Trop J Nat Prod Res, July 2025; 9(7): 3021 - 3025 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Cytotoxic Activity of N-Hexane, Ethyl Acetate, and Ethanol Extract of *Hibiscus tiliaceus* against A549 Lung Cancer Cells

Nilsya F. Zebua[1], Nerdy Nerdy[1]*, Abdi W. Septama[2], Des Suryani[3], Jesika R. Sipayung[1], Nazwa N.E. Purba[1], Mhd A.T. Mardia[1], Elisabeth P. Nahulae[1], Iksen Iksen[4], Suthasinee Seephan[5]

[1]*Department of Pharmacy, Faculty of Pharmacy and Health, Universitas Tjut Nyak Dhien, Medan, Indonesia, 20123*
[2]*Research Center for Pharmaceutical Ingredient and Traditional Medicine, National Research and Innovation Agency, Banten, Indonesia, 15314*
[3]*Islamic Medical Unit, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, Medan, Indonesia, 20219*
[4]*Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, -Chulalongkorn University, Bangkok, Thailand, 10330*
[5]*Department of Pharmacology and Physiology, Institute of Natural Medicine, University of Toyama, Toyama, Japan, 9300887*

ARTICLE INFO

Article history:
Received 01 March 2025
Revised 31 March 2025
Accepted 23 May 2025
Published online 01 August 2025

ABSTRACT

Lung cancer mortality is a leading cause of cancer-related deaths worldwide, with limited treatment efficacy and high risk of side effects posing significant challenges in its management. Exploration of alternative therapies with better efficacy and lower toxicity is crucial. This study investigates various parts of the *Hibiscus tiliaceus* plant, including the roots, stems, leaves, and flowers, using different solvents (n-hexane, ethyl acetate, and ethanol) for extraction to assess their cytotoxic potential. Human lung cancer A549 cells were used as the model and tested using the MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) to measure cell viability based on the metabolic activity of these extracts. The concentrations ranged from 31.25 µg/mL to 1000 µg/mL, with doxorubicin as the positive control. Absorbance was measured at a wavelength of 595 nm using a microplate reader. From these extracts, data were obtained to calculate the half-maximal inhibitory concentration (IC_{50}) and cell viability. The ethanol extract demonstrated the most potent cytotoxic effect, with an IC_{50} value of 18.7634 µg/mL, categorized as highly toxic according to cytotoxicity classification standards. These findings suggest that ethanol extracts from *Hibiscus tiliaceus*, particularly from specific plant parts, exhibit promising anticancer activity and have the potential for further development as a lung cancer agent with minimal side effects.

Keywords: Cytotoxic, *Hibiscus tiliaceus*, Solvent Polarity, Lung Cancer, Half Maximal Inhibitory Concentration .

Introduction

Cancer is characterized by uncontrolled cell growth in the body, making it difficult to cure and often leading to death. Cancer can affect the blood and lymphatic system and spread throughout the body.[1] The mortality rate of lung cancer is between 75% and 85% of all cancer cases worldwide. This makes lung cancer the deadliest type of cancer in the world.[2] According to data from the Global Cancer report calculated by the International Agency for Research on Cancer, in 2020, lung cancer accounted for 18% of the total cancer-related deaths worldwide, which reported the morbidity of lung cancer at 14.1% of all cancer cases in Indonesia. This case is suspected to occur more frequently in men, with a rate of 19.4% per 100,000 people. In terms of oncology-related deaths, lung cancer still has the highest mortality rate, at 13.2%.[3] The lack of effective treatments is one of the reasons for the increasing number of cancer-related deaths each year. Treatments commonly given for lung cancer cases, such as chemotherapy, radiotherapy, and molecular target therapy, often have limited effectiveness due to drug resistance and severe side effects.[4]

*Corresponding author. Email: nerdy@utnd.ac.id,
Tel: +628126300888

Citation: Zebua NF, Nerdy N*, Septama AW, Suryani D, Sipayung JR, Purba NNE, Mardia MAT, Iksen I, Seephan S. Cytotoxic Activity of Hibiscus tiliaceus N-Hexane, Ethyl Acetate, and Ethanol Extracts against A549 Lung Cancer Cells. Trop J Nat Prod Res. 2025; 9(7): 3021 – 3025 https://doi.org/10.26538/tjnpr/v9i7.9

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Therefore, searching for alternative treatments with low toxicity and high effectiveness is crucial in cancer research. Herbal plants have been a part of Indonesia's cultural heritage for many years. Local communities have practiced the traditional use of herbal plants for generations,[5] and the potential of active compounds found in various plants as anticancer agents. Many studies have explored this potential.[6] Bioactive compounds in herbal plants are considered a new alternative for cancer treatment due to their simple preparation and minimal side effects. The biodiversity of herbal plants offers potential for developing complementary therapies for cancer treatment.[7] *Hibiscus tiliaceus* is a promising plant for potential anticancer agents due to its active compounds such as flavonoids, sterols, and tannins.[8] The roots, stems, leaves, and flowers of *Hibiscus tiliaceus* have potential as medicine due to their active compounds.[9] The roots of *Hibiscus tiliaceus* contain tannins, saponins, and flavonoids. The leaves of *Hibiscus tiliaceus* contain polyphenols, saponins, and flavonoids. Cytotoxic effects have been reported as a result of flavonoid and phenolic compounds against lung and breast cancer.[10] The active compounds are predicted to inhibit cell proliferation, disrupt the cell cycle in cancer cells, and induce apoptosis.[11] This study aimed to investigate the cytotoxic activity of different parts of the *Hibiscus tiliaceus* plant against A549 Lung Cancer Cells.

Materials and Methods

Materials

Analytical grade solvents, n-hexane, ethyl acetate, and ethanol, were used to extract compounds from different parts (roots, stems, leaves,

and flowers) of *Hibiscus tiliaceus*. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay assessed their cytotoxic activity.

Plant collection and preparation
The sample (*Hibiscus tiliaceus*) was collected in August 2024 from Hilisimaetano Balaekha Village, Lahusa District, Nias Selatan Regency (0.760615, 97.848742), 22874, Sumatera Utara, Indonesia. The plant was identified by Prof. Etti Sartina Siregar at Herbarium Medanense, Universitas Sumatera Utara, Medan City, 20155, Sumatera Utara, Indonesia.

Extraction
The dried plant materials were extracted by maceration using the method of Nerdy and Ritarwan (2019),[12] with slight modification. The roots, stems, leaves, and flowers of *Hibiscus tiliaceus* were collected freshly, washed using running water, and drained at room temperature. The samples were then dried at 60°C and ground using a blender. Three solvents were used to successively extract the plant material with n-hexane, ethyl acetate, and ethanol. A total of 50 g of each sample was soaked in 375 mL of each solvent separately. This process lasted for 5 days, with stirring performed every 6 hours. The mixture was then filtered to obtain Filtrate 1 and Residue 1. Then, an additional extraction was conducted by soaking Residue 1 separately in 125 mL of each solvent. This second extraction lasted 2 days, stirring every 6 hours. The mixture was again filtered to obtain Filtrate 2 and Residue 2. The filtrates obtained were combined and evaporated to dryness using a rotary evaporator, resulting in the n-hexane, ethyl acetate, and ethanol extracts of *Hibiscus tiliaceus* [12,13]

Cytotoxic Activity Test
The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) solution was used for the cytotoxicity assay. A 96-well microplate was used to culture A549 lung cancer cells. The A549 lung cancer cells were incubated with 5% CO_2 at 37°C. Doxorubicin was used as the positive control. The extract was dissolved in a microtube using dimethyl sulfoxide (DMSO) as the solvent, homogenized, vortexed, and then serially diluted twofold to obtain a series of concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, and 31.25 µg/mL. Doxorubicin was also dissolved in a microtube using DMSO, homogenized, vortexed, and serially diluted twofold to obtain a series of concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, and 3.125 µg/mL. MTT solution was added to each well after 24 hours of treatment. The plate was then incubated for 4 hours. Afterwards, sodium dodecyl sulfate (SDS) solution was added, and the plate was stored in the dark overnight. Absorbance was measured using the Enzyme-Linked Immunosorbent Assay (ELISA) Reader BioTek 800 TS Absorbance Reader (Agilent, USA) at a wavelength of 595 nm. Each test was performed in triplicate. The half maximal inhibitory concentrati22on (IC_{50}) value represents the percentage of cell growth inhibition. The IC_{50} value indicates the ability of the compound to inhibit 50% of cell growth.[14,15]

Ethics approval
Ethical approval for this study was granted by the Health Research Ethics Commission of Universitas Prima Indonesia, located in Sei Putih Barat, Medan Petisah, Medan, 20118, North Sumatra, Indonesia, under approval number 074 / KEPK / UNPRI / XII / 2024, dated December 23 2024.

Results and Discussion

The data shows that the anticancer activity of *Hibiscus tiliaceus* extract depends on the dose used for the A549 cells, the solvent used, and the part of the plant used (Figure 1). Different viability level of A549 cells with each concentration of 1000 µg/mL was observed with different extract treatments. The treatment with n-hexane solvent extract showed the highest cell inhibition percentage (%) value of 92.39% from the root, which was followed by 84.80% from the flower, 76.65% from the stem, and 73.10% from the leaf. The treatment with ethyl acetate solvent extracts showed the highest cell inhibition percentage (%) value of 77.37% for the flowers, followed by the stem with 76.93%, the leaf with 51.09%, and the root with 43.13%. The highest cell inhibition percentage (%) with ethanol extract treatment was shown with the flowers (79.26%), followed by the stem (41.79%), the root (41.24%), and the leaf with 40.03%. In A549 cells treated with Doxorubicin at a concentration of 100 (ng/mL), the cell inhibition percentage was 57.25%.
The results obtained from the determination of percentage inhibition of the plant extracts against the cell line and control were expressed in terms of the IC_{50} value, which is affected by the type of extraction solvents and plant parts used are shown in Table 1. For example, the roots showed an IC_{50} value of 18.7634 µg/mL; the stem part was 63.9342 µg/mL; the leaf part was 4649.5909 µg/mL, and the flower part was 31.8742 µg/mL. The ethyl acetate extract showed that the IC_{50} value of the root part was 2086.1765 µg/mL; the stem part was 329.5479 µg/mL; the leaf part was 898.2886 µg/mL; and the flowers part was 57.1627 µg/mL. The ethanol extract of the leaves had an IC_{50} value of 4115.7431 µg/mL, the stem part (3212.3498 µg/mL), the root part (3739.5275 µg/mL), and the flower (61.7258 µg/mL). Doxorubicin showed a very low IC_{50} value at 37.3507 ng/mL (0.0373 µg/mL). The MTT assay uses a quantitative colourimetric cell method to assess the cytotoxicity of *Hibiscus tiliaceus* extracts on A549. The inhibition of A549 lung cancer cell proliferation was observed in this method. The MTT method is widely favoured because it is safe, inexpensive, easy, and suitable for use on cells.[16] Cytotoxic compounds during drug development can be screened using this method. During preclinical studies, this method is used to observe cytotoxic effects. A salt change occurs in this method, specifically the conversion of tetrazolium salts into formazan crystals. This change occurs in living cells, particularly in active mitochondria. [17] The results of the cytotoxicity test from each part of the *Hibiscus tiliaceus* plant with 3 types of solvents are shown in Figure 1. Increasing the extract concentration affected the percentage of inhibition of A549 lung cancer cell proliferation. The higher the concentration of the extract, the fewer the number of A549 lung cancer cells, and the greater the damage to the A549 lung cancer cell structure.[18] Hafiza *et al*., 2023, stated that the concentration of the extract used can influence the cytotoxic effects; thus, determining the appropriate extract concentration requires careful consideration.[13] A sample can inhibit 13% of Cell growth by 50% at a specific concentration. Cell growth inhibition can be calculated using the Half Maximal Inhibitory Concentration (IC_{50}) value.[18] The IC_{50} value of 57.13 µg/mL is categorized as moderate for the ethyl acetate extract of *Hibiscus* tiliaceus flowers.

Table 1: IC_{50} values of N-Hexane, Ethyl Acetate, and Ethanol extracts of *Hibiscus tiliaceus* with various concentrations against A549 lung cancer cells by MTT assay method

Solvent	Parts	IC_{50} (µg/mL)
N-hexane	Leaves	4640.5909
	Stem	63.9342
	Roots	18.7634
	Flowers	31.8742
Ethyl Acetate	Leaves	897.2886
	Stem	329.5479
	Roots	2086.1765
	Flowers	57.1626
Ethanol	Leaves	4116.7431
	Stem	3212.3498
	Roots	3739.5275
	Flowers	61.7258
Positive control	Doxorubicin	0.0373

.

The IC_{50} value of 61.70 µg/mL is categorized as moderate for the ethanol extract of *Hibiscus tiliaceus* flowers. The category is considered highly active if IC_{50} is less than or equal to 20 µg/mL, and moderately active if IC_{50} is greater than or equal to 21 µg/mL and less than or equal to 200 µg/mL. It is considered weak if IC_{50} is greater than or equal to 201 µg/mL and less than 500 µg/mL, and inactive if IC_{50} is greater than 500 µg/mL.[19]The polarities of extracting solvents affect the spectrum of phytochemicals that can be extracted.[20] Ethanol, ethyl acetate, and n-hexane are three commonly used solvents, with each solvent having a different ability to extract different compounds. N-hexane tends to be effective for extracting non-polar compounds such as steroids and terpenoids, while ethyl acetate and ethanol are more effective for semi-polar to polar compounds such as flavonoids and phenolics. The solvent used must be able to dissolve the substance to be extracted, have a low boiling point, and be non-toxic.[21] Terpenoids are one of the compounds with the potential as anticancers, while most phenolic compounds are found in polar solvents. Still, depending on the structure of phenolic compounds, in *Turbinaria conoides*, the total phenol content of the n-hexane extract is higher than that of the methanol extract.[22] Based on qualitative analysis with the FTIR instrument, the spectrum of *Hibiscus tiliaceus* shows the presence of several functional groups, namely alcohol, phenol, alkane, alkyne, alkyl halide, aldehyde, carboxylic acid, amine, aromatic and nitrogen compounds.[23] Zebua *et al*, 2024 reported that *Hibiscus tiliaceus* N-hexane extract showed positive for flavonoid and phenolic compounds, with ethyl acetate solvent positively contains alkaloids, tannins, and phenolic compounds, and with ethanol solvent compounds saponins, tannins, flavonoids, and phenolic compounds.[24]

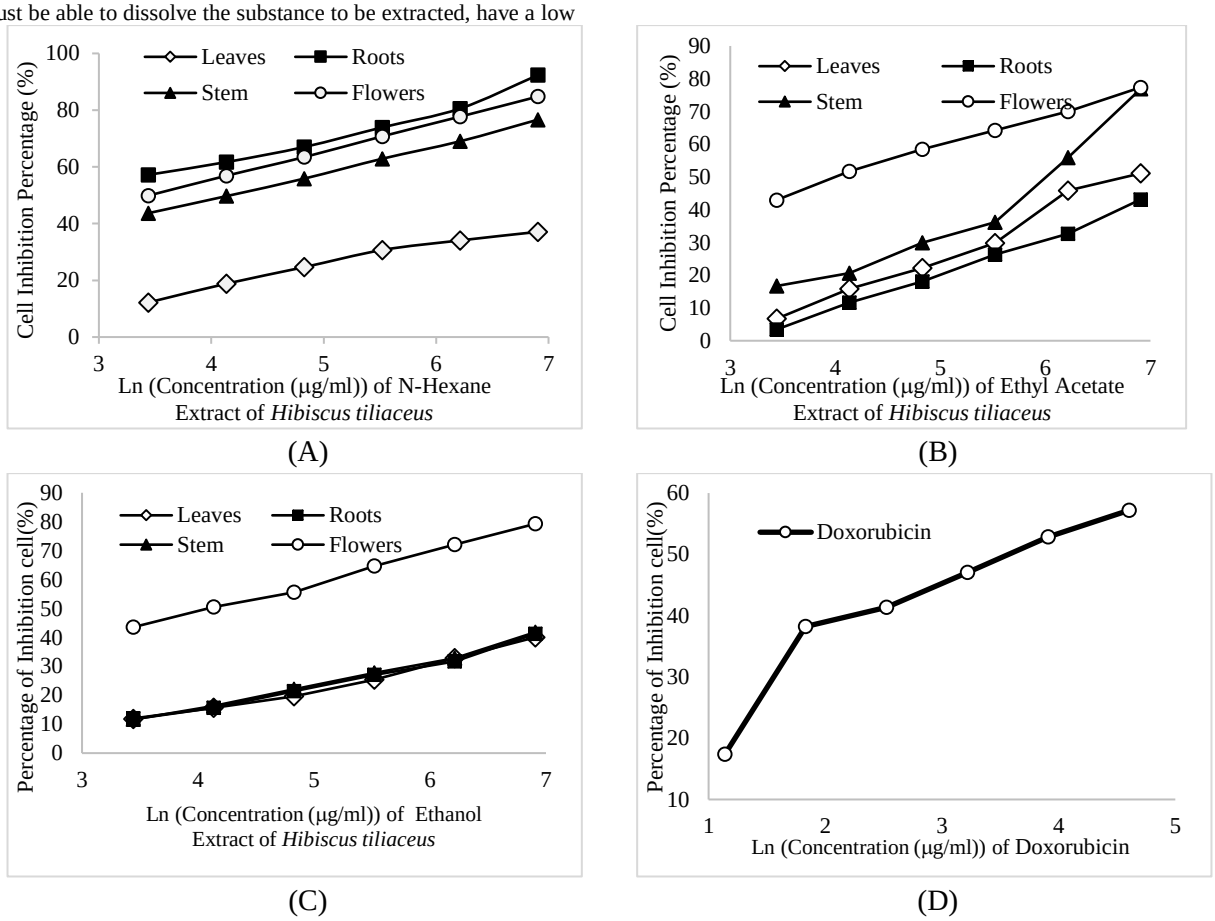

Figure 1: The Cell Inhibition Percentage of A549 cells (A) after being treated with *Hibiscus tiliaceus* ethyl acetate extract (B) after being treated with *Hibiscus tiliaceus* ethanol extract (C) after being treated with *Hibiscus tiliaceus* n-hexane extract, (D) after being treated with Doxorubicin

Hibiscus tiliaceus also contains hibiscusamide, N-transferuloyltiramine, and N-cisferuloyltiramine, which have anti-cancer effects against colon cancer.[25] Active compounds are also contained in the leaves, stems, flowers, and roots of *the Hibiscus tiliaceus* plant, which are extracted with water, including tannins, phlobatannins, coumarins, terpenoids, saponins, flavonoids, alkaloids, quinones, cardiac glycosides, anthraquinone glycosides, steroids, and phytosterols [25]

Non-polar active compounds can be extracted using n-hexane, which can serve as an anticancer agent, such as terpenoids.[26] Active compounds, such as tannins, saponins, flavonoids, and phenolics, have also shown anticancer activity. These compounds can be extracted using n-hexane as a solvent. These compounds have mechanisms involved in the induction of apoptosis, cell cycle inhibition, or cancer cell proliferation.[27] Both polar and non-polar active compounds can be extracted simultaneously using ethanol, making it a universal solvent.[28] Therefore, ethanol solvent is commonly used in research to extract active compounds from plants.[29] Research on *Phyllanthus emblica* (Malacca fruit) showed that ethanol extracts have high antioxidant activity due to their ability to solubilize large amounts of phenolics.[30] However, ethyl acetate solvents often extract certain semi-polar compounds more efficiently; for example, semi-polar flavonoids such as quercetin and alkaloids with high pharmacological potential are more soluble in ethyl acetate than ethanol solvents.[27] The IC_{50} value obtained from n-hexane extract was better than that obtained from extraction with ethyl acetate and ethanol solvents. Previous studies have also concluded that extraction with n-hexane solvent is more effective than extraction with ethyl acetate and ethanol solvents.[31] In addition to the solubility of the solvent, many other things can affect the process of extracting active substances from plants, such as the instability of secondary metabolites, the type of metabolic compounds targeted, the ratio, time, temperature, and extraction method used. High temperatures in the extraction process can affect the solubility of the

compounds to be extracted. Maintaining a balance in extracting temperature is key to obtaining important bioactive compounds.[32] The active compounds in *Hibiscus tiliaceus* leaves are polyphenols, flavonoids, terpenoid polyphenols, steroids and glycosides.[33,34] Various studies have shown the role of flavonoids as agents that can reduce cancer risk and as chemotherapy agents. Flavonoid quercetin has been shown to have a cytotoxic effect on breast cancer.[35] Other studies have shown that quercetin can inhibit cell migration.[36]

Hibiscus tiliaceus flowers contain flavonoids, phenolic compounds, saponins, alkaloids, tannins, steroids, and triterpenoids.[37] Flavonoids such as quercetin, kaempferol, genistein, and apigenin have shown anticancer potential by inhibiting cancer cell cycle, apoptosis, and angiogenesis. Flavonoids also have antioxidant properties that protect normal cells from oxidative damage, enhanced the effectiveness of conventional cancer therapies by increasing apoptosis, inhibiting drug resistance, and improving cancer cell sensitivity to radiation.[38] It has been shown that the n-hexane extract of *Boswellia carteii* has cytotoxic activity against Breast cancer.[39] Phenolic compounds, including gallic acid, vanillic acid, and p-coumaric acid, are also present. They are compounds with aromatic structures containing hydroxyl groups with anticancer and antioxidant activities. Steroids and terpenoids work by inducing apoptosis and inhibiting cancer cell proliferation.[40] The mechanism of triterpenoid compounds as anticancer agents involves inhibiting cell proliferation and inducing apoptosis.[41] This study did not isolate the active compounds from any part of the *Hibiscus tiliaceus* plant extracts. Further research on isolating anticancer agents from *Hibiscus tiliaceus* flowers is highly recommended.

Conclusion

The study revealed promising anticancer potential from the n-hexane extract of *Hibiscus tiliaceus* flowers against lung cancer. The extract is believed to exhibit selective apoptotic effects and in vitro prevention of proliferation in lung cancer cells. However, the isolation of active compounds is recommended for better results.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors gratefully acknowledge the Ministry of Education, Culture, Research, and Technology for providing financial support through the basic research scheme, based on the master contract dated June 11 2024 (contract number 103/E5/PG.02.00.PL/2024) and the derivative contract dated June 20 2024 (contract number 032/LL1/AL.04.03/2024). The authors also thank Universitas Tjut Nyak Dhien for the technical assistance provided.

Funding

This research was supported by a grant from the Minister of Education, Culture, Research, and Technology through the basic research scheme. The master contract was finalized on June 11, 2024, under the number 103/E5/PG.02.00.PL/2024, while the derivative contract was established on June 20, 2024, with the contract number 032/LL1/AL.04.03/2024.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–249.
2. Araghi M, Mannani R, Heidarnejad maleki A, Hamidi A, Rostami S, Safa SH, Faramarzi F, Khorasani S, Alimohammadi M, Tahmasebi S, Akhavan-Sigari R. Recent Advances in Non-small Cell Lung Cancer Targeted Therapy: An Update Review. Cancer Cell Int. 2023;23(1):1–26. Doi: 10.1186/s12935-023-02990-y
3. Asmara OD, Tenda ED, Singh G, Pitoyo CW, Rumende CM, Rajabto W, Ananda NR, Trisnawati I, Budiyono E, Thahadian HF, Boerma EC. Lung Cancer in Indonesia. J Thoracic Oncology. 2023;18(9):1134–1145.
4. Bray F, Laversanne M, Weiderpass E, Soerjomataram I. The Ever-Increasing Importance of Cancer as a Leading Cause of Premature Death Worldwide. Cancer. 2021;127(16):3029–3030. Doi: 10.1002/cncr.33587
5. Zebua NF, Fujiko M, Dachi K, Nerdy N, Septama AW. Antibacterial, Antioxidant and Total Flavonoid Content of *Adenostemma levenia* (L.). Natural J. 2024;24(2):73–81. Doi: 10.24815/jn.v24i2.36396
6. Okolie NP, Falodun A, Davids O. Evaluation of the Antioxidant Activity of Root Extract of Pepper Fruit (*Dennetia tripetala*), and It's Potential for the Inhibition of Lipid Peroxidation. Afr J Tradit Complement Altern Med. 2014;11(3):221-227.
7. Engel N, Falodun A, Kühn J, Kragl U, Langer P, Nebe B. Pro Apoptotic and Anti Adhesive Effects of Four African Plant Extracts on the Breast Cancer Cell Line MCF-7. BMC Complement Altern Med. 2014;14:334.
8. Surana AR, Kumbhare MR, Gunjal AR, Goswami SS, Ghuge DM. Chemical Characterization, Thrombolytic and Antioxidant Activity of *Hibiscus tiliaceu*s L. Leaves. Nat Prod Res. 2022;35(23):6106–6110. Doi: 10.1080/14786419.2022.2051705
9. Zebua NF, Nerdy N, Dachi K, Fujiko M, Septama AW. Ethnomedicine In Nias Island. Pharmacogn J. 2024;16(1):186–194.
10. Nguyen C, Baskaran K, Pupulin A, Ruvinov I, Zaitoon O, Grewal S, Scaria B, Mehaidli A, Vegh C, Pandey S. Hibiscus Flower Extract Selectively Induces Apoptosis in Breast Cancer Cells and Positively Interacts With Common Chemotherapeutics. BMC Complement Altern Med. 2019;19(1):1–14.
11. Saber A, Abedimanesh N, Somi MH, Khosroushahi AY. Anticancer Properties of Red Beetroot Hydro-alcoholic Extract and Its Main Constituent , Betanin, on Colorectal Cancer Cell Lines. BMC Complement Med Ther. 2023;7:1–12. Doi: 10.1186/s12906-023-04077-7
12. Nerdy N, Ritarwan K. Hepatoprotective Activity and Nephroprotective Activity of Peel Extract from Three Varieties of the Passion Fruit (*Passiflora sp.*) in the Albino Rat. Maced J Med Sci. 2019;7(4):536–542.
13. Yusuf H, Novia H, Fahriani M. Cytotoxic Activity of Ethyl Acetate Extract of *Chromolaena odorata* on MCF7 and T47D Breast Cancer Cells. Narra J. 2023;3(3)(e326):1–9.
14. Mahardika RS, Putri FR, Azzahra L, Maliza R, Ilham K, Ilham K. Effectiveness of Bioactive Compounds Against T47D Breast Ductal Carcinoma Cell Line : A Systematic Review. J Biol Trop. 2024;24(4):1–23.
15. Malacrida A, Erriquez J, Hashemi M, Rodriguez-Menendez V, Cassetti A, Cavaletti G, Miloso M. Evaluation of Antitumoral Effect of *Hibiscus Sabdariffa* Extract on Human Breast Cancer Cells. Biochem Biophys Reports. 2022;32:101353. Doi: 10.1016/j.bbrep.2022.101353
16. Nga NTH, Ngoc TTB, Trinh NTM, Thuoc TL, Thao DTP. Optimization and application of MTT assay in Determining Density of Suspension Cells. Anal Biochem. 2020;610:113937. Doi: 10.1016/j.ab.2020.113937
17. Rekha S, Anila. EI. In Vitro Cytotoxicity Studies of Surface Modified CaS Nanoparticles on L929 Cell Lines Using MTT Assay. Mater Lett. 2019;2019(236):637-639. Doi: 10.1016/j.matlet.2018.11.009.
18. Surahmaida, Rachmawati A, Handayani E. Chemical Composition of Waru Leaf (*Hibiscus tiliaceus)* in Lingkar Timur Area, Sidoarjo. J Pharm Sci. 2020;5(2):39–42.

19. Andriani Y, Sababathy M, Amir H, Sarjono PR, Syamsumir DF, Sugiwati S, Kassim MNI. The Potency of *Hibiscus tiliaceus* Leaves as Antioxidant and Anticancer agents Via Induction of Apoptosis against MCF-7 Cells. IOP Conf Ser Mater Sci Eng. 2020;959(1):012022. Doi: 10.1088/1757-899x/959/1/012022
20. Kamalia AZ, Tunjung WAS. Efficacy of Different Solvents in the Extraction of Bioactive Compounds and Anticancer Activities of *Thymus vulgaris* Leaves and Twigs. Indones J Pharm. 2023;34(3):419–430.
21. 21.Asmah N, Suniarti DF, Margono A, Mas'ud ZA, Bachtiar EW. Identification of Active Compounds in Ethyl Acetate, Chloroform, and N-hexane Extracts from Peels of *Citrus aurantifolia* from Maribaya, West Java, Indonesia. J Adv Pharm Technol Res. 2020;11(3):107–112.
22. Aavula T, Narasimman V, Ramachandran S, Murugan R, Ponnusamy M, Pazhani GP, Sivaleela G. Antioxidant and Teratogenic Activities of Formulated Agar Extracted from Brown Seaweed *Turbinaria conoides* against Zebrafish Larvae. Evidence-Based Complement Altern Med. 2022;2022:1–10.
23. Rawool PP, Parulekar BC. Phytochemical screening of *Hibiscus tiliaceus* by FTIR Spectroscopic Analysis. Int J Pharm Biol Sci. 2019;3(9):1308–1319.
24. Zebua NF, Nerdy, Bahrianur R, Suwailim S, Alfajar SH. Antioxidant And Antimicrobial Activities Of Leaves Of Medicinal Plant *Hibiscus tiliaceus* L. Forte J. 2024;04(02):302–313.
25. Ali M, Wani SU, Salahuddin M, Sn M, Mruthunjaya K, Dey T, Zargar MI, Singh J. Recent Advances of Herbal Medicines in Cancer - A Molecular Approach. Heliyon. 2023;9(2):e13684. Doi: 10.1016/j.heliyon.2023.e13684.
26. Zhiqin X, Cai Y, Ma Q, Zhao Z, Yang. D, Xu X. Optimization of Extraction of Bioactive Compounds from *Baphicacanthus cusia* Leaves by Hydrophobic Deep. Molecules. 2021;26(1729):1–13. Doi: 10.3390/molecules26061729
27. Li Y, Pan Z, Wang B, Yu W, Song S, Feng H, Zhao W, Zhang J. Ultrasound-assisted Extraction of Bioactive Alkaloids from *Phellodendri amurensis* Cortex Using Deep Eutectic Solvent Aqueous Solutions. New J Chemisrty. 2020;2020(22):9172–9178. Doi: 10.1039/D0NJ00877J
28. Rohmah J, Saidi IA, Rini CS, Masyitha DA, Ramadhani DN, Wulandari HP. Antioxidant Activity of Ethanol, Ethyl Acetate, and n-Hexane Extracts of Turi Putih (*Sesbania grandiflora* (L.) Pers.) dengan Metode Dpph (1,1-Diphenyl-2-Picrylhydrazyl). J Kim Ris. 2020;5(1):67–85.
29. Edison AD, Ariani NM, Ilza M. The Bioactive Components and Antioxidant Activity of the Crude Extract of *Sargassum plagyophyllum.* JPHPI. 2020;23(1):58–66.
30. Nazaruddin, Rosmaidar, Siregar TN, Wahyuni S, Sutriana A. Phytocomponents Analysis and Antioxidant Activity of Malacca fruit extract (*Phyllanthus emblica*) using Three Different Solvents. BIODIVERSITAS. 2024;25(5):1911–1919. Doi: 10.13057/biodiv/d250507
31. Amin A, Gani AP, Murwanti R. Cytotoxic Activities of (*Graptophyllum pictum* (L.) Griff) Ethanolic Extract and Its Fractions on Human Colon Cancer Cell WiDr.Trad Med J. 2020;25(April):29–33. Doi:10.22146/mot.48189
32. Ozdemir M, Gungor V, Melikoglu M, Aydiner C. Solvent selection and Effect of Extraction Conditions on Ultrasound-Assisted Extraction of Phenolic Compounds From Galangal (*Alpinia officinarum*). J Appl Res Med Aromat Plants. 2024;38(February):100525. Doi: 10.1016/j.jarmap.2023.100525
33. Samsudin MS, Andriani Y, Sarjono PR, Syamsumir DF. Study on *Hibiscus tiliaceus* Leaves as Antibacterial and Antioxidant Agents. ALOTROP, J Pendidik Dan Ilmu Kim. 2019;3(2):123–131.
34. Sangkal A, Supriati HS, Jamal AS. Identification of Secondary Metabolite Compounds of Turi Leaf Extract (*Sesbania grandiflora* L.) Using Ethanol, Ethyl Acetate and Hexane Solvents. J Sains dan Kesehat. 2021;5(1):42-48.
35. Zhou Y, Chen D, Xue G, Yu S, Yuan C, Huang M, Jiang L. Improved Therapeutic Efficacy of Quercetin-Loaded Polymeric Nanoparticle on Triple Negative Breast Cancer by Inhibiting uPA. RSC Adv. 2020;10:34517–34526. Doi: 10.1039/D0RA04231E
36. Gonçalves CF, Hecht F, Cazarin J, Fortunato RS, Vaisman M, de Carvalho DP, Ferreira AC. The Flavonoid Quercetin Reduce Cell Migration and Increases NIS and E-Cadherin mRNA in the Human Thyroid Cancer Cell Line BCPAP. Mol Cell Endocrinol. 2021;5(21):111–266.
37. Matsumoto T, Imahori D, Achiwa K, Saito Y, Ohta T, Yoshida T, Kojima N, Yamashita M, Nakayama Y, Watanabe T. Fitoterapia Chemical Structures and Cytotoxic Activities of the Constituents Isolated from *Hibiscus tiliaceus*. Fitoterapia. 2020;142:104524.
38. Hidayah H, Widyaningsih A, Pangestu AD, Dewi SR. Literatur Review: Flavonoid Activity as an Anti-Cancer Compound. Mahesa Malahayati Heal Student J. 2023;3(5):1255–1263.
39. Xie J, Huang H, Wei X, Tan P, Ouyang L, Wang L, Liu D, Wang F, Wang Z, Tu P, Li J. *Boswellia carterii* N - Hexane Extract Suppresses Breast Cancer Growth Via Induction of Ferroptosis by Downregulated GPX4 and Upregulated Transferrin. Sci Rep. 2024;14(14307):1–12.
40. Chen DL, Chen MY, Hou Y, Wang CH, Sun ZC, Yang Y, Liang HQ, Ma GX, Xu XD, Wei JH. Cadinane-Type Sesquiterpenoids with Cytotoxic Activity from the Infected Stems of the Semi-mangrove *Hibiscus tiliaceus.* J Nat Prod. 2022;85(1):127–135.
41. Hidayah H, Fatmawati F, Khairunnisa J, Putri MH. Triterpenoid Activity as Anticancer Compounds. Innov J Soc Sci Res. 2023;3(2):10168–10183.