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In silico and Histochemical Analysis of Soursop Leaves (*Annona muricata*) Against Alpha Estrogen Receptor

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Abstract

Soursop leaves (*Annona muricata*) are known as a plant that has the potential to treat breast cancer, which has a high mortality rate in women in Indonesia. The receptor that plays the most important role in breast cancer development is the Estrogen Receptor Alpha (ERα). This study aims to identify active compounds in soursop leaves that have the potential to inhibit breast cancer cell growth through in silico analysis using molecular docking of the ERα receptor using KNApSAck, PASS Online, PubChem, PDB ID, PyRx, and Discovery Studio software and Histochemical analysis was performed using fresh soursop leaves by making an incision on the lower part of the leaf and adding reagents AlCl, FeCl, Na2CO, $CuSO_4$, glycerin, Wagner reagent, immersion oil, and ethanol, then observed microscopically. The in silico results showed the presence of specific compounds from soursop leaves and one reference compound (OHT600). Cis-Solamin had the highest binding affinity (-9.3 kcal/mol), better than the native ligand (-7.6 kcal/mol), and interacted with the amino acid Glutamine 532 (GLU532). Histochemical analysis showed the content of flavonoids, phenols, tannins, terpenoids, and alkaloids, accompanied by color changes in trichome and stomatal tissues. Based on the research data, it was concluded that soursop leaves can inhibit the growth of breast cancer cells through the content of secondary metabolites that actively inhibit Erα receptors.

Keywords: Breast cancer; Erα; Histochemistry; In Silico; Soursop leaf.

INTRODUCTION

According to the 2010 Pathological Based Registration in Indonesia, breast cancer is the most common type of cancer suffered by Indonesian women, with a relative frequency of 18.6%, and carries a high risk of mortality. In 2022, according to the World Health Organization (WHO), nearly 10 million deaths were caused by cancer, making it the leading cause of death in 2020, with 685,000 of these deaths being caused by breast cancer. Breast cancer is generally treated with surgery to remove cancerous tissue. However, this still has the potential for new cancerous tissue to grow if the removal is not perfect. Furthermore, chemotherapy and radiotherapy can also be used to kill cancer cells, but normal cells can also be damaged (Pertiwi, 2020). Chemotherapy will not only affect cancer cells but also other body cells that share characteristics with tumor cells with high cell division rates, such as hair follicles, bone marrow, gastrointestinal tract cells, and reproductive cells, making these cells the most affected by the cytotoxic effects of chemotherapy (Prieto et al., 2020). To overcome the side effects of chemotherapy, a combination of natural bioactive compounds can be used (Dewayani et al, 2023).

Phytochemical studies on soursop leaves (*Annona muricata*) indicate that they are a major source of ethnomedicinal compounds used to suppress tumor and cancer growth. Furthermore, soursop leaf extract has a cytotoxic effect on lung carcinoma cells, pancreatic cancer cells, and breast cancer cells, which has been demonstrated in vivo in animal models (Dewayani et al., 2023). To determine the medicinal uses of active compounds in plant tissue, in silico analysis using molecular docking is necessary. Computational molecular docking aims to predict how a compound or ligand will interact with a particular protein by calculating its binding affinity (Asari et al., 2023). In silico analysis using the molecular docking method uses the alpha estrogen receptor, which is commonly found in breast cancer cells and is the receptor that plays the most important role in breast cancer progression (Mubarakati et al., 2019). In addition to in silico analysis, this can also be confirmed by histochemical laboratory analysis. The purpose of histochemical analysis is to identify bioactive compounds found in plant tissue (Nurhasanah et al., 2019). The tissue used for detection is the epidermis, which functions to protect the cells within it and store secondary metabolite compounds (Maghfiroh et al., 2018).

Given the abundant potential as a traditional herbal medicine found in the soursop plant is needed, particularly to identify the active compounds in the leaves of the soursop plant that can be used as cancer-fighting agents. Therefore, to determine the bioactivity of these compounds against breast cancer, in silico analysis using molecular docking is necessary. Furthermore, histochemical analysis can also be performed to determine the secondary metabolite content in soursop leaves.

This study aims to identify specific compounds in soursop leaves, then analyze the interaction of these active compounds with the estrogen receptor alpha using molecular docking and identify secondary metabolites in soursop leaves. This research is expected to serve as a basis for further research and as a reference for biotechnology development and preserving medicinal plants, particularly soursop (*Annona muricata*).

MATERIALS AND METHODS

Materials

This research was conducted at the Integrated Laboratory of Universitas Islam Malang and was conducted from December 2023 to February 2024. The tools used were: beakers, petri dishes, measuring cups, clamps, dropper pipettes, tweezers, object glass, cover glass, test tubes, test tube racks, stationery, labels, cutters, cameras, microscopes, and a set of computers with several software namely, PubChem, Pass online, Protein Data Bank (PDB ID), KNApSAcK, Pyrx, Discovery studio Visualizer. In addition, the materials used were: fresh soursop leaves (*Annona muricata*) from the 5th node, taken from the Batu City area, glycerin, Wagner reagent, Na_2CO_3, a 5% $CuSO_4$ solution, an $AlCl_3$ solution, a 10% $FeCl_3$ solution, 85% ethanol, 70% alcohol, and immersion oil, for molecular docking using a receptor with the PDB code 3ERT downloaded through RSCB PDB and several active compound ligands downloaded through PubChem.

Methods

This research employed a quantitative descriptive research method. The data obtained were analyzed using in silico and qualitative methods to determine the results of the histochemical analysis. In silico analysis was carried out using several software, including PubChem, PASS online, Protein Data Bank (PDB ID), KNApSAcK, PyRx, and Discovery Studio Visualizer. Meanwhile, histochemical analysis was performed using various specialised reagents according to the type of analysis. Several types of histochemical analysis were carried out, including tests for flavonoids, phenols, tannins, terpenoids, and alkaloids.

Procedures

Preparation and Testing In Silico

Bioactivity and 3D Structure Testing

The chemical structures of secondary metabolites were analyzed in silico using PubChem software (https://pubchem.ncbi.nlm.nih.gov/), and their bioactivity was determined using PASS Online software (http://www.way2drug.com/PASSOnline/index.php).

Molecular docking test

3ERT protein preparation

The 3ERT protein was downloaded using PDB software (http://www.rcsb.org/pdb). The target protein was then selected in its active form that binds to its native ligand. The 3ERT protein, which had bound to its native ligand, was then separated using Discovery Studio Virtualiser software for validation.

Validation with Molecular Docking

Validation of the molecular docking method was performed by re-docking the native ligand and the target protein, which had been separated from the native ligand, using PyRx software. If the Root Mean Square Deviation (RMSD) value obtained is $\leq$ 3 Å, the method is considered valid, allowing docking of the test compound and its target protein; however, according to research (Manno & Utami, 2023).

Preparation of Active Compounds

Test compounds were downloaded from the PubChem software (https://pubchem.ncbi.nlm.nih.gov/) and prepared using the Discovery Studio Virtualiser software, utilizing PDB files for docking adjustments.

Docking of Active Compounds and 3ERT Protein

Docking of the test compounds with 3ERT, whose native ligand had been separated, using PyRx software, yielded a docking result that confirmed the lowest binding energy and was related to the target protein.

Molecular docking data analysis

Molecular docking data analysis was based on the binding energy results, Root Mean Square Deviation (RMSD) values, and the bond types generated by the molecular docking. The bond energy value indicated the bond strength between the test compound and its receptor. The lower the bond energy value, the stronger the bond between the compound and the receptor.

Preparation and Histochemical Testing of Soursop Leaves

Histochemical analysis was performed using fresh soursop leaves (*Annona muricata*), which were sterilized by thoroughly washing them with running water. Then, an incision was made on the underside of the leaf (abaxial) for preparation. The fresh slides were placed on a glass slide and treated with prepared immersion oil,

then observed under a microscope. The secondary metabolite detection method is as follows,

Flavonoid Compound Analysis
Flavonoid compounds were detected by adding a solution of $AlCl_3$ in 85% ethanol. If there is a color change to yellow or blue, the sample is positive for flavonoid compounds (Rupa, 2015; Pratiwi, 2020).

Phenol Compound Analysis
Phenol compound analysis was performed by immersing a sample slice in a 10% $FeCl_3$ solution with several Na_2CO_3 pellets and allowing it to stand at room temperature for approximately 15 minutes. If there is a color change to dark green or even black, the sample is positive for phenol compounds (Andriya, 2016; Pratiwi, 2020).

Tannin Compound Analysis
Tannin compound analysis was performed by adding a 10% $FeCl_3$ solution to the sample incision. A color change to dark brown indicated the sample contains tannin compounds (Rupa, 2015; Pratiwi, 2020).

Terpenoid Compound Analysis
Terpenoid compounds were detected by immersing the sample in a 5% $CuSO_4$ solution for approximately 24 hours. Then, a drop of glycerin was added and observed under a microscope. A positive result for terpenoids was indicated by a color change to brownish-yellow (Andriya, 2016; Pratiwi, 2020).

Alkaloid Compound Analysis
Alkaloid compounds was detected by soaking the sample in Wagner's reagent for 48 hours. Then, they were observed under a microscope. A positive result for alkaloid compounds was indicated by a color change to reddish-brown (Andriya, 2016; Pratiwi, 2020).

Data Analysis
Data obtained from the in silico method were analyzed quantitatively based on values generated from the molecular docking process. Meanwhile, the histochemical method was analyzed qualitatively based on research documentation, which showed specific colour changes.

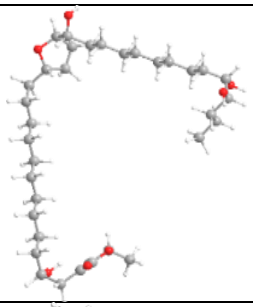
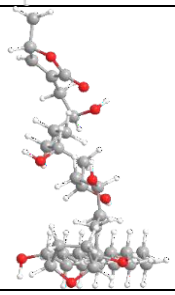
RESULTS AND DISCUSSION

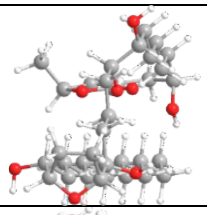
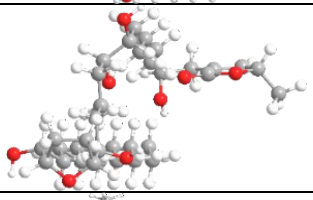
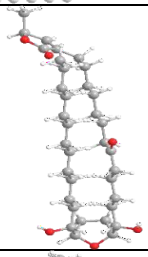
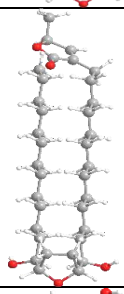
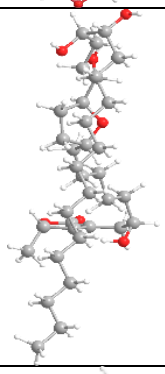
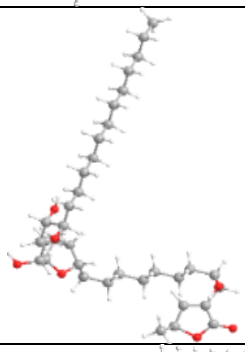
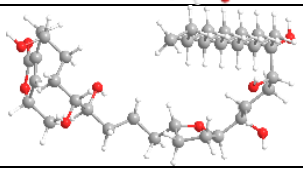
RESULTS

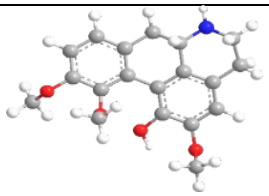
Identification of Specific Compounds in Soursop Leaves (*Annona muricata*) and Their Bioactivity through In Silico Analysis

Based on the in silico analysis conducted, several compounds were identified as being specific to soursop leaves, and common compounds were also detected in several other plant species. The results of the active compound search and 3D structure are shown in Table 1.

Table 1. KNApSAcK Results of Active Compounds and 3D Structure of Soursop Leaves (*Annona muricata*).

No	Molecular Formula	Structural Formula	Compound Name	Compound Classes	Information
1	$C_{35}H_{64}O_7$		Annocatalin	Flavonoid	Specific
2	$C_{35}H_{64}O_9$		Annohexocin	Flavonoid	Specific

No	Molecular Formula	Structural Formula	Compound Name	Compound Classes	Information
3	$C_{35}H_{64}O_8$		Annomuricin E	Flavonoid	Specific
4	$C_{35}H_{64}O_8$		Annomuricin A	Flavonoid	Specific
5	$C_{35}H_{62}O_6$		Cis-Corossolone	Flavonoid	Specific
6	$C_{35}H_{64}O_5$		Cis-Solamin	Flavonoid	Specific
7	$C_{35}H_{64}O_7$		Gigantetrocin A	Flavonoid	Detected in 4 species: *Annona muricata, Annona glabra, Goniothalamus giganteus, Xylopia aromatica*
8	$C_{35}H_{64}O_7$		Muricatetrocin A	Flavonoid	Specific
9	$C_{35}H_{64}O_9$		Murihexocin C	Fenol	Specific

No	Molecular Formula	Structural Formula	Compound Name	Compound Classes	Information
10	$C_{19}H_{21}NO_4$		Norcorydine	Alkaloid	Detected in 8 species: *Annona muricata, Annona reticulate, Annona squamous, Miliusa velutina, Trivalvaria macrophylla, Xylopia danguyella, Xylopia pancheri, Croton hemiargyreus*

The active compounds of soursop leaves obtained from the results of the KNApSAcK program search include Annocatalin, Annohexocin, Annomuricin A, Annomuricin E, Cis-corossolane, Cis-Solamin, Gigantetrocin A, Muricatetrocin A, a derivative of acetogenin, Norcorydine, a derivative of alkaloids, and Murihexocin C, a derivative of phenolics. Some of these compounds are specific compounds that are only found in soursop leaves, including Annocatalin, Annomuricin A, Annomuricin E, Annohexocin, Muricatetrocin A, Murihexocin C, Cis-corossolane, and Cis-Solamin.

Their potential as breast cancer anticancer agents was then assessed, and the bioactivity values were obtained in the form of probable to be active (Pa) values for each compound, presented in Table 2.

Table 2. Percentage of Bioactivity of Active Compounds in Soursop Leaves (*Annona muricata*) as Candidates for Breast Cancer Drugs.

No	Compound Name	Bioactivity Percentage (%)
1	*Annocatalin*	56,2
2	*Annohexocin*	46,7
3	*Annomuricin E*	63,5
4	*Annomuricin A*	63,5
5	*Cis-Corossolone*	63,4
6	*Cis-Solamin*	41,8
7	*Gigantetrocin A*	56,2
8	*Muricatetrocin A*	56,2
9	*Murihexocin C*	50,6
10	*Norcorydine*	19

Based on Table 2, the probable to be active (Pa) values were converted into percentages. The results show that several compounds have quite high activity, namely annomuricin E (63.5%), annomuricin A (63.5%), cis-corossolone (63.4%), gigantetrocin A (56.2%), muricatetrocin A (56.2%), annocatalin (56.2%), and murihexocin (50.6%). Meanwhile, annohexocin, cis-solamine, and norcorydine are classified as compounds with low activity with values of 46.6%, 41.8%, and 19%, respectively.

Results of Molecular Interaction of Active Compounds in Soursop Leaves (*Annona muricata*) with the Alpha Estrogen Receptor

The detailed profile of 3ERT obtained through RSCB PDB software data is as follows (Figure 1).

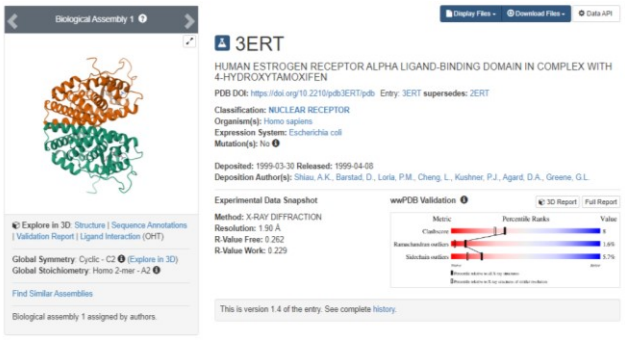


Figure 1. 3ERT Profile.

The molecular docking resulted in interactions indicated by the type of bond to amino acid residues. The results of the interaction between the active compounds in soursop leaves and the 3ERT protein are presented in the following figure (Figure 2-12).

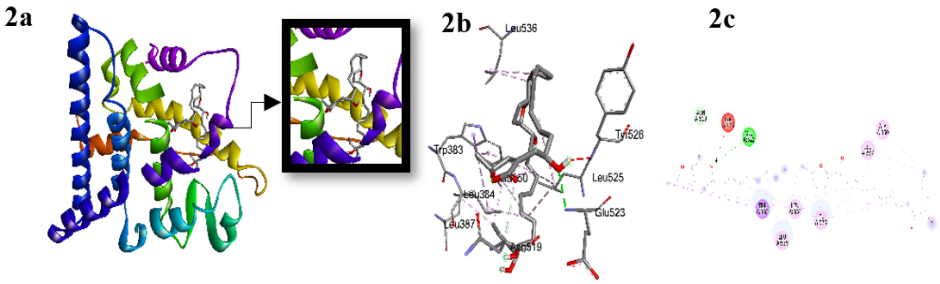


Figure 2. Molecular docking simulation results of Estrogen Alpha (PDB ID: 3ERT) with the active compound *Annonacatalin* (a) Protein-ligand complex; (b) 3-dimensional structure interaction; and (c) 2-dimensional structure interaction.

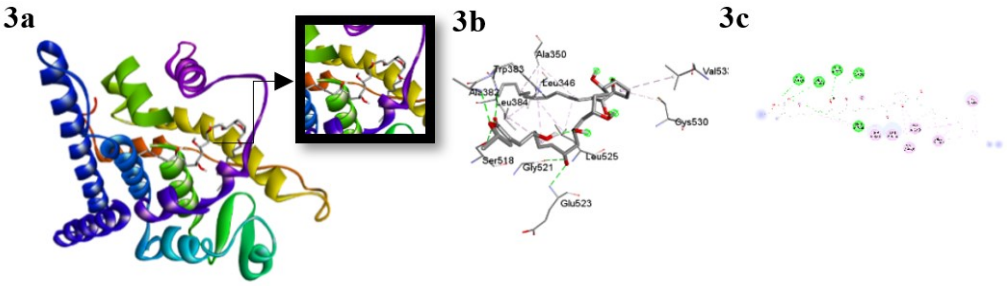


Figure 3. Molecular docking simulation results of Estrogen Alpha (PDB ID: 3ERT) with the active compound *Annohexocin* (a) Protein-ligand complex; (b) 3-dimensional structure interaction; and (c) 2-dimensional structure interaction.

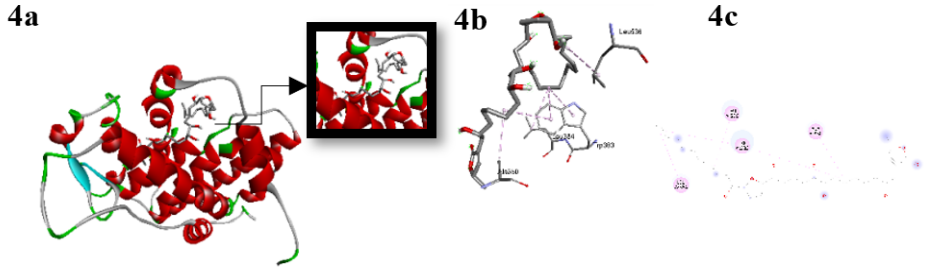


Figure 4. Molecular docking simulation results of Estrogen Alpha (PDB ID: 3ERT) with the active compound *Annomuricin E* (a) Protein-ligand complex; (b) 3-dimensional structure interaction; and (c) 2-dimensional structure interaction.

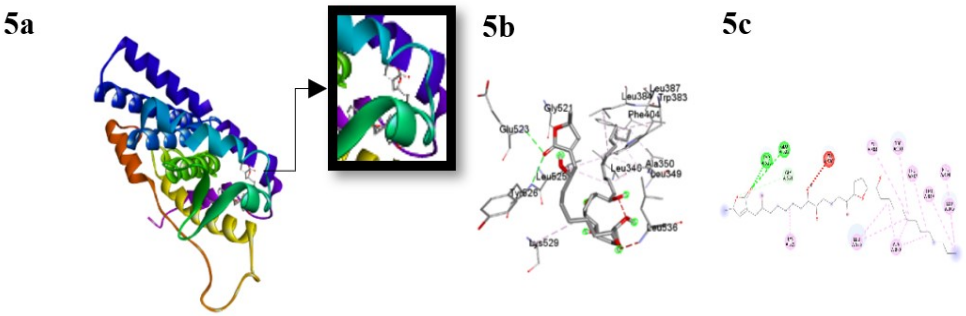


Figure 5. Molecular docking simulation results of Estrogen Alpha (PDB ID: 3ERT) with the active compound *Annomuricin A* (a) Protein-ligand complex; (b) 3-dimensional structure interaction; and (c) 2-dimensional structure interaction.

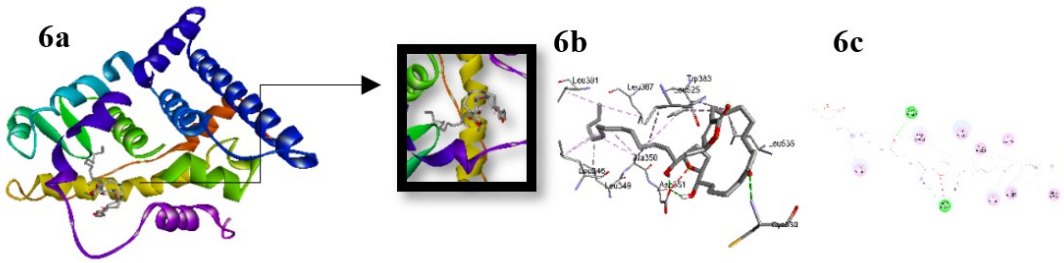


Figure 6. Molecular docking simulation results of Estrogen Alpha (PDB ID: 3ERT) with the active compound *Cis-Corossolone* (a) Protein-ligand complex; (b) 3-dimensional structure interaction; and (c) 2-dimensional structure interaction.

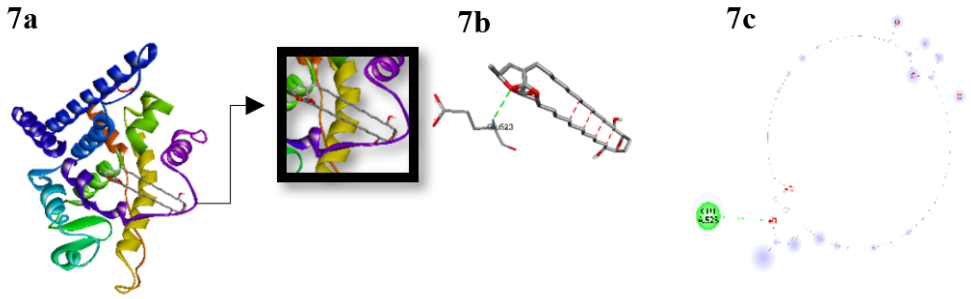


Figure 7. Molecular docking simulation results of Estrogen Alpha (PDB ID: 3ERT) with the active compound *Cis-Solamin* (a) Protein-ligand complex; (b) 3-dimensional structure interaction; and (c) 2-dimensional structure interaction.

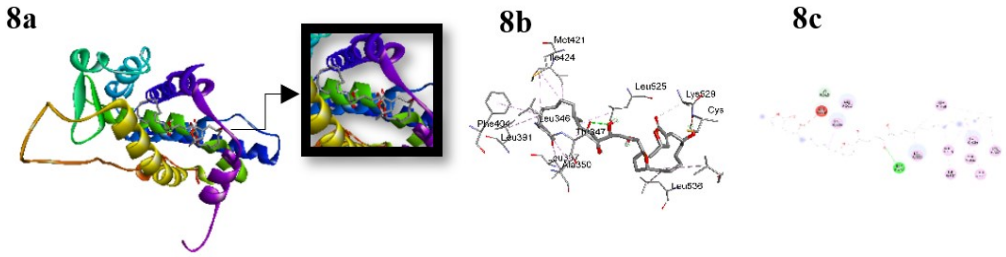


Figure 8. Molecular docking simulation results of Estrogen Alpha (PDB ID: 3ERT) with the active compound *Gigantetrocin A* (a) Protein-ligand complex; (b) 3-dimensional structure interaction; and (c) 2-dimensional structure interaction.

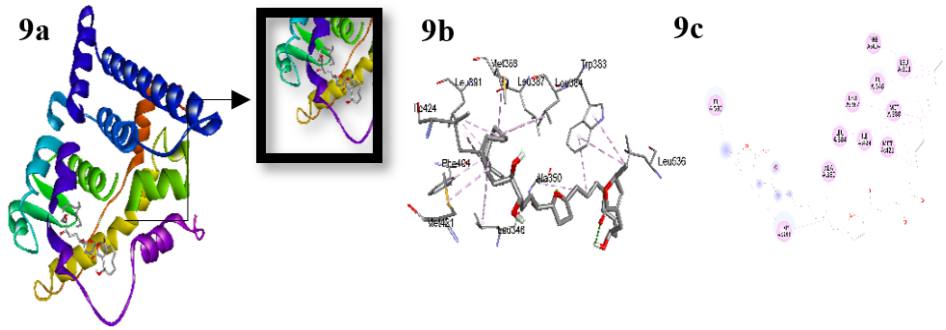


Figure 9. Molecular docking simulation results of Estrogen Alpha (PDB ID: 3ERT) with the active compound *Muricatetrocin A* (a) Protein-ligand complex; (b) 3-dimensional structure interaction; and (c) 2-dimensional structure interaction.

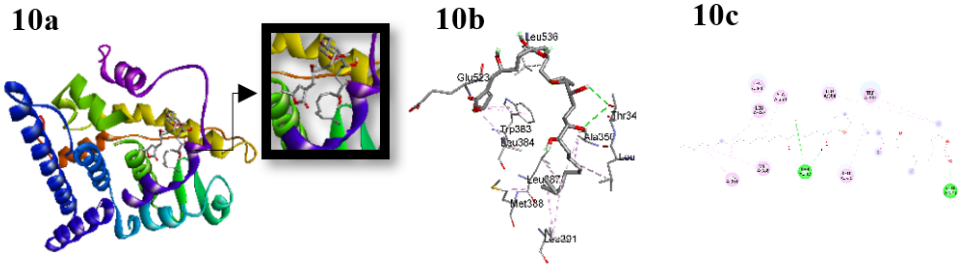


Figure 10. Molecular docking simulation results of Estrogen Alpha (PDB ID: 3ERT) with the active compound *Murihexocin C* (a) Protein-ligand complex; (b) 3-dimensional structure interaction; and (c) 2-dimensional structure interaction.

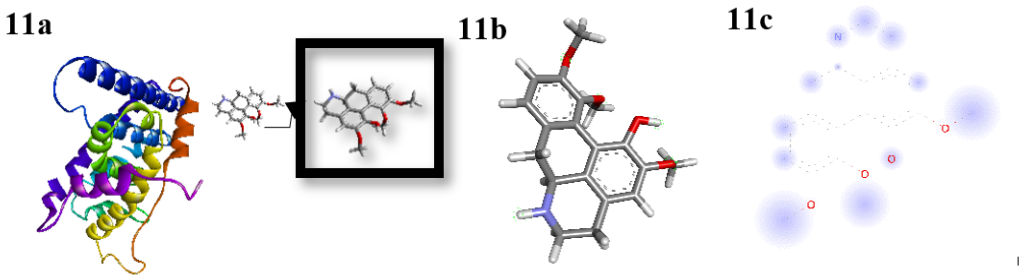


Figure 11. Molecular docking simulation results of Estrogen Alpha (PDB ID: 3ERT) with the active compound *Norcorydine* (a) Protein-ligand complex; (b) 3-dimensional structure interaction; and (c) 2-dimensional structure interaction.

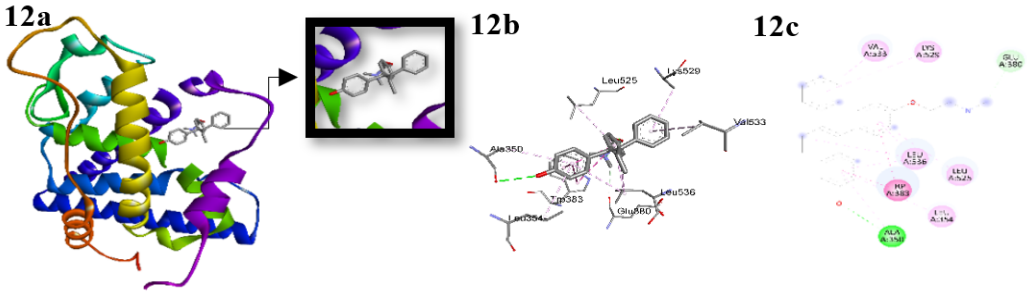


Figure 12. Molecular docking simulation results of Estrogen Alpha (PDB ID: 3ERT) with the active compound *OHT600* (Native ligand/control) (a) Protein-ligand complex; (b) 3-dimensional structure interaction; and (c) 2-dimensional structure interaction.

Molecular docking results show the binding affinity values obtained, as well as interactions with amino acid residues of various bond types. The binding values of the 11 active compounds tested, along with the bound amino acids, are presented in Table 3.

Table 3. Binding Affinity Value and Interaction of Active Compounds in Soursop Leaves (*Annona muricata*) as a Candidate for Breast Anticancer Drugs.

No.	Compound	Binding Affinity (kcal/mol)	RMSD Lower bond	RMSD Upper Bond	Interaction type
1.	*Annonacatalin*	-6,6	1,888	3,418	Hydrogen Bonds: GLU523, ASN519 Hydrophobic: TRP383, ALA350, LEU384, LEU525, LEU536, LEU384, LEU387 Unfavorable: TYR526
2.	*Annohexocin*	-6,6	1,848	2,477	Hydrogen Bonds: ALA382, LEU384, SER518, GLU523, GLY521 Hydrophobic: ALA350, LEU525, CYS530, VAL533, LEU384, LEU384, LEU346, TRP383
3.	*Annomuricin E*	-6,1	2,484	5,271	Hydrophobic: ALA350, LEU536, LEU384, LEU384
4.	*Annomuricine A*	-6,3	3,070	8,751	Hydrogen Bonds: GLY521, TYR526, GLU523 Hydrophobic: ALA350, LYS529, LEU525, LEU384, LEU525, LEU387, LEU346, LEU349, TRP383, PHE404 Unfavorable: LEU536
5.	*Cis-corossolone*	-6,7	1,929	3,087	Hydrogen Bonds: CYS530, ASP351 Hydrophobic: ALA350, LEU525, LEU346, LEU349, LEU391, TRP383 Unfavorable: ASP351

No.	Compound	Binding Affinity (kcal/mol)	RMSD Lower bond	RMSD Upper Bond	Interaction type
6.	*Cissolamin*	-9,3	1,673	2,590	Hydrogen Bonds: GLU523
7.	*Gigantetrocin A*	-6,4	0,906	2,226	Hydrogen Bonds: THR347, LYS529 Hydrophobic: ALA350, LEU525, VAL533, LEU536, MET421, ILE424, ILE424, LEU346, LEU391, LEU387, PHE404 Unfavorable: CYS530
8.	*Muricatetrocin A*	-6,4	1,427	1,980	Hydrophobic: ALA350, LEU384, LEU387, MET388, LEU391, LEU346, MET421, ILE424, LEU536, TRP383 PHE404
9.	*Murihexocin C*	7,1	1,367	2,170	Hydrogen Bonds: THR347, GLU523 Hydrophobic: LEU346, ALA350, LEU536, LEU387, LEU391, MET388, LEU384, TRP383
10.	*Norcorydine*	-6,4	2,980	5,835	No Interaction
11.	*OHT600*	-7,6	0,681	1,528	Hydrogen Bonds: ALA350, GLU380 Hydrophobic: TRP383, LEU536, ALA350, LEU354, LEU525, LYS529, VAL533

Based on the docking results of Estrogen Alpha with 10 active compounds in soursop leaves, binding affinity, Root Mean Square Deviation (RMSD) and hydrogen bond values were obtained. Then, filtering based on the validity of the RMSD data, five valid compounds were identified:

- Annohexocin with a binding affinity value of -6.6 kcal/mol, a lower bond RMSD of 1.848 Å and an upper bond RMSD of 2.477 Å. This compound forms hydrogen bonds with the amino acids ALA382, LEU384, SER518, GLU523, and GLY521, as well as hydrophobic bonds with the amino acids ALA350, LEU525, CYS530, VAL533, LEU384, LEU346, and TRP383.
- Cis-Solamin with a binding affinity value of -9.3 kcal/mol, with a Lower bond RMSD value of 1.673 Å and an Upper Bond RMSD of 2.590 Å. This compound forms Hydrogen Bonds with the amino acid GLU523.
- Gigantetrocin A has a binding affinity value of -6.4 kcal/mol with a Lower bond RMSD value of 0.906 Å and an Upper Bond RMSD of 2.226 Å. This compound forms Hydrogen Bonds with the amino acids THR347 & LYS529, Hydrophobic bonds with the amino acids ALA350, LEU525, VAL533, LEU536, MET421, ILE424, ILE424, LEU346, LEU391, LEU387 & PHE404, and Unfavorable bonds with the amino acid CYS530.
- Muricatetrocin A has a binding affinity of -6.4 kcal/mol with a lower bond RMSD of 1.427 Å and an upper bond RMSD of 1.980 Å. This compound forms hydrophobic bonds with the amino acids ALA350, LEU384, LEU387, MET388, LEU391, LEU346, MET421, ILE424, LEU536, TRP383 & PHE404.
- Murihexocin C has a binding affinity of -7.1 kcal/mol with a lower bond RMSD of 1.367 Å and an upper bond RMSD of 2.170 Å. This compound forms hydrogen bonds with amino acids THR347 & GLU523 and hydrophobic bonds with amino acids LEU346, ALA350, LEU536, LEU387, LEU391, MET388, LEU384 & TRP383.

Results of Identification of Secondary Metabolite Compounds in Soursop Leaves (*Annona muricata*) Through Histochemical Analysis

Histochemical analysis was used to qualitatively determine the secondary metabolite content of plant tissue. This study used soursop leaf samples collected in Batu City. Only the fifth node from the shoot or leaf tip was used as the research boundary. The results of the histochemical analysis are shown in Table 4..

Table 4. Results of detection of secondary metabolite compounds in soursop leaves (*Annona muricata*) through histochemical analysis.

Secondary metabolite compounds	Detection reagent	Positive detection and color change results
Flavonoid	$AlCl_3$ and ettanol 85%	+ (Reddish yellow)
Fenol	$FeCl_3$ 10% and Na_2CO_3	+ (Dark green)
Tanin	$FeCl_3$ 10%	+ (Dark brown)
Terpenoid	$CuSO_4$ 5% and gliserin	+ (Brownish yellow)
Alkaloid	Wagner	+ (Brownish red)

The results of histochemical analysis indicate that soursop leaves contain secondary metabolite compounds, including flavonoids, phenols, tannins, terpenoids, and alkaloids.

Microscopic observations of soursop leaf epidermal cells revealed the presence of secondary metabolites in the trichomes and stomatal tissues. Flavonoid secondary metabolites were identified by the color change from clear to reddish-yellow in the trichome tissue, as seen in Figure 13.

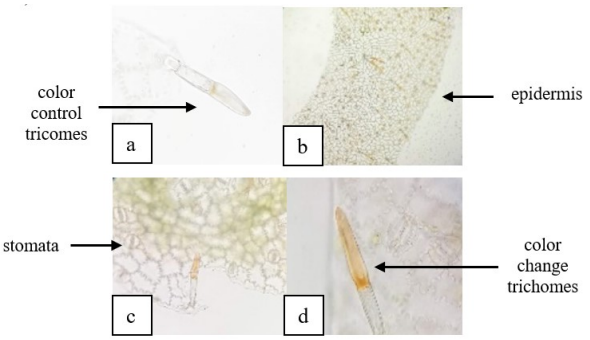


Figure 13. Histochemical Micrograph of Abaxial Longitudinal Section of Soursop Leaf Before Treatment (a) magnification 1000x, After Flavonoid Detection Treatment (b) 100x, (c) 400x, (d) 1000x.

The results of microscopic observations of the epidermal cells in soursop leaves reveal the presence of phenolic compounds, as indicated by a change from clear to dark green in the trichome tissue, as shown in Figure 14.

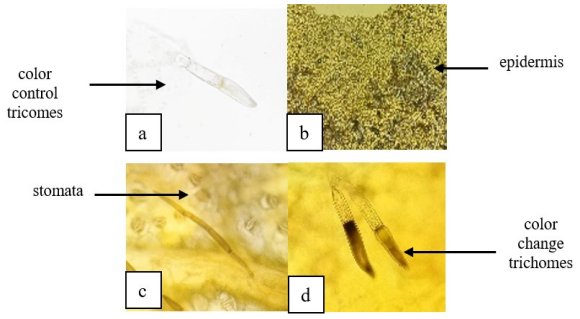


Figure 14. Histochemical Micrograph of Abaxial Longitudinal Section of Soursop Leaf Before Treatment (a) magnification 1000x, After Phenol Detection Treatment (b) 100x, (c) 400x, (d) 1000x.

The results of microscopic observations of the epidermal cells in soursop leaves reveal the presence of tannin compounds, with a transition from clear to dark brown in the trichome and stomata tissue, as shown in Figure 15.

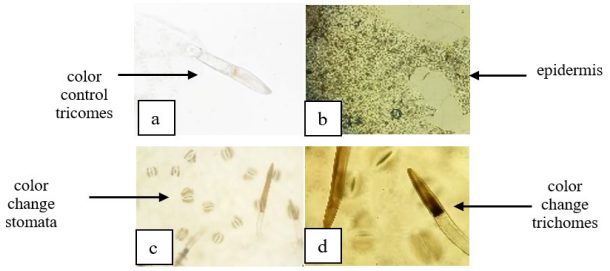


Figure 15. Histochemical Micrograph of Abaxial Longitudinal Section of Soursop Leaf Before Treatment (a) magnification 1000x, After Tannin Detection Treatment (b) 100x, (c) 400x, (d) 1000x.

The results of microscopic observations of soursop leaf epidermal cells revealed the presence of terpenoid compounds, as indicated by a change from clear to brownish-yellow in the trichome tissue, as shown in Figure 16.

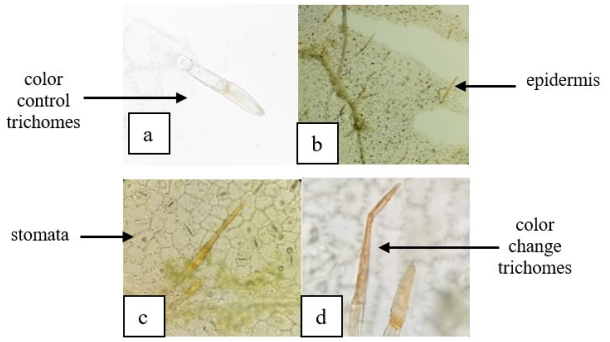


Figure 16. Histochemical Micrograph of Abaxial Longitudinal Section of Soursop Leaf Before Treatment (a) magnification 1000x, After Terpenoid Detection Treatment (b) 100x, (c) 400x, (d) 1000x.

Microscopic observations of soursop leaf epidermal cells revealed the presence of alkaloid compounds, characterised by a colour change from clear to brownish-red in the trichome and stomata tissue, as shown in Figure 17.

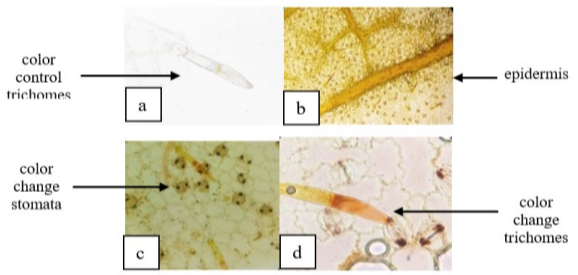


Figure 17. Histochemical Micrograph of Abaxial Longitudinal Section of Soursop Leaf Before Treatment (a) magnification 1000x, After Alkaloid Detection Treatment (b) 100x, (c) 400x, (d) 1000x.

DISCUSSION

Identification of Specific Compounds in Soursop Leaves (*Annona muricata*) and Their Bioactivity through In Silico Analysis

In silico research results identified 10 active compounds in soursop leaves that have the potential to act as anti-breast cancer agents, including Annocatalin, Annohexocin, Annomuricin A, Annomuricin E, Cis-Corossolane, Cis-Solamin, Gigantetrocin A, Muricatetrocin A, Murihexocin C, and Norcorydine. Gigantetrocin A and Norcorydine compounds are not specifically found only in soursop leaves, but are also found in several other species. This is in line with research by Qomaliyah (2022), which suggests that soursop leaves contain active secondary metabolite compounds with potential bioactivity due to the presence of flavonoid compounds, acetogenin derivatives, and other groups, including alkaloids and phenols. Another study, according to Siswarni et al. (2016), states that soursop has an acetogenin chemical content that is useful as an anticancer agent, and its use in the health sector continues to increase. The known compounds were analysed for their bioactivity and potential, followed by molecular docking to determine their binding affinity to the alpha estrogen receptor protein. The bioactivity of the compounds in soursop leaves was analysed using the PASS Online web server to evaluate their biological potential as potential drug candidates (Istiqomah et al., 2023).

Based on Table 2, the probable to be active (Pa) value has been converted into a percentage. The (Pa) value indicates the probability of the compound's activity in soursop leaves as an anti-breast cancer agent. If the Pa value is higher than 0.7 (Pa > 0.7), the compound exhibits very high biological activity, and the results are not significantly different from those of laboratory tests. If the Pa value is between 0.5 and 0.7 (0.5 < Pa < 0.7), the compound exhibits quite high biological activity in in vitro or in vivo tests and has potential as a new drug candidate for anticancer applications. If the Pa value is less than 0.5 (Pa < 0.5), the compound has low biological activity both in the laboratory and computationally (Malikhana et al., 2021). The results show that several compounds have quite high activity, namely annomuricin E, annomuricin A, cis-corossolone, gigantetrocin A, muricatetrocin A, annocatalin, and murihexocin. Meanwhile, annohexocin, cis-solamine, and norcorydine are classified as compounds with low activity. Compounds with low Pa values do not necessarily mean they have low activity; however, these compounds have not been widely studied in the laboratory or computationally, so further research is needed to explore them as potential drug candidates.

Molecular Interaction of Active Compounds in Soursop Leaves (*Annona muricata*) with the Alpha Estrogen Receptor

Molecular docking was performed using the Estrogen Alpha receptor protein with PDB ID: 3ERT. The crystal structure with the code 3ERT is ERα that binds tamoxifen (Xue et al., 2019). ERα is a receptor in cells that binds the female hormone estrogen and other natural and synthetic ligands. Its role is very important in normal development, physiological function, and diseases related to the endocrine system. ERα belongs to a large family of nuclear receptors and transcription factors, regulating the expression of its target genes depending on the type of ligand bound (Candelaria et al., 2013). Molecular docking results indicate an interaction between the ligand compound and the receptor protein. This interaction produces binding affinity values and root mean square deviation (RMSD) values for the lower and upper bonds. Interactions with amino acid residues with various bond types are also observed.

The parameter used to validate molecular docking results is the Root Mean Square Deviation (RMSD), a measure of similarity widely used in macromolecular structure and dynamics analysis. When larger macromolecular systems are studied, dimensional effects such as the “curse of dimensionality” (a reduced ability to distinguish pairwise differences between conformations with increasing system size) may exist and have a significant impact on RMSD-based analysis (Sargsyan et al., 2017). The RMSD parameter is considered valid, and the interaction between the ligand and protein can be carried out if it has an RMSD value ≤ 3 Å (Yuniati et al., 2023). RMSD values <2 and <3 Å are normal or average; if the RMSD value is between 3 and 4 Å, it means there is a difference in protein folding, and if the RMSD value is more than 4 Å, it indicates that there is a problem in binding or is declared invalid (Lestari et al., 2019). In the study of Manno et al. (2023), RMSD is a two-pose measurement that compares the atomic positions between the experimental structure and the docking structure of the protein. The results are declared valid if the value obtained is greater than 3 Å, and the smaller the value obtained, the better the ligand pose.

Based on the results of the prediction stage of the interaction of active compounds in Soursop leaves with the Alpha Estrogen Receptor analysis molecule (Table 3), it was found that the specific soursop leaf compound that had the highest binding affinity and the strongest bond was the Cis-Solamin compound of -9.3 kcal/mol, Cis-Solamin has an RMSD value below 3 Å so that the data can be said to be valid, Cis-Solamin has a Lower bond RMSD of 1.673 Å and an Upper bond RMSD of 2.590 Å. While the native ligand or control compound, namely OHT600, has a binding affinity value of -7.6 with a Lower bond RMSD of 0.681 Å and an Upper bond RMSD of 1.528 Å. Based on molecular docking, it was found that the Cis-Solamin compound has a higher binding affinity value than the OHT600 compound,

which was used as a control in this study. This indicates that Cis-Solamin has a greater binding strength to the Alpha Estrogen receptor, making it a potential candidate for breast cancer drugs. The bonds formed by the active compound Cis-Solamin are Hydrogen Bonds with the amino acid GLU532, which share similarities with the native ligand, specifically hydrogen bonds with the amino acids ALA359 & GLU380.

Based on molecular docking data, the active compound Cis-Solamin was identified in soursop leaves, which has a binding affinity with the Alpha Estrogen receptor (PDB ID: 3ERT). So the active compound in soursop leaves has the potential to be an ERα inhibitor because it has a high binding affinity value of -9.3 kcal/mol, Cis-Solamin has an RMSD value below 3 Å so that the data can be said to be valid; the smaller the RMSD value, the better the ligand conformation. The Cis-Solamin compound shows a higher binding affinity than the OHT600 compound, which is the original ligand found in the 3ERT protein. Alpha estrogen is the main target in ER+ breast cancer therapy, and inhibiting ERα activity has been shown to inhibit the growth of ER+ breast cancer cells.

Identification of Secondary Metabolite Compounds in Soursop Leaves (*Annona muricata*) Through Histochemical Analysis

Histochemical analysis results indicate that soursop leaves contain secondary metabolites, including flavonoids, phenols, tannins, terpenoids, and alkaloids. This finding is similar to the research of Fertilita et al. (2020), which showed that the phytochemical screening results of soursop leaves contained secondary metabolites, including alkaloids, flavonoids, tannins, and steroids. This finding aligns with the research of Ashafani et al. (2022), which revealed that the phytochemical screening results of soursop leaves contained secondary metabolites, including alkaloids, terpenoids, steroids, saponins, flavonoids, phenols, and tannins. Secondary metabolites are widely used for nutritional therapy or the treatment of various diseases, one of which is breast cancer.

Flavonoid Compound

Flavonoids are chemical compounds derived from 2-phenyl-benzyl-γ-pyrone, whose biosynthesis occurs via the phenylpropanoid pathway. Flavonoids play a crucial role in imparting colour, flavour, and aroma to seeds, flowers, and fruits. These compounds are easily oxidized at high temperatures and are not heat-stable. Flavonoids exhibit various pharmacological effects, including antioxidant, anti-ageing, anti-inflammatory, and antiviral properties. Research has shown that by 2011, more than 9,000 flavonoids had been discovered, many of which have been used as health supplements. Flavonoid compounds include flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins, and chalcones (Ningsih et al., 2023). The principle of total flavonoid analysis using $AlCl_3$ is based on the formation of a complex between $AlCl_3$ and the keto group at the C-4 atom and the hydroxyl group at the adjacent C-3 or C-5 atoms in the flavone and flavonol structures. An $AlCl_3$ solution is used to form a coloured complex with flavonoids, which causes a shift in wavelength to the visible region, characterised by a change in the solution's colour to a more yellow hue (Lindawati & Ma'ruf, 2020).

Phenolic Compound

Phenolic compounds are compounds containing hydroxyl groups, which generally also have the potential to exhibit antioxidant activity. Antioxidant activity is directly proportional to the total phenol content of a natural ingredient (Berlinsyah et al., 2021). The principle of the phenol test using $FeCl_3$ is that coloured phenolic compounds form complexes with Fe^{3+} in $FeCl_3$, resulting in darker (black) spots, which indicate the presence of phenolic compounds (Nurmalasari et al., 2020). Research by Qomaliyah (2022) also indicated that soursop leaves contain phenolic compounds, which have potential cytotoxic bioactivity against cancer cells.

Tannin Compound

Tannins are highly complex compounds and are widely distributed in various plant species. They are present in almost every plant species. In the health sector, tannins possess several properties, including antidiarrheal, antioxidant, antibacterial, and astringent properties. Tannins are typically found in specific plant parts, including the fruit, leaves, stems, and bark (Sunani and Hendriani, 2023). Phytochemical tests using $FeCl_3$ can detect the presence of phenol groups. If phenolic compounds are detected, tannins are likely present, as tannins are polyphenolic compounds. The color change to blackish green occurs due to the formation of a complex between tannins and $FeCl_3$ (Ikalinus et al., 2015). Other research suggests that soursop leaves contain secondary metabolites in the form of tannins, which have the potential to inhibit cancer cell growth due to their cytotoxic activity as therapeutic agents (Qomaliyah, 2022).

Terpenoid Compound

Terpenoid compounds found in plants exhibit significant pharmacological activities, including antiviral, antibacterial, anti-inflammatory, antihypertensive, antimicrobial, cholesterol synthesis inhibition, and anticancer properties. Compounds in the triterpenoid group are phytochemical agents that can selectively kill breast cancer cells and protect normal cells from damage (Soliha et al., 2017). Qualitative terpenoid testing was conducted by exploiting the colour-forming ability of terpenoid compounds contained in the sample leaves using a 5% $CuSO_4$ solution and glycerin, resulting in a colour change in the trichome tissue from clear to

brownish-yellow. This is consistent with research by Qomaliyah (2022), which states that soursop leaves contain secondary metabolites in the form of terpenoids, making them potential pharmacophores for cancer therapy.

Alkaloid Compound

Alkaloids are organic bases containing nitrogen (N) and are generally derived from plants, exhibiting strong physiological effects on humans. In pharmacology, alkaloids are used to stimulate the nervous system, increase blood pressure, and fight microbial infections (Wullur et al., 2018). Alkaloid analysis is carried out using Wagner's reagent. In preparations treated with Wagner's reagent, a colour change is observed in the tissue under a microscope. The reaction of Wagner's reagent will precipitate protoplasts, and a positive alkaloid content is indicated by a colour change, marked by a reddish-brown colour in the tissue (Nurhasanah & Iriani, 2021). In the study by Tiara et al. (2024), it was also stated that the secondary metabolite profile test using Wagner's reagent showed positive results for alkaloid compounds.

In a study by Rahayu et al. (2021), it was stated that the content of secondary metabolites in plant tissue varies depending on the location of the tissue and environmental conditions, including temperature and humidity. Leaf samples were sliced transversely at the abaxial section. This study also utilised fresh preparations, which were immediately observed under a microscope after slicing without any preservation process. In each sample, the tissue colour changed from clear to several distinct colours, allowing the identification of secondary metabolite content. The plant tissues that showed colour changes were the epidermal tissue of trichomes and stomata, as these tissues store secondary metabolites in plants that function as a defence against pests or diseases, and as a means of self-protection from environmental stress. Several other studies also reported that the results of histochemical analysis revealed colour changes in epidermal tissue, including trichomes and stomata (Anisa et al., 2018; Rahayu et al., 2021).

Relationship Between In Silico Analysis and Histochemistry

In this study, in silico analysis was employed to identify the presence of specific active compounds unique to soursop leaves, representing an innovative research approach. The results of specific compounds in soursop leaves are Annocatalin, Annomuricin A, Annomuricin E, Annohexocin, Muricatetrocin A, Murihexocin C, Cis-corossolane, and Cis-Solamin. These active compounds were subjected to molecular docking to determine their interactions and bonds to the estrogen alpha receptor protein (3ERT). By knowing the valid bond value between the ligand compound and the protein, it is evident that there is an interaction between the active compound and the estrogen alpha receptor. The Cis-Solamin compound has a higher binding affinity value than the OHT600 compound, which is used as a control in the in silico analysis, indicating that Cis-Solamin has a greater binding strength to the Estrogen Alpha receptor, making it a candidate for breast cancer drugs. This is evidenced by histochemical analysis of soursop leaves, which show positive results for the content of secondary metabolite compounds, including flavonoids, phenols, tannins, terpenoids, and alkaloids.

CONCLUSIONS

Based on in silico and histochemical analysis of soursop (*Annona muricata*) leaves on estrogen receptor alpha, it can be concluded that active compounds specific to soursop leaves include Annocatalin, Annomuricin A, Annomuricin E, Annohexocin, Muricatetrocin A, Murihexocin C, Cis-corossolane, and Cis-solamine. Molecular docking revealed that the active compound Cis-Solamin has a higher binding affinity than the native ligand. The binding affinity of Cis-Colamin is (-9.3 kcal/mol), while OHT600 has a binding affinity of (-7.6 kcal/mol). The docking results show that the Cis-Solamin compound with the highest binding affinity is bound to the amino acid 3ERT, namely Glutamine 532 (GLU523). Histochemical analysis of soursop leaves shows the presence of secondary metabolites in the form of flavonoids (reddish yellow), phenols (dark green), tannins (dark brown), terpenoids (brownish yellow), and alkaloids (brownish red).

Authors' Contributions: Afidah Fajar Nuari, Gatra Ervi Jayanti & Majida Ramadhan designed the study. Afidah Fajar Nuari carried out the laboratory work. Afidah Fajar Nuari analyzed the data. Afidah Fajar Nuari, Gatra Ervi Jayanti & Majida Ramadhan wrote the manuscript. All authors read and approved the final version of the manuscript.

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REFERENCES

Andriya, N., Nurafifah, N.S., Yohana, C.R., & Otih. 2016. Analisis Struktur Anatomi dan Histokimia Tiga Varietas Kumis Kucing (Orthosiphon aristatus Miq.). *Skripsi.* Departemen Biologi: Institut Pertanian Bogor.

Anisa, K., Rahayu, T., & Hayati, A. 2018. Profil Metabolit Sekunder Daun Tin (*Ficus carica*) melalui Analisis Histokimia dan Deteksi Flavonoid dengan Metode Kromatografi Lapis Tipis (KLT). *Jurnal Ilmiah Sains Alami* 1(1): 104-110

Asari, D. I. F., Rafsanjani, R., Vikriansyah, M. F., Muzayyan, A. R., Mubarakati, N. J., & Jayanti, G. E. 2023. Detection of Secondary Metabolites in Cucumber (*Cucumis sativus*) Leaves

and Its Potential as Candidates for Acne Drug Using Histochemical Analysis and In Silico Study. Exhibition and Seminar on Science and Creative Technology: University of Al-Azhar Indonesia Proceeding

Ashafani., & Majidah, E.L. 2022. Morfoanatomi Daun dan Batang Sembung Rambat (*Mikania micrantha*) yang Terpapar Pb Pada Lahan Tempat Pemrosesan Akhir (TPA) Junrejo Batu. *Skripsi*, Sains dan Teknilogi: Universitas Islam Negeri Malik Ibrahim

Berliansyah, S. Z., Dewi, A. R., & Purnomo, Y. 2021. Penentuan Kadar Fenol Total dan Aktivitas Antioksidan Fraksi n-Butanol Daun Pulutan (Urena Lobata). *Jurnal Bio Komplementer Medicine,* 8(2), 1–8.

Dewayani, A.R., Ghalia, S., Parameswari, N., Pribadi, A.P.A., Ahadi, H.M., Aulifa, D.L., Elaine, A.A., & Sitinjak, B.D.P. 2023. Studi *In silico* Senyawa Daun Sirsak (*Annona muricara L.*) Sebagai Inhibitor BRAF V600E Kinase Pada Kanker Melanoma. *Jurnal Farmasi Udayana,* 11(2), 80-88

Fertilita, S., Sandhika, W., & Suprabawati, D.G.A. 2020. The Cytotoxic Activity Of Annona muricara Linn Leaves Ethanolic Extract (AMLEE) On T47D Breast Cancer Cell Line. *Medical Laboratory Technology Journal,* 6(1), 32-39.

Ikalinus, R., Widyastuti, S., & Eka Setiasih, N. 2015. Skrining Fitokimia Ekstrak Etanol Kulit Batang Kelor (*Moringa oleifera*). *Indonesia Medicus Veterinus*, 4(1), 77.

Istiqomah, N., Fatikasari, S., & Hutuba, A.H. 2023. Kajian *in silico* Daun Sungkai (*Peronema canescens*) dalam Menghambat *Enzim lanosterol 14-α demethylase* Jamur *Candida albicans*. *Indonesian Journalof Pharmaceutical Education (e-Journal),* 3(1), 131-142.

Lestari, W. E., Adnyani, K. D., Prabowo, H., Siaka, P. A., & Laksmiani N. P. 2019. Aktivitas dari Kuersetin Sebagai Agen Pencerah Kulit Secara *In Silico*. *Jurnal Kimia,* 13 (2), 207-212.

Lindawati, N.Y., & Ma'ruf, S.H. 2020. Penetapan Kadar Total Flavonoid Ekstrak Etanol Kacang Merah (*Phaseolus vulgaris* L.) Dengan Metode Kompleks Kolorimetri Secara Spektrofotometri Visibel. *Jurnal Ilmiah Manuntung,* 6(1), 83-91.

Manno, M. R., & Utami, D. 2023. Senyawa Aktif Kelautan Indonesia sebagai *Inhibitor Protease* Utama SARS-CoV-2:Studi *Molecular Docking*. *Jurnal Ilmiah Farmasi,* 19(2), 182-194.

Mubarakati, N. J., Oktavia, R. P., & Rahayu, T., Alik, M. 2019. *In silico* Exploration the Phenolic Compound of Olive Leaves as Acetylcholinesterase Enzyme (AChE) Inhibitor for Alzheimers Disease Theraphy. *Journal of Biological Researches,* 24(2).

Ningsih, I.S., Chatri, M., Advinda, L., & Violita. 2023. Flavonoid Active Compounds Found In Plants. *Serambi Biologi,* 8(2), 126-132

Nurhasanah T., Lukmayani Y., & Kodir R.A. 2019. Karakterisasi Simplisia dan Ekstrak serta Identifikasi Histokimia Daun Pulutan *(Urena lobata L.). Prosiding Farmasi,* 5(1), 29-35.

Nurhasanah., & Iriani, D. 2021. Jurnal Biologi Tropis Histochemical Test of root, petiole and leaf of Kelembak (*Rheum officinale* Baill). *Jurnal Biologi Tropis,* 21(3), 726–733.

Nurmalasari, E.Y., Luliana, S., & Wahdaningsih, S. 2020. Identifikasi Senyawa Fenol Dan Flavonoid Dari Berbagai Bagian Tanaman Senggani (*Melastoma malabathricum* L.) Menggunakan Metode Kromatografi Lapis Tipis. *Jurnal Fakultas Kedokteran Universitas Tanjungpura* 1-5.

Pertiwi W., Dessy A., & Linosefa. 2020. Pengaruh Ekstrak Daun Sirsak (*Annona muricate Linn*) Terhadap Viabilitas Cell Line Kanker Payudara T47D Secara In Vitro. *Jurnal Kesehatan Andalas,* 9(15), 165-170.

Prieto, C, B., Rivera, F., Romero, A., Romero, M., Gómez S, J., & Ruiz F, C. 2020. Relationship between chemotherapy-induced adverse reactions and health-related quality of life in patients with breast cancer. *Medicine* 99(33), 21695. doi.org/10.1097/MD.0000000000021695

Qomaliyah, E.N. 2022. Etnofarmakologi dan Potensi Bioaktivitas Daun dan Buah Sirsak (*Annona muricata*). *BIOCITY Journal of Pharmacy Bioscience and Clinical Community,* 1(1), 36-55.

Rahayu, T., Pratiwi, R. I. A., & Mubarakati, N. J. 2021. Profil Metabolit Daun Kesambi (Schleichera oleosa) Berdasarkan Analisis Histokimia dan In Silico. *Metamorfosa: Journal of Biological Sciences,* 8(1), 156-165.

Rupa, D. 2015. Indentifikasi Struktur Sekretori dan Analisis Histokimia dan Uji Fitokimia Tumbuhan Obat Antiinfeksi di Kawasan Taman Nasional Bukit Dua Belas Jambi. *Tesis*. Institut Pertanian Bogor.

Sargsyan, K., Grauffel, C., & Lim, C. 2017. How Molecular Size Impacts RMSD Applications in Molecular Dynamics Simulations. *Journal of Chemical Theory and Computation, 13*(4), 1518-1524.

Siswarni, M.Z., Nurhayani, & Sinaga, S.D. 2016. Ekstraksi Acetogenin dari Daun Dan Biji Sirsak (*Annona muricata* L) dengan Pelarut Aseton. *Jurnal Teknik Kimia USU*, 5(2), 1-4.

Soliha, I., Widiyantoro, A., & Destiarti, L. 2017. Karakterisasi Terpenoid Dari Fraksi Diklorometana Bunga Nusa Indah (*Mussaenda erythrophylla*) Dan Aktivitas Sitotoksiknya Terhadap Sel Kanker Payudara T47D. *JKK* 6(4): 10–14.

Sunani, S., & Hendriani, R. 2023. Classification and Pharmacological Activities of Bioactive Tannins. *Indonesian Journal of Biological Pharmacy,* 3(2), 130-136

Tiara., Faisal., & Ramadhan, M. 2024. Profil Metabolit Sekunder dari Ekstrak Etanol Buah Delima Putih (*Punica granatum* L.). *JIMSUM,* 2(2), 156-162.

Wullur, A.C., Schaduw, J., & Wardhani, A.N.K. 2018. Identifikasi kandungan pada daun sirsak (*Annona muricata* L.). *JIF-Jurnal Ilmiah,* 3(2), 54–56.

Xue, M, Zhang K, Mu K, Xu, J., Yang, H., Liu, Y., Wang, B., Wang, Z., Li, Z., Kong, Q., Li, X., Wang, H., Zhu, J., & Zhuang, T. 2019. Regulation of Estrogen Signaling and Breast Cancer Proliferation by An Ubiquitin Ligase TRIM56. *Oncogenesis*, 8(30), 1-14.

Yuniati, N. I., Islamiyati, D., Khasanah, N. A., & Husen F. 2023. Perbandingan Senyawa Kuersetin dan Kaemferol pada reseptor COX-2 Sebagai gen antikanker kolorektal secara *in silico*. *Jurnal Bina Cipta Husada,* 19 (01), 98-107.