thus relies on caspase-7 or caspase-independent mechanisms for apoptosis. In this context, the Bcl2 gene family plays a pivotal role in regulating intrinsic apoptosis. This family includes both anti-apoptotic members (e.g., BCL2, BCL-XL, BCL-W) and pro-apoptotic members (e.g., BAX, BAK, BID, BIM), which together control mitochondrial outer membrane permeabilization (MOMP), a critical step in the initiation of programmed cell death (Warren *et al.* 2019; Shenoy and Abdul Salam 2024).

Gene expression analysis following treatment with *L. amara* extract at its IC_{50} concentration revealed significant downregulation of the anti-apoptotic gene Bcl2 and upregulation of the pro-apoptotic gene Bax, resulting in an increased Bax/Bcl2 ratio from 1.0 to 4.8. This shift is a well-recognized trigger for MOMP, leading to cytochrome c release, apoptosome formation, and activation of downstream caspases (Zhang *et al.* 2024; Angel-Lerma *et al.* 2025; Wurlina *et al.* 2025). The observed modulation of gene expression supports the hypothesis that *L. amara* extract induces apoptosis via the intrinsic pathway. Secondary metabolites such as flavonoids and alkaloids are known to influence apoptotic pathways by modulating Bax and Bcl2 expression (Sinnarkar *et al.* 2024). Previous studies have reported that *L. amara* contains bioactive quinoline alkaloids, including lunine, lunamarine, and hydroxylunine, which have shown pharmacological activity in various biological systems (Saputra *et al.* 2024). These compounds are likely contributors to the observed pro-apoptotic effects.

To further explore their potential, molecular docking studies were conducted to evaluate the interaction of these compounds with Bcl2 and Bax proteins. Docking results revealed that lunamarine and lunine exhibited strong binding affinities with both targets, with values approaching that of lapatinib, a known anticancer drug.

These interactions were particularly notable at key residues within the Bcl2 binding site (e.g., GLU209 and LEU201), which are critical for ligand stabilization. Compounds such as hydroxylunine, lunacrine, and pteleprenine showed lower binding affinities, consistent with their limited interaction with these high-affinity residues. For Bax, lunamarine and lunine again demonstrated favorable interactions, notably with residues GLU90, ARG94, and PHE93—sites also involved in lapatinib binding. Although other compounds like piperlongumine and pteleprenine shared some of these interactions, their weaker binding affinities suggest less effective receptor engagement.

Taken together, these findings highlight lunamarine and lunine as the most promising candidates among the tested compounds. Their dual interaction with both anti-apoptotic and pro-apoptotic proteins suggests a balanced mechanism of action that could suppress survival signals while promoting apoptotic pathways. Furthermore, LC-HRMS profiling confirmed that *L. amara* stem bark is rich in quinolines and other bioactive compounds, potentially contributing to its anticancer activity (Saputra *et al.* 2024). These results support the continued investigation of *L. amara* and its constituent compounds as potential leads for the development of plant-based anticancer therapeutics.

In conclusion, *L. amara* ethanol extract significantly modulated the expression of apoptosis-related genes by downregulating Bcl2 and upregulating Bax, leading to an increased Bax/Bcl2 ratio indicative of activation of the intrinsic apoptotic pathway. These findings provide mechanistic insight into the anticancer potential of quinoline alkaloids present in *L. amara* and support its potential as a natural source of pro-apoptotic agents for breast cancer therapy. Further research is needed to isolate and characterize the active compounds responsible for these effects and to validate their therapeutic potential through in vivo and clinical studies.

Acknowledgements

The authors gratefully acknowledge the financial support provided by Universitas Nusa Cendana through the DIPA Universitas Nusa Cendana funding scheme.

References

- Adriani, Noorhamdani, Winarsih, S., Ardyati, T., 2019. Molecular docking study from lunacridine, scopoletin and skimmianine as antidiabetes through α -glucosidase inhibitor. *J. Phys.: Conf. Ser.* 1374, 012026. <https://doi.org/10.1088/1742-6596/1374/1/012026>

- Al-Mahayri, Z.N., Patrinos, G.P., Ali, B.R., 2020. Toxicity and pharmacogenomic biomarkers in breast cancer chemotherapy. *Front. Pharmacol.* 11, 445. <https://doi.org/10.3389/fphar.2020.00445>
- Angel-Lerma, L.E., Carrillo-Campos, J., Siañez-Estrada, L.I., Siqueiros-Cendón, T.S., León-Flores, D.B., Espinoza-Sánchez, E.A., Arévalo-Gallegos, S., Iglesias-Figueroa, B.F., Rascón-Cruz, Q., 2025. Molecular docking of lactoferrin with apoptosis-related proteins insights into its anticancer mechanism. *Int. J. Mol. Sci.* 26, 2023. <https://doi.org/10.3390/ijms26052023>
- Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., the International Natural Product Sciences Taskforce, Supuran, C.T., 2021. Natural products in drug discovery: advances and opportunities. *Nat. Rev. Drug. Discov.* 20, 200–216. <https://doi.org/10.1038/s41573-020-00114-z>
- Bunga, E.V., Farid, N., Hasriadi, H., Ilyas, I.L., 2024. Investigation of the role of *Lunasia amara* Blanco in the treatment of malaria through network pharmacology analysis. *J. Herb. Med.* 44, 100857. <https://doi.org/10.1016/j.hermed.2024.100857>
- Chen, Z., Li, Z., Li, H., Jiang, Y., 2019. Metabolomics: a promising diagnostic and therapeutic implement for breast cancer. *Oncotargets Ther.* 12, 6797–6811. <https://doi.org/10.2147/OTT.S215628>
- Dar, A.M., Mir, S., 2017. Molecular docking: approaches, types, applications and basic challenges. *J. Anal. Bioanal. Tech.* 8, 1–3. <https://doi.org/10.4172/2155-9872.1000356>
- Dhani Putri, M., Bakar Tawali, A., 2024. Bioactive compounds of sanrego (*Lunasia amara* Blanco.) extracted using different methods: a review. *BIO Web Conf.* 96, 01012. <https://doi.org/10.1051/bioconf/20249601012>
- Draguet, A., Tagliatti, V., Colet, J.M., 2021. Targeting metabolic reprogramming to improve breast cancer treatment: an *in vitro* evaluation of selected metabolic inhibitors using a metabolomic approach. *Metabolites.* 11, 556. <https://doi.org/10.3390/metabol11080556>
- Gambaro, K., Groleau, M., McNamara, S., Awan, A., Salem, M., Abdelsalam, M., St-Hilaire, E., Vincent, F., Carrier, J., MacKay, H., Provencher, L., Boudreau, D., Hamilou, Z., Saad, F., Ferrario, C., Batist, G., Marques, M., 2023. Third-line treatment patterns in HER2-positive metastatic breast cancer: a retrospective analysis of real-world data in Canada. *J. Pharm. Sci.* 26, 12078. <https://doi.org/10.3389/jpps.2023.12078>
- Hadisaputri, Y.E., Soekamto, N., Pudjiastuti, P., Aristokrat, A., Bahrin, B., Abdullah, F., Okino, T., 2024. Antimicrobial and cytotoxic effect of bornetella nitida green algae isolate on MCF-7 breast cancer. *Breast Cancer: Targets Ther.* 16, 555–565. <https://doi.org/10.2147/BCTT.S469036>
- Holliday, D.L., Speirs, V., 2011. Choosing the right cell line for breast cancer research. *Breast Cancer Res.* 13, 215. <https://doi.org/10.1186/bcr2889>
- Hsu, C.Y., Rajabi, S., Hamzeloo-Moghadam, M., Kumar, A., Maresca, M., Ghildiyal, P., 2024. Sesquiterpene lactones as emerging biomolecules to cease cancer by targeting apoptosis. *Front. Pharmacol.* 15, 1371002. <https://doi.org/10.3389/fphar.2024.1371002>
- Indriwati, I., Mariya, S., Andrianto, V., Iskandriati, D., 2022. Apoptosis of human breast cancer cells (MCF-7) induced by *Psidium guajava* simplisia extract. *Ind. J. Prim.* 1, 33–41. <https://doi.org/10.29244/primatology.1.01.33-41>
- Jeibouei, S., Akbari, M.E., Kalbasi, A., Aref, A.R., Ajoudanian, M., Rezvani, A., Zali, H., 2019. Personalized medicine in breast cancer: pharmacogenomics approaches. *PGxPM.* 12, 59–73. <https://doi.org/10.2147/PGPM.S167886>
- Kashyap, D., Garg, V.K., Goel, N., 2021. *Intrinsic and Extrinsic Pathways of Apoptosis: Role in Cancer Development and Prognosis*, fourth ed. Academic Press, Cambridge. <https://doi.org/10.1016/bs.apcsb.2021.01.003>
- Lazovic, A., Markovic, B.S., Corovic, I., Markovic, T., Andjelkovic, M., Stojanovic, B., Jovanovic, I., Mitrovic, M., 2025. Unlocking the molecular mechanisms of anticancer and immunomodulatory potentials of cariprazine in triple negative breast cancer. *Biomed. Pharmacother.* 184, 117931. <https://doi.org/10.1016/j.biopha.2025.117931>
- Liu, Z., Shen, Y., Ni, L., Jiang, X., Tang, Z., Xie, J., Zheng, Z., 2024. Study on the mechanism of yadanza oil in treating lung cancer based on network pharmacology and molecular docking technology. *ACS Omega.* 9, 19117–19126. <https://doi.org/10.1021/acsomega.3c10105>
- Macabeo, A.P.G., Aguinaldo, A.M., 2008. PHCOG REV.: plant review chemical and phytomedicinal investigations in *Lunasia amara*. *Phcog. Rev.* 2, 10, 317–325.
- O'Brien, M.A., Kirby, R., 2008. Apoptosis: a review of pro-apoptotic and anti-apoptotic pathways and dysregulation in disease. *J. Vet. Emerg. Crit. Care.* 18, 572–585. <https://doi.org/10.1111/j.1476-4431.2008.00363.x>
- Pakrashi, S., Mandal, P.K., Nanda Goswami, J., Dey, S.K., Maiti Choudhury, S., Bhattacharya, B., Emmerling, F., Alasmay, F.A., Dolai, M., 2022. Bioinformatics and network pharmacology of the first crystal structured clerodin: anticancer and antioxidant potential against human breast carcinoma cell. *ACS Omega.* 7, 48572–48582. <https://doi.org/10.1021/acsomega.2c07173>
- Pandey, P., Ramniwas, S., Verma, M., Sharma, N., Upadhye, V.J., Khan, F., Shah, M.A., 2024. A study to investigate the anticancer potential of carvacrol via targeting Notch signaling in breast cancer. *Open Chem.* 22, 1–9. <https://doi.org/10.1515/chem-2024-0008>
- Permana, S., Nurzaidah, L., Widodo, E., Anita, K.W., Nugraheni, R.W., Kawamoto, Y., Endrawati, H., Endharti, A.T., 2024. Anticancer activity of *Hedyotis corymbosa* nanoliposomes targeting estrogen receptor-alpha in breast cancer cells: *in silico* and *in vitro* studies. *J. Pharm. Pharmacogn. Res.* 12, 303–322. https://doi.org/10.56499/jppres23.1783_12.2.303
- Pistritto, G., Trisciuglio, D., Ceci, C., Garufi, A., D'Orazi, G., 2016. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. *Aging.* 8, 603–619. <https://doi.org/10.18632/aging.100934>
- Polyak, K., 2011. Heterogeneity in breast cancer. *J. Clin. Invest.* 121, 3786–3788. <https://doi.org/10.1172/JCI60534>
- Prescott, T.A.K., Sadler, I.H., Kiapranis, R., Maciver, S.K., 2007. Lunacridine from *Lunasia amara* is a DNA intercalating topoisomerase II inhibitor. *J. Ethnopharmacol.* 109, 289–294. <https://doi.org/10.1016/j.jep.2006.07.036>
- Putra, A., Riwanto, I., Putra, S., Wijaya, I., 2020. Typhonium flagelliforme extract induce apoptosis in breast cancer stem cells by suppressing survivin. *J. Can. Res. Ther.* 16, 1302. https://doi.org/10.4103/jcrt.JCRT_85_20

- Saddam, Md., Paul, S.K., Habib, M.A., Fahim, Md.A., Mimi, A., Islam, S., Paul, B., Helal, M.M.U., 2024. Emerging biomarkers and potential therapeutics of the BCL-2 protein family: the apoptotic and anti-apoptotic context. *Egypt. J. Med. Hum. Genet.* 25, 12. <https://doi.org/10.1186/s43042-024-00485-7>
- Saputra, A., Wientarsih, I., Rafi, M., Sutardi, L.N., Mariya, S., 2024. LC-HRMS metabolite profiling of *Lunasia amara* stem bark and *in silico* study in breast cancer receptors. *Indones. J. Pharm.* 35, 116–125.
- Septaningsih, D.A., Suparto, I.H., Achmadi, S.S., Heryanto, R., Rafi, M., 2024. Untargeted metabolomics using UHPLC-Q-Orbitrap HRMS for identifying cytotoxic compounds on MCF-7 breast cancer cells from ANNONA MURICATA Linn leaf extracts as potential anticancer agents. *Phytochem. Anal.* 35, 1418–1427. <https://doi.org/10.1002/pca.3373>
- Shenoy, T.N., Abdul Salam, A.A., 2024. Therapeutic potential of dietary bioactive compounds against anti-apoptotic Bcl-2 proteins in breast cancer. *Crit. Rev. Food Sci. Nutr.* 65, 4915–4940. <https://doi.org/10.1080/10408398.2024.2398636>
- Sinnarkar, S., Suryawanshi, P., Dilip, A., Bhawalkar, J., Ladke, V., 2024. Galangin promotes apoptosis by upregulating the pro-apoptotic gene BAX in triple-negative breast cancer. *J. Egypt. Natl. Canc. Inst.* 36, 1–18. <https://doi.org/10.1186/s43046-024-00246-y>
- Suo, F., Wu, Y., Zhou, Q., Li, L., Wei, X., 2024. BIRC3–HSP90B1 interaction inhibits non-small cell lung cancer progression through the extracellular signal-regulated kinase pathway. *ACS Omega.* 9, 19148–19157. <https://doi.org/10.1021/acsomega.3c10274>
- Takahashi, N., Subehan, Kadota, S., Tezuka, Y., 2012. Mechanism-based CYP2D6 inactivation by acridone alkaloids of Indonesian medicinal plant *Lunasia amara*. *Phytother.* 83, 774–779. <https://doi.org/10.1016/j.fitote.2012.03.011>
- Tanaka, N., Kashiwada, Y., 2021. Phytochemical studies on traditional herbal medicines based on the ethnopharmacological information obtained by field studies. *J. Nat. Med.* 75, 762–783. <https://doi.org/10.1007/s11418-021-01545-7>
- Wang, X., Semba, T., Phi, L.T.H., Chainitikun, S., Iwase, T., Lim, B., Ueno, N.T., 2020. Targeting signaling pathways in inflammatory breast cancer. *Cancers.* 12, 2479. <https://doi.org/10.3390/cancers12092479>
- Warren, C.F.A., Wong-Brown, M.W., Bowden, N.A., 2019. BCL-2 family isoforms in apoptosis and cancer. *Cell Death Dis.* 10, 1–12. <https://doi.org/10.1038/s41419-019-1407-6>
- Wurlina, W., Meles, D., Mustofa, I., Khairullah, A., Putra, D., Suwasanti, N., Akintunde, A., Utama, S., Mulyati, S., Wasito, W., Raissa, R., Ahmad, R., Julaeha, J., Ekawasti, F., 2025. Alkaloid fraction of *Achyranthes aspera* linn triggers breast cancer apoptosis in mice (*Mus musculus*) model. *Open Vet. J.* 15, 1279–1288. <https://doi.org/10.5455/OVJ.2025.v15.i3.19>
- Youssif, B.G.M., Morcoss, M.M., Bräse, S., Abdel-Aziz, M., Abdel-Rahman, H.M., Abou El-Ella, D.A., Abdelhafez, E.S.M.N., 2024. Benzimidazole-based derivatives as apoptotic antiproliferative agents: design, synthesis, docking, and mechanistic studies. *Molecules.* 29, 446. <https://doi.org/10.3390/molecules29020446>
- Zasheva, D., Mladenov, P., Zapryanova, S., Gospodinova, Z., Georgieva, M., Alexandar, I., Velinov, V., Djilianov, D., Moyankova, D., Simova-Stoilova, L., 2024. Cytotoxic effects of plant secondary metabolites and naturally occurring bioactive peptides on breast cancer model systems: molecular mechanisms. *Molecules.* 29, 1–28. <https://doi.org/10.3390/molecules29225275>
- Zhang, Z., Hou, L., Liu, D., Luan, S., Huang, M., Zhao, L., 2024. Directly targeting BAX for drug discovery: therapeutic opportunities and challenges. *Acta Pharm. Sin. B.* 14, 2378–2401. <https://doi.org/10.1016/j.apsb.2024.02.010>
- Zubair, M.S., Subehan, S., 2010. Molecular docking of lunacridine from *Lunasia amara* to DNA: its inhibition and interaction study correlated with the cytotoxic activity on P388 murine leukemia cells. *Indones. J. Cancer Chemoprev.* 1, 108–117. <https://doi.org/10.14499/indonesianjcanchemoprev1iss2pp108-117>
- Zubair, M.S., Anam, S., Lallo, S., 2016. Cytotoxic activity and phytochemical standardization of *Lunasia amara* blanco wood extract. *Asian Pac. J. Trop. Biomed.* 6, 962–966. <https://doi.org/10.1016/j.apjtb.2016.04.014>