OPEN ACCESS
Journal of Multidisciplinary Applied Natural Science Vol. 5 No. 1 (2025) https://doi.org/10.47352/jmans.2774-3047.246
Check for updates
Research Article

Potential of Bioactive Compounds In *Coleus amboinicus*, Lour., Leaves Against Breast Cancer By Assessment Using A Network Pharmacology Approach and Cytotoxic Test

Winarto Haryadi*, Kasta Gurning, Jajah Fachiroh, and Endang Astuti

Received : September 18, 2024	Revised : November 25, 2024	Accepted : November 25, 2024	Online : January 26, 2025

Abstract
Breast cancer is a disease that significantly contributes to global women death. The study aims to conduct *in vitro* activity testing and assessment with a bioinformatics approach using a pharmacological network of bioactive compounds from bangun-bangun (*Coleus amboinicus*) leaves extract as a breast cancer drug. The methods used are extraction of bioactive compounds by maceration and partition, identification and analysis of bioactive compounds using the Liquid Chromatography High-Resolution Mass Spectrometry (LC-HRMS) instrument, cytotoxic testing of breast cancer cells (MCF-7) and normal cells (CV-1) with the MTT method, and assessment with a bioinformatics approach through a network pharmacology. The results of the cytotoxic test of ethyl acetate extract provided better activity with IC_{50} value of 102.30 and 457.09 µg/mL against MCF-7 cancer cells and CV-1 normal cells. The selectivity index value of 4.23 indicates the potential for further development in the treatment of breast cancer. The results of the analysis of chemical compound content show various types of potential bioactive compounds as breast cancer anticancer; assessment of the bioinformatics approach through networks pharmacology with pathways in cancer provides predictions of signal transducer and activator of transcription 3 (STAT3) protein as the main therapeutic mechanism target in breast cancer treatment. This study provides initial information for further research on testing and utilizing bioactive compounds from *C. amboinicus* leaves as an alternative treatment for breast cancer.

Keywords: *Coleus amboinicus*, bioinformatics, pharmacology network, breast cancer, MCF-7, STAT3

1. INTRODUCTION

Breast cancer is the most common type of disease diagnosed in women. Over the past two decades, breast cancer has continued to increase and become the highest contributor to women death [1]-[3]. More than two million women are diagnosed each year in various age ranges, and it is a common and high-occurring disease in more than 100 countries in the world [4]. In developed countries, the age of diagnosis is 68 years and in Indonesia, it is diagnosed around the age of 48 years, and around 5,000 women are diagnosed under the age of 50 years [5][6]. Based on geographical areas, the incidence in developed areas is higher than in developing countries [7][8]. Risk factors for the occurrence are known, such as environmental factors, lifestyle behavior, genetics, metabolomic factors, eating habits, and others [9]. Genetic factors such as family members who have breast or ovarian cancer, which causes congenital mutations in BRCA1 and BRCA2, contribute to 5–10% of cases [4]. In 2020, the incidence and mortality rates of age-standardized breast cancer ranked first and second among all cancers [10].

Understanding the high-risk factors, incidence, and mortality associated with breast cancer, prevention, and control are very important [10]. Cancer events due to genetic factors that encode proteins in the cell cycle or because of somatic mutations in upstream cell signaling make conventional therapy or treatment unsuccessful. This incident is also exacerbated by the occurrence of cancer cell metastasis, malignancy, and resistance to chemotherapy and radiotherapy, resulting in failure of healing [11]. Research and development of various cancer treatments continue to be pursued with the aim of obtaining effective and efficient drugs and minimizing the side effects they cause. The side effects of chemotherapy and radiotherapy have been reported to increase the risk of cardiovascular disease, and this occurs acutely, during the treatment process, or decades after treatment [12]. Treatment with targeted immunotherapy is an alternative that continues to be developed for patients with early-stage and metastatic breast cancer. Treatment with

Table 1. Testing the activity of bioactive compounds.

No.	Sample	IC_{50} (µg/mL)		Selectivity Index (SI)
		Breast cancer (MCF-7)	Normal (CV-1)	
1.	Initial EtOH extract	154.80	501.19	3.24
2.	*n*-C_6H_{12} extract	217.20	630.96	2.91
3.	CH_3Cl extract	102.30	189.10	1.85
4.	EtOAc extract	108.00	457.09	4.23[b]
5.	Residual EtOH extract	396.40	531.00	1.34
6.	Cisplatin	53[a]		

Description: [a]Concentrations are expressed in µM or equivalent to 15.96 ppm, and [b]selected extracts against breast cancer.

immunotherapy agents or in combination is considered promising for breast cancer patients with certain conditions. Therefore, the integration of biomarkers and consideration of dynamic signs of early response or resistance provide optimal information in the investigation of immunotherapy and its clinical practice integration [13]. Treatment with the development of new therapeutic drugs that are specifically in the treatment of breast cancer continues to be carried out, one of which is by utilizing natural products such as those sourced from plants [14]-[17].

Plants have various groups of bioactive compounds such as terpenoids, flavonoids, phenolics, polyphenols, and alkaloids that provide cytotoxic activity *in vitro* and *in vivo* against breast cancer cells with various mechanisms in inhibiting intrinsic and extrinsic apoptosis pathways, autophagy activity, and stopping cancer cells [14]. Bangun-bangun (*Coleus amboinicus*) plant has 76 volatile compounds and 30 non-volatile compounds such as the monoterpenoid, diterpenoid, triterpenoid, sesquiterpenoid, phenolic, flavonoid, alcohol, and aldehyde esters [18]-[20]. The study of various possible mechanisms of various bioactive compounds in natural product products was carried out through a bioinformatics approach through pharmacology networks. This study aims to reveal the mechanism of bioactive compound content in *C. amboinicus* leaf extract with a bioinformatics approach through pharmacology networks and cytotoxic testing against breast cancer cells (MCF-7).

2. MATERIALS AND METHODS

2.1. Materials

Materials in the preparation process, analysis of bioactive compounds, and testing of *C. amboinicus* leaf extract on MCF-7 cancer cells refer to the research data we conducted previously [21]. The materials used for molecular docking based on the results of pharmacological studies are computers (Intel Core I5 with Windows 10 operations), PyMol 2.3, PyRx 0.8, Auto Dock Tools 1.5.6, GaussView 5.0 (MDL Information System, Inc.), and Discovery Studio 21.0 Client (DSV 19.0).

2.2. Establishment of Compounds Information

The extraction process of *C. amboinicus* leaf samples using ethanol solvents was then carried out in stages with *n*-hexane, chloroform, and ethyl acetate solvents, and finally the residue (ethanol and water) was obtained. Each extract was tested for anticancer activity against MCF-7 cancer cells using the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method and against normal cells (CV-1). The results of the activity test were calculated as the selectivity index (SI) value using the formula 1 [22][23].

$$SI = \frac{IC_{50}\ value\ for\ normal\ cell}{IC_{50}\ value\ for\ breast\ cancer\ cell} \quad (1)$$

The extract with the best anti-breast cancer activity (or lowest IC_{50} value) and a greater selectivity index for cancer was continued with the analysis of bioactive compounds using the Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) instrument. Bioactive compounds were filtered, and ten dominant compounds were selected based on kastan the

highest area and were considered to have the potential to be breast anticancer.

2.3. Assessment of Active Compounds Against Target Cancers Using Pharmacological Networks

2.3.1. Pharmacological Identification of Active Compounds Against Target Cancers

Ten bioactive compounds (target compounds) were drawn using ChemDraw to obtain SMILE data, then uploaded to the SWISS target prediction database (http://www.swisstargetprediction.ch/). The gene data for each target compound was downloaded in CSV format, filtered, and integrated using Microsoft Excel. This data provides information on the target proteins of drug candidates that are used to identify therapeutic protein targets for diseases. The therapeutic target of the disease was set as "breast cancer," obtained from the National Center for Biotechnology Information GeneCards database (https://www.genecards.org/), Therapeutic Target Database (TTD; https://db.idrblab.net/ttd/), Online Mendelian Inheritance in Man (OMIM; https://www.omim.org/), and DisGeNET (https://www.disgenet.org/) [15][24][25]. The gene information of bioactive compounds and therapeutic proteins of cancer targets was integrated using the VENNY diagram (https://www.biotools.fr/misc/venny) to obtain protein targets.

2.3.2. PPI between Bioactive Compounds and Breast Cancer Target Proteins

The Protein-Protein Interaction (PPI) network between selected bioactive compounds of *C. amboinicus* extract and breast cancer therapeutic proteins was constructed and analyzed using the STRING database (https://string-db.org/) with the "homo sapiens" setting and a confidence level of 0.7. PPI interactions are expressed by Node1 and Node2, and the combined score of the imported export file was constructed using Cytoscape 3.10.2 software [26]. The results of the analysis to

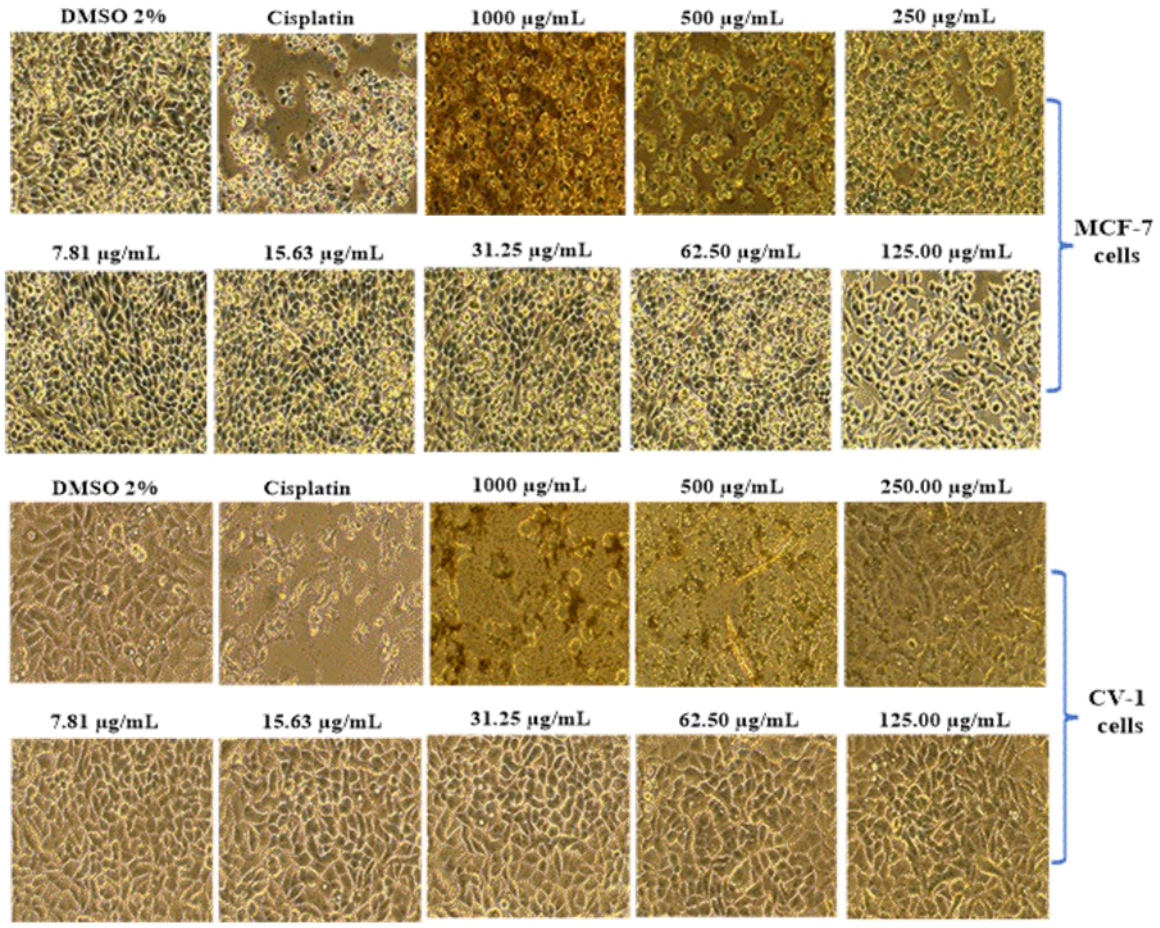


Figure 1. Morpology of cytotoxic testing of EtOAc extract against breast cancer and normal cells.

Table 2. Potential bioactive compounds from the EtOAc extract against breast cancer.

No.	Compounds name	Formula	RT (min)	Ionisasi (FTMS)	Area	Molecular weight (g/mol)		Pubchem ID	Ref
						Obs	Calc		
1	Resolvin D1	$C_{22}H_{32}O_5$	14.075	+	19855216920	376.224	376.50	44251266	[34]-[38]
2	Gibberellin A24	$C_{20}H_{26}O_5$	14.117	-	3531256616	346.178	346.40	443454	[39][40]
3	Hexyl 2-furoate	$C_{11}H_{16}O_3$	6.222	+	2206344168	196.110	196.24	61984	[41]
4	13(*S*)-HOTrE	$C_{18}H_{30}O_3$	12.239	-	1803807869	294.219	294.40	16061072	[42]-[44]
5	Arachidonic acid	$C_{20}H_{32}O_2$	14.518	+	1022268949	304.240	304.50	444899	[45]-[48]
6	4-Indolecarbaldehyde	C_9H_7NO	6.442	+	553570860	145.053	145.16	333703	[49]-[52]
7	Asiatic acid	$C_{30}H_{48}O_5$	11.209	-	505413139	488.353	488.70	119034	[53]-[55]
8	9(*S*)-HpOTrE	$C_{18}H_{30}O_4$	12.605	-	403834858	310.214	310.40	6450029	[56]
9	Caffeic acid	$C_9H_8O_4$	4.229	-	281564542	180.042	180.16	689043	[57]-[61]
10	Arctiopicrin	$C_{19}H_{26}O_6$	11.770	-	269776327	350.173	350.40	5281423	[48][62][63]

determine the main protein targets using the CytoHubba plugin.

2.3.3. GO and KEGG

GO and KEGG enrichment pathway analysis was explored from the overlapping of each gene of bioactive compounds in *C. amboinicus* leaves extract with breast cancer genes using bioinformatic data (ShinyGO 0.80) with the "Human" setting to obtain combined targets. GO enrichment studies are expressed in biological functions, biological processes, cellular components, and KEGG pathways of potential targets of bioactive compounds in breast cancer treatment. The target protein studied by molecular docking analysis is one of the main target proteins found in the pharmacological network of cancer pathways.

2.4. Molecular Docking

Molecular docking is a method of designing active drug compounds by exploring interactions with receptors, where the active compound acts as a ligand and the receptor is the main target protein. This study is a theoretical simulation that focuses on intermolecular interactions, predictions of bond types, and their affinities [26]. First, each active compound that has been determined is optimized for its molecular structure to obtain its ideal geometric conformation using Gaussian 09W software with the DFT method, B3LYP hybrid function, and 3-21G basis set. The target protein is obtained from the Protein Data Bank (PDB; http://www.rcsb.org/) database. Molecular docking between the active compound (ligand) and the target protein is performed using Autodock Vina software. The results of molecular docking are visualized using AutoDock Tools and Discovery Studio Visualizer.

3. RESULTS AND DISCUSSIONS

3.1. Selection of C. amboinicus Leaves Extract

The *C. amboinicus* leaves were extracted using the maceration method (solid–liquid) using an ethanol solvent, filtered using Whatman No. 1 filter paper, and an ethanol extract of *C. amboinicus* leaves (the initial extract). The ethanol extract (initial extract) obtained was then partitioned (liquid–liquid) in stages using a separating funnel

with *n*-hexane, chloroform, and ethyl acetate solvents, so that five extracts were obtained. The addition of water in the partition process was carried out to clarify the boundary between extracts in the separating funnel. Each liquid extract obtained was concentrated using a rotary vacuum evaporator to obtain a thick extract, and activity testing was continued against breast cancer cells (MCF-7) and normal cells (CV-1) using the MTT method. The results of the activity test against cancer and normal cells are shown in Table 1. The results of the activity test were determined by the selectivity index value.

Ethyl acetate (EtOAc) extract was selected as the best activity for breast cancer. The selected extracts were further analyzed using an LC-HRMS instrument to obtain ten potential bioactive compounds against the target cancer. The National Cancer Institute (NCI) classifies the anticancer agent based on cytotoxic activity (IC_{50}) values, i.e., IC_{50} < 20 µg/mL in the high category, IC_{50} 20–100 µg/mL in the medium category, IC_{50} 101–500 µg/mL in the weak category, and IC_{50} > 500 µg/mL in the inactive category [27]-[29]. The anticancer activity of all *C. amboinicus* leaves extracts against MCF-7 cells is included in the weak category. Each extract activity value is stronger against cancer cells compared to normal cells, and the activity of cisplatin as a drug is included in the high category (53 µM or 15.93 µg/mL) (Figure 1). The concentration of EtOAc extract between 62.50 and 125.00 µg/mL clearly shows the difference in the growth or density of MCF-7 cancer cells (it has begun to loosen or decrease), indicating that growth inhibition has occurred. This is in line with the IC_{50} value of cytotoxic EtOAc extract against MCF-7 cancer cells (102.30 µg/mL), which is included in the weak category. Increasing the activity of therapeutic drugs or natural compounds can generally be done by making them nanoparticles with ideal size and shape engineering results, as well as increasing their solubility, biodistribution, and blood circulation half-life [30][31]. In normal cells at concentrations between 250 and 500 µg/mL, a clear difference is seen in that the density of cell growth has been inhibited, and it is suspected that there is damage to the integrity of the cell membrane (lysis process), as indicated by the increasing loss of black marks (cell membranes) in that concentration range. This is in line with the

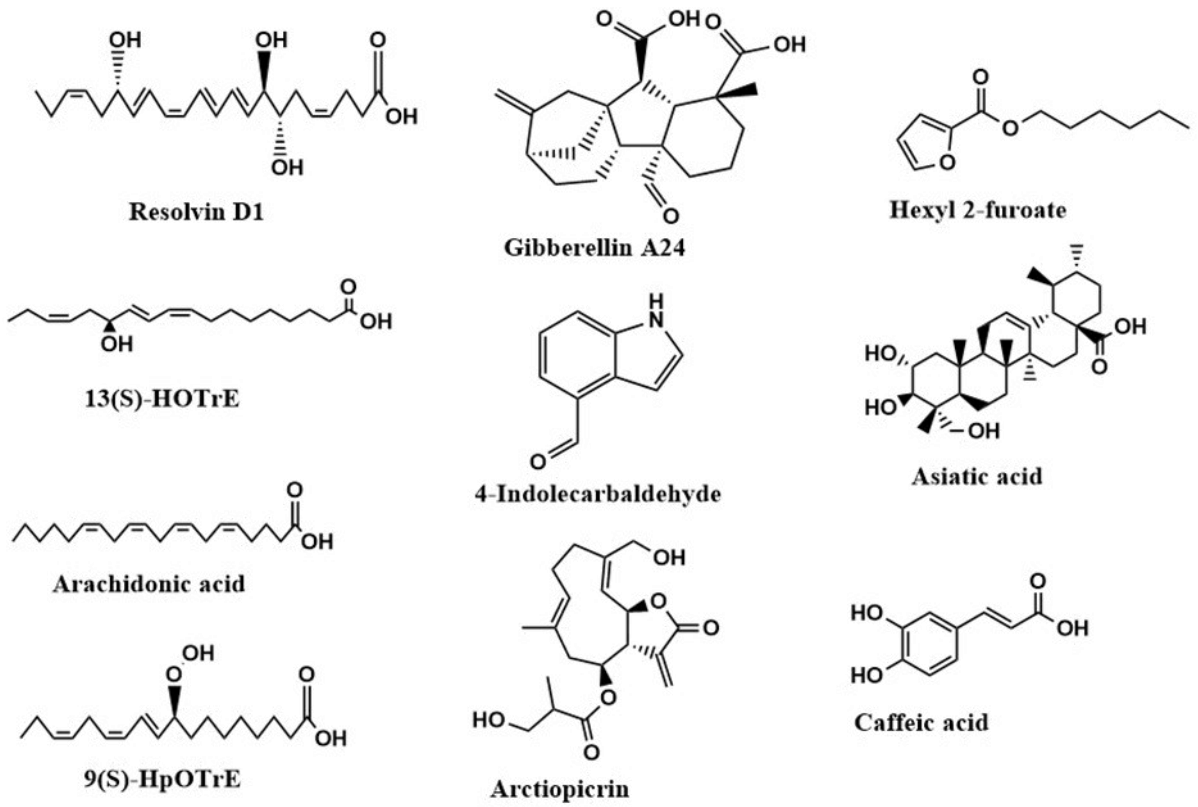


Figure 2. Structure of potential bioactive compounds from the EtOAc extract against breast cancer.

IC_{50} value of ethyl acetate extract against normal cells at a concentration of 457.09 µg/mL. The activity of the selected extract showed stronger cytotoxicity against cancer cells compared to normal cells and had an SI value > 3 [32][33], so it is worthy of further analysis to identify the content of bioactive compounds as an alternative in the treatment of breast cancer.

The potential anti-breast cancer activity of bioactive compounds from EtOAc extract of *C. amboinicus* leaves has not shown more promising activity compared to the activity of bioactive compounds from various plants such as cruciferous vegetables (benzyl isothiocyanate, isothiocyanate), blueberries (resveratrol, phytoestrogens, polyphenols), and soybeans (genistein, phytoestrogens) that have been successfully isolated. However, the results of this study provide important initial information in the exploration of bioactive compound content (single compounds) for the development of therapeutic drugs for breast cancer in the future. The existence of diverse bioactive compound content in the extract provides its own complexity in understanding the mechanism of action (synergistic or antagonistic) of each bioactive compound in treating the disease.

Ten target bioactive compounds from EtOAc extract were further investigated for their anti-breast cancer activity (Table 2 and Figure 2). The selection of ten bioactive compounds from EtOAc extract was based on the highest area and potential anti-breast cancer activity from literature studies and PASS online database sources (https://www.way2drug.com/passonline/predict.php). The selected bioactive compounds were predicted by ADMET using databases (http://www.swissadme.ch/ and https://tox.charite.de/protox3/index.php?site=home) (Table 3). The predicted bioactive compounds were then subjected to correlation analysis against breast cancer proteins through a pharmacological network pathway approach. The pharmacological pathway approach chosen was the pathway in cancer, and then validation of experimental data on the potential of bioactive compounds against key proteins from the pathway in cancer was carried out with molecular docking studies using computation.

Table 3. ADMET and PASS online predictions of bioactive compounds of the EtOAc extract.

No.	Compounds	Physicochemical Properties					Drug-Likeness	Toxicity Prediction		PASS online	
		MW (g/mol)	HBA	HBD	TPSA (Å^2)	Log P		LD_{50} (mg/kg)	Class	Pa	Activity
1.	Resolvin D1	376.49	5	4	97.99	3.46	-	3,389	5	0.931	CYP2J substrate
2.	Gibberellin A24	145.16	1	1	32.86	1.66	Yes	1,250	4	0.739	CYP2F1 substrate
3.	Hexyl 2-furoate	196.24	3	0	39.44	2.77	Yes	1,500	4	0.737	CYP2J substrate
4.	13(*S*)-HOTrE	310.43	4	2	66.76	5.13	-	10,000	6	0.921	CYP2J substrate
5.	Arachidonic acid	180.16	4	3	77.76	0.93	Yes	2,980	5	0.887	CYP2J substrate
6	4-Indolecarbaldehyde	350.41	6	2	93.06	1.77	Yes	452	5	0.866	Apoptosis agonist
7	Asiatic acid	304.47	2	1	37.30	6.22	-	10,000	6	0.951	PPI*
8	9(*S*)-HpOTrE	346.42	5	3	91.67	2.45	Yes	2500	5	0.783	Apoptosis agonist
9	Caffeic acid	488.70	5	4	97.99	4.45	Yes	2,000	4	0.982	Hepatoprotectant
10	Arctiopicrin	294.43	3	2	57.53	4.63	-	650	4	0.951	CYP2J substrate

*Phosphatidylglycero phosphatase inhibitor

3.2. Identification of Bioactive Compounds Against Target Cancer

Based on the investigation of ADMET prediction, online PASS and toxicology showed all bioactive compounds in the ethyl acetate extract of *C. amboinicus* leaves have the potential to be anti-breast cancer. Prediction of genes (proteins) of bioactive compounds for Resolvin D1 (NA), Gibberellin A24 (33 genes), Hexyl 2-furoate (43 genes), 13(S)-HOTrE (102 genes), Arachidonic acid (110 genes), 4-Indolecarbaldehyde (10 genes), Asiatic acid (69 genes), 9(S)-HpOTrE (110 genes), Caffeic acid (49 genes), and Arctiopicrin (NA) (Table S1). Breast cancer genes filtered results 14,180 genes. The total bioactive compound genes from ethyl acetate extract are 526 potential genes and are filtered by removing duplicate genes to obtain 282 target genes. These genes are analyzed to find genes that overlap with breast cancer genes (Figure 3).

3.3. PPI Network Analysis of Bioactive Compounds with Target Cancer

The intersecting target genes from each were imported into the STRING database to build potential relationships, which were investigated using a confidence score of 0.7. The results of STRING Data analysis of bioactive compounds of *C. amboinicus* leaves EtOAc extract on breast cancer obtained the number of nodes 265, the number of edges 1,013, the average node degree 7.65, the average local clustering coefficient 0.527, the expected number of edges 325, and the enrichment p value $<10e^{-16}$. Cytoscape analysis showed the main clusters related to the PPI network based on edge count, average shortest path length, betweenness centrality, closeness centrality, and clustering coefficient. PPI interaction STRING data, major clusters associated with the PPI network, and 15 potential target proteins from the cancer pathway of bioactive compounds of EtOAc extract against breast cancer are shown in Figures 4 (a)-(c) (Table S2).

3.4. GO and KEGG Pathway Analysis

Gene ontology (GO) analysis conducted includes GO biological process, GO cellular component, GO molecular function, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) of each gene (protein) that intersects with bioactive compounds against target cancer using the ShinyGO 0.80 database. ShinyGO 0.8 database uses a confidence score of 0.05 by setting a special “homo sapiens." The results of the GO and KEGG analyses of the bioactive compounds of the EtOAc extract of *C. amboinicus* leaves in the treatment of breast cancer are shown in Figures 5(a)–(d).

Bioinformatic study of GO biological processes provides prediction of specific genetic analysis of molecular sequences to produce certain gene products from an organism. GO cellular molecule provides prediction of location description relative to compartment and cell structure where molecular function occurs, while GO molecular function

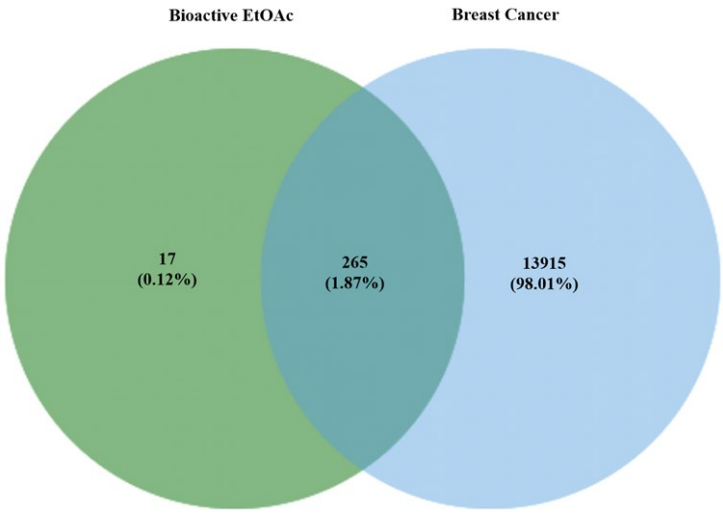


Figure 3. Venn diagram depicting the overlap between bioactive compounds of *C. amboinicus* leaves ethyl acetate extract and breast cancer.

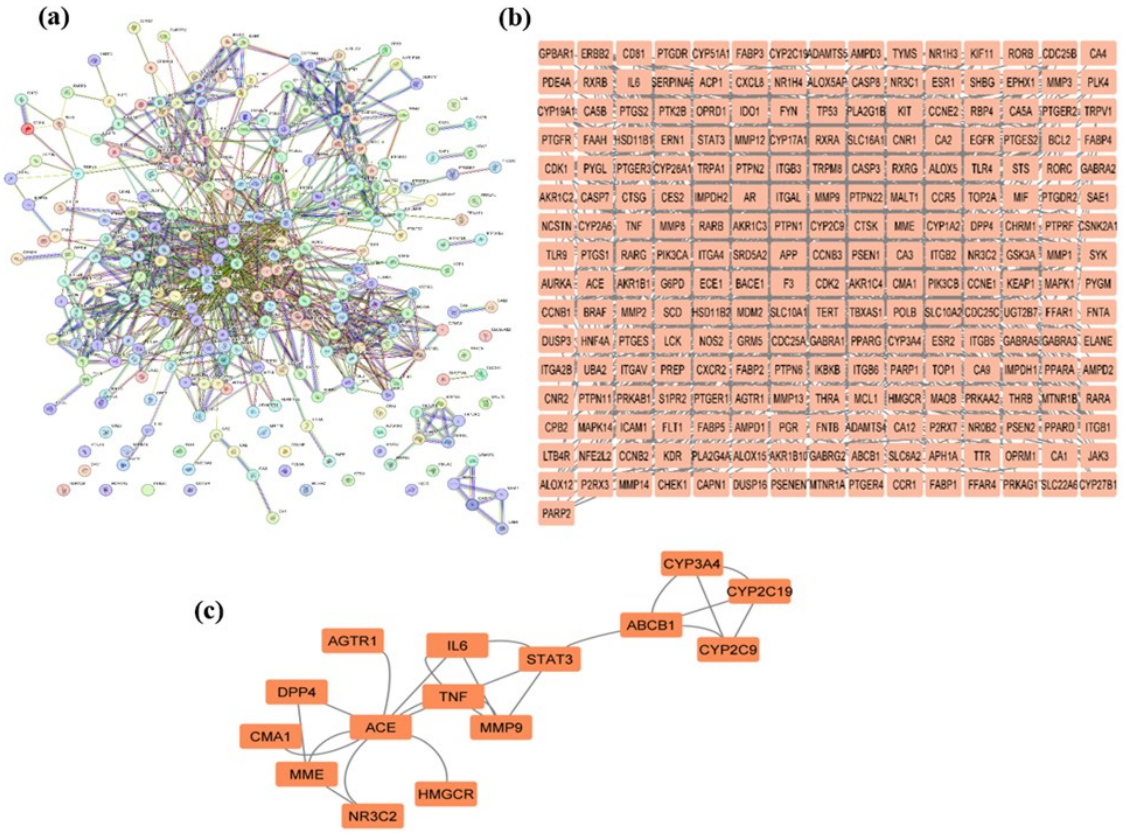


Figure 4. PPI analysis of bioactive compounds of *C. amboinicus* EtOAc extract against breast cancer. (a) PPI network from the STRING database; (b) PPI network built using the cytoHubba 3.10.2 plug-in target. STRING database; (c) 15 potential gene targets in the PPI network from network analysis using the CytoHubba 3.10.2 plug-in.

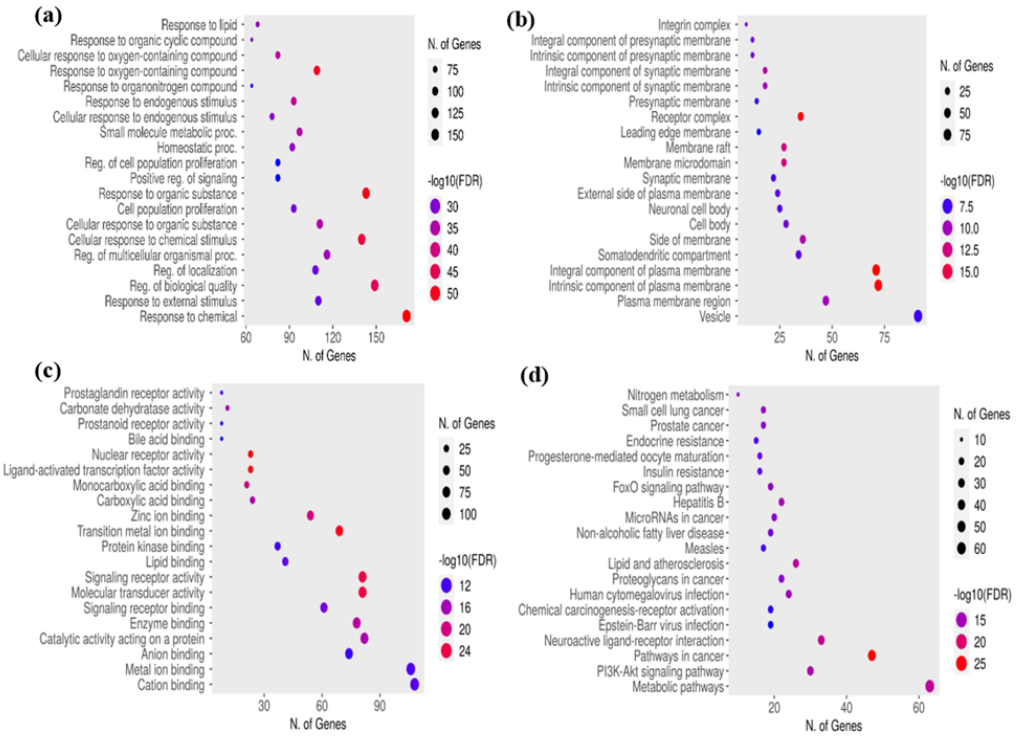


Figure 5. GO and KEGG analysis of bioactive compounds of EtOAc extract in breast cancer treatment. (a) biological process; (b) cellular component; (c) molecular function; and (d) potential KEGG of bioactive compounds.

studies the process carried out by macromoleculars through direct interaction with other molecular entities, including biochemical activity and role as a component in a larger molecular process system [64]. KEGG provides biological interpretation, including cellular and organism-level functions. Various molecular-level data sets containing gene sets in complete genomes are generally widely used in the approach to uncovering pharmacological pathways [65]. This study revealed 20 possible components of the GO biological process: the GO cellular molecule, the GO molecular function, and the KEGG of bioactive compounds against target cancer diseases. Bioinformatic studies such as this are part of the development of targeted and rational active compounds for the discovery of alternative new drugs by utilizing active compounds sourced from natural materials. Utilizing biological integration and polypharmacology bases with various considerations of network interactions between potential bioactive compounds against target diseases makes it possible to find potential new drugs for various diseases [66]. This study reveals the correlation of the results of cytotoxic testing of the dominant bioactive compound content of the EtOAc extract of *C. amboinicus* leaves against breast cancer cells with a pharmacological network approach in cancer pathways. Proteins involved in the cancer pathway network are shown in Figures 6(a) and 6(b), showing the relationship between pathways in cancer and other pharmacological pathways of bioactive compound activity (Table S3).

Based on the results of the analysis of GO and KEGG in the general cancer pathway network, the main protein to continue the molecular docking analysis is the STAT3 protein. The STAT3 protein is the third potential protein in the three PPI analysis results. Constitutively, the Signal Transducer and Activator of Transcription 3 (STAT3) protein is almost always activated in various types of cancer, including more than 40% of breast cancers [34]. STAT3 is a protein that is a cytoplasmic transcription factor that mediates intracellular signaling on the surface of receptor cells to send signals to the nucleus when activated by cytokines and growth factors [67][68]. Under normal conditions, STAT3 protein activation occurs quickly and temporarily and functions to regulate nuclear genes that control bassic biological processes such as proliferation, sursvival, and cell development [68]. The cellular functions of STAT 3 proteins include protein phosphorylation by specific kinases, dimerization that drives phsosphorylation, and gene expression activity by phosphorylated dimers [69]. Under abnormal conditions, STAT3 signaling promotes the initiation and development of cancer cells by inhibiting apoptosis or inducing cell proliferation, invasion, angiogenesis, and metastasis. Therefore, suppression of STAT3 activity in cancer patients results in the induction of apoptosis in tumor cells or cancer cells and

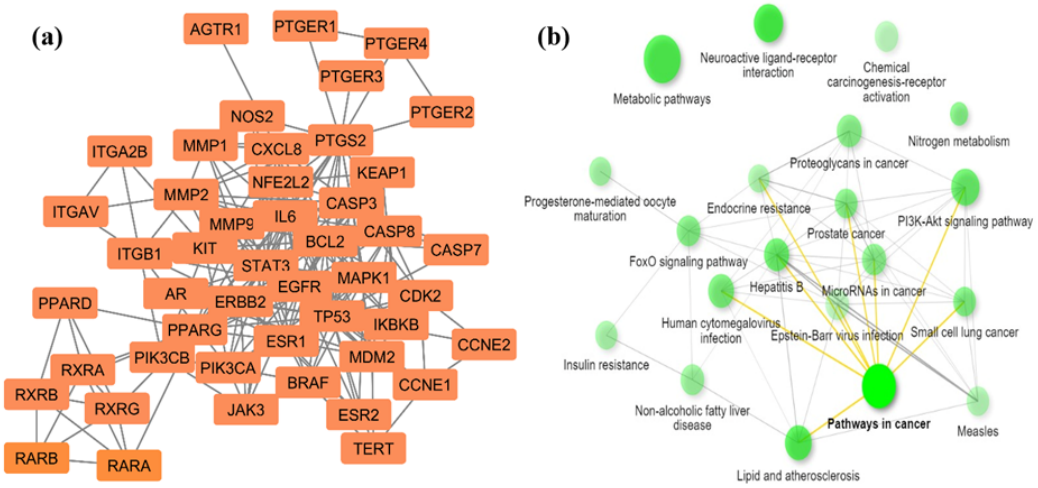
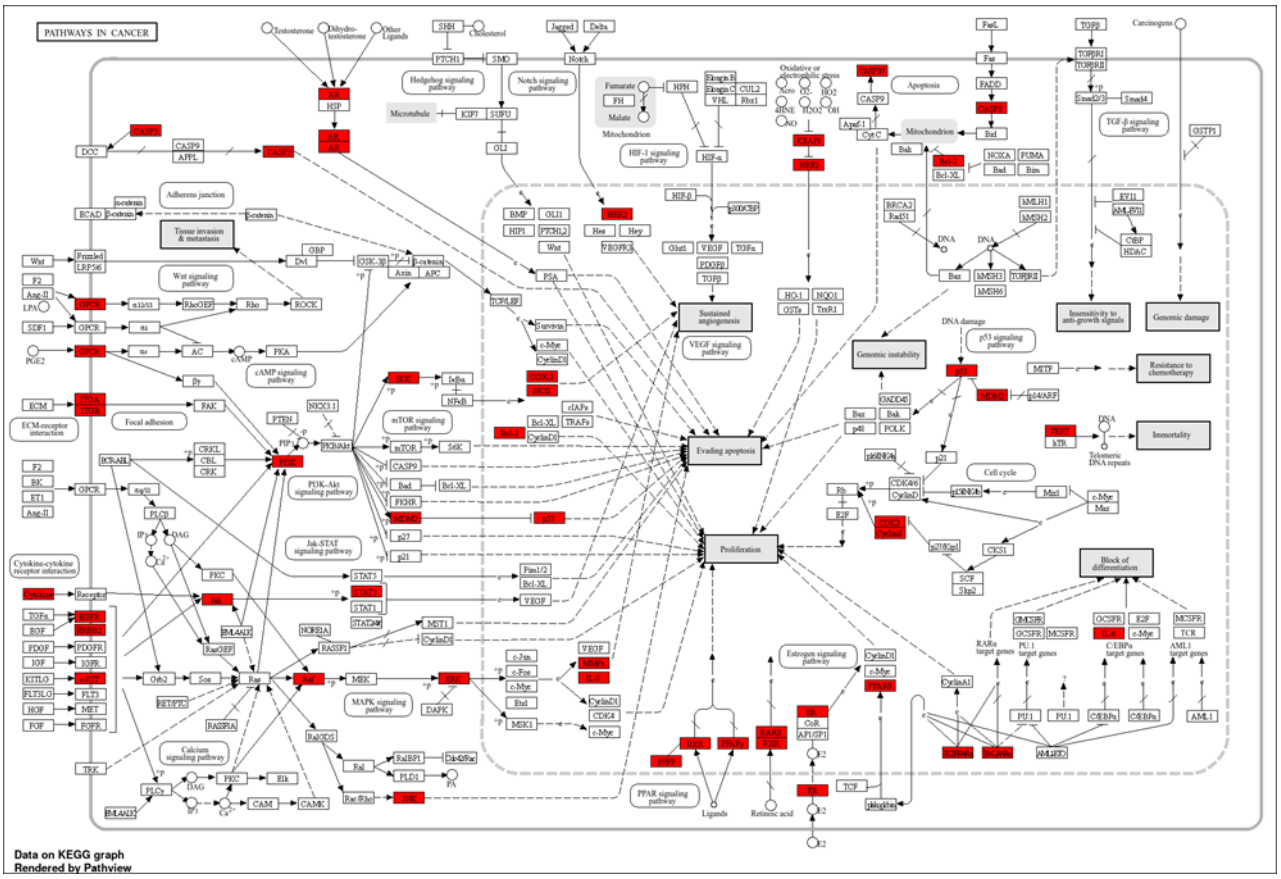


Figure 6. (a). PPIs involved in the cancer pathway network, and (b) prediction of the relationship of the cancer pathway network with other pathways included in the top 20 KEGG. This analysis uses the ShinyGO 0.80 database with an FRD cutoff of <0.05.



[S]**Figure 7.** Prediction of the cancer pathway network of bioactive compounds in the ethyl acetate extract of *C. amboinicus* leaves using the ShinyGO 0.8 database with an FDR cutoff of 0.05.

encourages pharmacological modulation by tyrosine kinase inhibitors, antisense, decoy nucleotides, oligonucleotides, dominant negative proteins, RNA interference, and chemopreventive agents in suppressing breast cancer cell proliferation that occurs [70][71]. Literature-based identification results show the interaction of bioactive compounds with STAT3 protein in cancer pathways, including resolvin D1 and hexyl 2-furoate compounds, which play a role in inhibiting tumor inflammation [72] [73]; gibberellin A24 plays a role in inhibiting FGFR 1 activation and KDR activation [40]; 13(*S*)-HOTrE and 9(*S*)-HpOTrE compounds play a role in apoptosis and anti-inflammation [42][56]; arachidonic acid plays a role in suppressing growth, migration, and invasion [74]; 4-indolecarbaldehyde plays a role in apoptosis [75]; asiatic acid plays a role in inhibiting phosphorylation, inducing cell death, and reducing tumor growth and metastasis [53][55]; caffeic acid plays a role in the process of inhibiting proliferation and migration [76]; and arctiopicrin plays a role in anti-inflammatory and proliferative inhibition [62]. Prediction of cancer pathways from bioactive compounds of *C. amboinicus* leaves EtOAc extract from tissue pharmacology studies is shown in Figure 7.

3.5. Analysis of The Interaction of Bioactive Compounds with The Main Target Cancer Proteins by Molecular Docking

Each potential bioactive compound as an anti-breast cancer was optimized for energy using GaussView5.0.8 by selecting jobtype optimization, ground state DFT method, and basis set 3-21G. The results of energy optimization for bioactive compounds are shown in Table 4. The GaussView program is an application that is able to determine and position each atom in space in its stable position so that the resulting molecular structure is in ideal conditions with minimum repulsion [77].

Energy optimization of bioactive compounds aims to obtain the ideal conformation form of the active molecule before conducting a molecular docking study with the protein, thus optimizing the interaction between the active compound and the receptor to produce better therapeutic effects [78].

With optimization, it is expected that the molecular structure is in an ideal condition and almost resembles its 3D form when in the body. This method is part of a semi-empirical approach involving computational assistance. The compound resulting from energy optimization is then docked to the STAT3 protein as the main target protein. The STAT3 protein was downloaded from the PDB database to analyze the binding of the STAT3 phosphotyrosine peptide [79]. The results of the molecular docking of various bioactive compounds are shown in Table 5. The STAT3 protein expressed in cancer by pro-inflammatory cytokines gp130 and interleukin 6 and 11 [80], in excess greatly encourages cell survival, including proliferation, cell cycle development, migration, anti-apoptosis, invasion, angiogenesis, chemotherapy drug resistance, immunosuppression, and stem cell differentiation, by regulating the expression of its downstream target genes. Thus, targeted therapy by suppressing STAT3 expression is considered very effective in the treatment and therapy of breast cancer [81][82]. Targeted therapy of STAT3 protein is considered effective because drug compounds act as inhibitors by blocking the phosphorylation and dimerization of its active site. STAT3 protein (PDB ID: 6NUQ) studies and reports phosphorylation peptide binding activity. Native ligand forms six hydrogen bonds with Ser509, Trp510, Gly253, Ser513, Thr526, and Trp501. Bioactive compounds of the EtOAc extract of *C. amboinicus* leaves provide no hydrogen bond interactions that are the same as native. The bioactive compound arctiopicrin does not show any hydrogen bond interactions. Other bioactive compounds provide sixteen hydrogen bonds, including Gln247, Ala241 (resolvin D1), Pro333 (gibberellin A24), Gln232, Thr236 (hexyl 2-furoate), Cys251, Gln326, Glu324 (13(*S*)-HOTrE), Asp334, Cys251 (arachidonic acid), Gln232, Asn315 (4-indole-carbaldehyde), Cys251 (asiatic acid), Ser540, Tyr539, Asp502 (9(*S*)-HpOTrE), and Asp334, Gln326 (caffeic acid). Therefore, the molecular docking of bioactive compounds in the EtOAc extract of *C. amboinicus* leaves does not provide inhibitors by blocking phosphorylation optimally.

4. CONCLUSIONS

The EtOAc extract of *C. amboinicus* leaves has various bioactive compounds that have the potential to be breast cancer anticancers. The results of *in vitro* activity testing showed that the IC_{50} given was included in the weak category (102.30 µg/mmL) against MCF-7 cancer cells and against normal cells (CV-1) (457.09 µg/mL). The selectivity value of the cytotoxic test index is 4.23, indicating the presence of potential bioactive compounds in *C. amboinicus* leaves EtOAc extract against breast cancer cells and that it is relatively safe for normal cells. The results of the molecular docking study with a pharmacological network approach on the cancer pathway provide information on the content of bioactive compounds targeting the STAT3 protein, where this protein is an important protein in

Table 4. Energy optimization of bioactive compounds.

No.	Compounds	Optimization	
		Energy (kJ/mol)	Dipole moment (Debye)
1.	Resolvin D1	-1,226.865	3.111
2.	Gibberellin A24	-1,147.590	4.247
3.	Hexyl 2-furoate	-650.886	1.457
4.	13(*S*)-HOTrE	-918.527	2.308
5.	Arachidonic acid	-926.707	1.281
6	4-Indolecarbaldehyde	-474.511	5.707
7	Asiatic acid	-1,539.783	2.463
8	9(*S*)-HpOTrE	-999.268	1.467
9	Caffeic acid	-645.079	2.120
10	Arctiopicrin	-1,184.421	4.842

Table 5. Molecular docking of bioactive compounds of the EtOAc extract of *C. amboinicus* leaves to STAT3 protein.

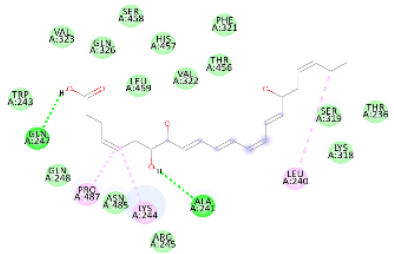
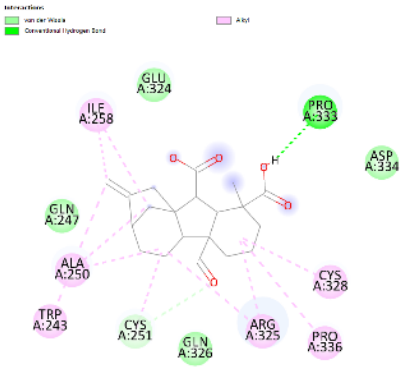
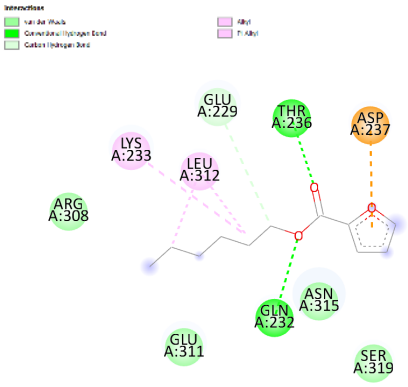
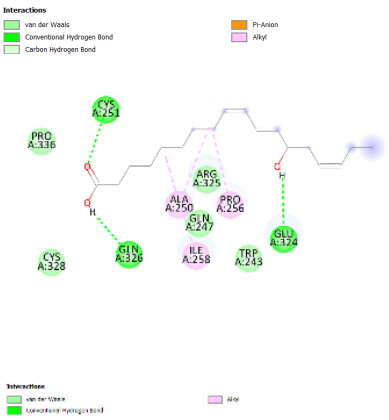
Compound	STAT3 protein		
	H-Bond	Other Bond	2D interaction
Resolvin D1	Gln247, Ala241	Pro487, Lsy244, Leu240	
Gibberellin A24	Pro333	Ile258, Ala250, Trp243, Arg325, Pro336, Cys328, Cys251,	
Hexyl 2-furoate	Gln232, Thr236	Lys233, Leu312, Glu229, Asp237	
13(*S*)-HOTrE	Cys251, Gln326, Glu324	Ala250, Ile258, Pro256	

Table 5. *Cont.*

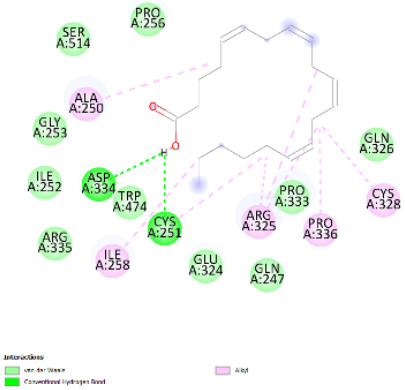
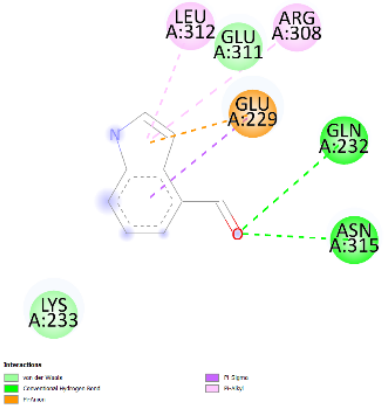
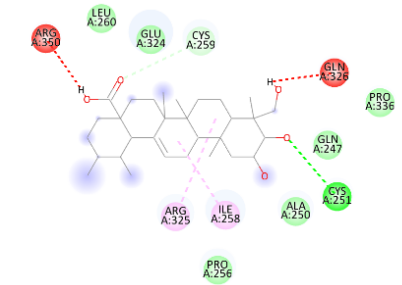
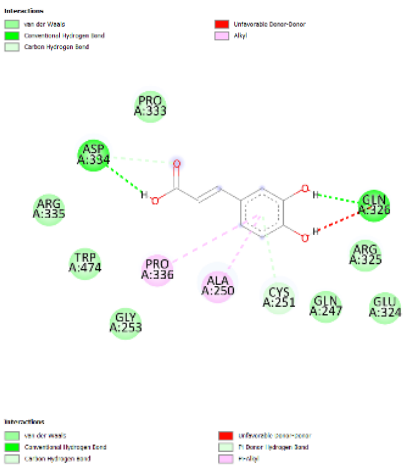
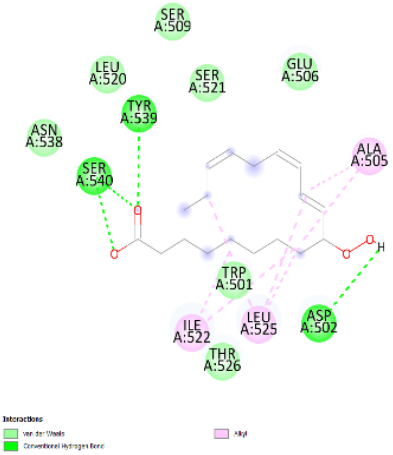
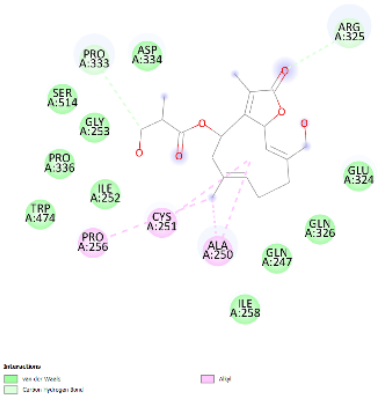
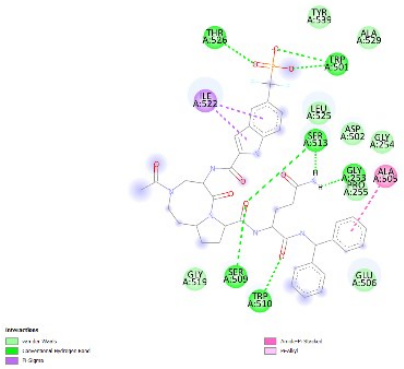
Compound	STAT3 protein		
	H-Bond	Other Bond	2D interaction
Arachidonic acid	Asp334, Cys251	Ala250, Ile258, Cys251, Arg325, Pro336, Cys328	
4-Indole-carbaldehyde	Gln232, Asn315	Leu312, Arg308, Glu229	
Asiatic acid	Cys251	Cys259, Arg325, Ile258, Arg350, Gln326	
Caffeic acid	Asp334, Gln326	Asp334, Gln326, Pro336, Ala250	

Table 5. *Cont.*

Compound	STAT3 protein		
	H-Bond	Other Bond	2D interaction
9(*S*)-HpOTrE	Ser540, Tyr539, Asp502	Ala505, Ile522, Leu525	
Arctiopicrin	-	Pro333, Arg325, Pro256, Cys251, Ala250	
Native ligand	Ser509, Trp510, Gly253, Ser513, Thr526, Trp501	Ala505, Ile522	

initiating and controlling various basic biological process conditions, including proliferation, survival, and cell development in normal conditions. Abnormal conditions will encourage the initiation and development of cancer cells by inhibiting apoptosis or inducing cell proliferation, invasion, angiogenesis, and metastasis, so that the application of the content of bioactive compounds in *C. amboinicus* leaves EtOAc extract in the treatment of breast cancer targets the suppression of STAT3 protein activity. Further testing is needed to validate the activity of the bioactive compound content of the EtOAc extract of *C. amboinicus* leaves against breast cancer *in vivo* or pre-clinically, so that in the future it can be developed as a potential alternative breast cancer drug.

AUTHOR INFORMATION

Corresponding Author

Winarto Haryadi — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia);
orcid.org/0000-0001-7970-1528
Email: wnrt_haryadi@ugm.ac.id

Authors

Kasta Gurning — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia); Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Medan-20141 (Indonesia);
orcid.org/0000-0002-0676-0030

Jajah Fachiroh — Department of Histology and Cell Biology, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia);
orcid.org/0000-0002-2650-1941

Endang Astuti — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia);
orcid.org/0000-0002-6401-2719

Author Contributions

Conceptualization, W. H., and K. G.; Methodology, W. H., J. F.; and E. A.; Software, W. H.; Formal Analysis, K. G.; Investigation, K. G.; Resources, W. H.; Data Curation, W. H., J. F.; and E. A.; Writing – Original Draft Preparation, W. H. and K. G.; Writing – Review & Editing, K. G.; W. H., J. F.; and E. A.; Funding Acquisition, W. H.

Conflicts of Interest

All authors declare that there is not any conflicts of interest.

SUPPORTING INFORMATION

Supplementary data associated with this article can be found in the online version at doi: 10.47352/jmans.2774-3047.246.

ACKNOWLEDGEMENT

The author would like to thank the research funds and support facilities from the Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada Yogyakarta. In addition, the author would like to thank the Education Fund Management Institute (LPDP) and the Higher Education Fund Management Center (BPTP) for providing a full scholarship (Doctoral Education) to Kasta Gurning based on Decree No. 00788/J5.2.3/BPI.06/9/2022.

REFERENCES

[1] N. Azamjah, Y. Soltan-Zadeh, and F. Zayeri. (2019). "Global Trend of Breast Cancer Mortality Rate: A 25-Year Study". *Asian Pacific Journal of Cancer Prevention.* **20** (7): 2015-2020. 10.31557/APJCP.2019.20.7.2015.

[2] Y. S. Sun, Z. Zhao, Z. N. Yang, F. Xu, H. J. Lu, Z. Y. Zhu, W. Shi, J. Jiang, P. P. Yao, and H. P. Zhu. (2017). "Risk Factors and Preventions of Breast Cancer". *International Journal of Biological Sciences.* **13** (11): 1387-1397. 10.7150/ijbs.21635.

[3] C. E. DeSantis, J. Ma, M. M. Gaudet, L. A. Newman, K. D. Miller, A. Goding Sauer, A. Jemal, and R. L. Siegel. (2019). "Breast cancer statistics, 2019". *CA: A Cancer Journal for Clinicians.* **69** (6): 438-451. 10.3322/caac.21583.

[4] Z. Chen, L. Xu, W. Shi, F. Zeng, R. Zhuo, X. Hao, and P. Fan. (2020). "Trends of female and male breast cancer incidence at the global, regional, and national levels, 1990-2017". *Breast Cancer Research and Treatment.* **180** (2): 481-490. 10.1007/s10549-020-05561-1.

[5] S. Anwar, A. Almatroudi, M. A. Alsahli, M. A. Khan, A. A. Khan, and A. H. Rahmani. (2020). "Natural Products: Implication in Cancer Prevention and Treatment through Modulating Various Biological Activities". *Anti-Cancer Agents in Medicinal Chemistry.* **20** (17): 2025-2040. 10.2174/1871520620666200705220307.

[6] S. M. Lima, R. D. Kehm, and M. B. Terry. (2021). "Global breast cancer incidence and mortality trends by region, age-groups, and fertility patterns". *EClinicalMedicine.* **38** : 100985. 10.1016/j.eclinm.2021.100985.

[7] S. Lei, R. Zheng, S. Zhang, R. Chen, S. Wang, K. Sun, H. Zeng, W. Wei, and J. He. (2021). "Breast cancer incidence and mortality in women in China: temporal trends and projections to 2030". *Cancer Biology and Medicine.* **18** (3): 900-9. 10.20892/j.issn.2095-3941.2020.0523.

[8] S. Lei, R. Zheng, S. Zhang, S. Wang, R. Chen, K. Sun, H. Zeng, J. Zhou, and W. Wei. (2021). "Global patterns of breast cancer incidence and mortality: A population-based cancer registry data analysis from 2000 to 2020". *Cancer Communication (Lond).* **41** (11): 1183-1194. 10.1002/cac2.12207.

[9] Y. Xu, M. Gong, Y. Wang, Y. Yang, S. Liu, and Q. Zeng. (2023). "Global trends and forecasts of breast cancer incidence and deaths". *Scientific Data.* **10** (1): 334. 10.1038/s41597-023-02253-5.

[10] C. Luo, N. Li, B. Lu, J. Cai, M. Lu, Y. Zhang, H. Chen, and M. Dai. (2022). "Global and regional trends in incidence and mortality of female breast cancer and associated factors at national level in 2000 to 2019". *Chinese medical journal (English).* **135** (1): 42-51. 10.1097/CM9.0000000000001814.

[11] E. B. Yahya and A. M. Alqadhi. (2021). "Recent trends in cancer therapy: A review on the current state of gene delivery". *Life Sciences.* **269** : 119087. 10.1016/j.lfs.2021.119087.

[12] J. B. Vo, C. Ramin, A. Barac, A. Berrington de Gonzalez, and L. Veiga. (2022). "Trends in heart disease mortality among breast cancer survivors in the US, 1975-2017". *Breast Cancer Research and Treatment.* **192** (3): 611-622. 10.1007/s10549-022-06515-5.

[13] M. J. Elliott, B. Wilson, and D. W. Cescon. (2022). "Current Treatment and Future Trends of Immunotherapy in Breast Cancer". *Current Cancer Drug Targets.* **22** (8): 667-677. 10.2174/1568009622666220317091723.

[14] M. I. Khan, A. Bouyahya, N. E. L. Hachlafi, N. E. Menyiy, M. Akram, S. Sultana, G. Zengin, L. Ponomareva, M. A. Shariati, O. A. Ojo, S. Dall'Acqua, and T. C. Elebiyo. (2022). "Anticancer properties of medicinal plants and their bioactive compounds against breast cancer: a review on recent investigations". *Environmental Science and Pollution Research.* **29** (17): 24411-24444. 10.1007/s11356-021-17795-7.

[15] W. Haryadi, K. Gurning, and E. Astuti. (2024). "Molecular target identification of two Coleus amboinicus leaf isolates toward lung cancer using a bioinformatic approach and molecular docking-based assessment". *Journal of Applied Pharmaceutical Science.* 10.7324/japs.2024.164753.

[16] K. Gurning and W. Haryadi. (2022). "Potential antioxidants of secondary metabolite isolates ethyl acetate fraction Coleus amboinicus Lour. Leaves". *ScienceRise: Pharmaceutical Science.* **5** (39): 100-105. 10.15587/2519-4852.2022.266401.

[17] K. Gurning, W. Haryadi, and H. Sastrohamidjojo. (2021). "Isolation And Characterization Of Antioxidant Compounds Of Bangun-Bangun (Coleus Amboinicus, L.) Leaves From North Sumatera, Indonesia". *Rasayan Journal of Chemistry.* **14** (01): 248-253. 10.31788/rjc.2021.1416077.

[18] K. Gurning. (2020). "Identification of secondary metabolic and test of activity ethyl acetate fraction of bangunâ¬- bangun (coleus amboinicus lour.) Leaves as antioxidant". *BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan).* **7** (1): 117-122. 10.31289/biolink.v7i1.3732.

[19] K. Gurning. (2020). "Determination antioxidant activities methanol extracts of bangun-bangun (Coleus amboinicus L.) Leaves with DPPH method". *Jurnal Pendidikan Kimia.* **12** (2): 62-69. 10.24114/jpkim.v12i2.19397.

[20] S. Slusarczyk, A. Cieslak, Y. R. Yanza, M. Szumacher-Strabel, Z. Varadyova, M. Stafiniak, D. Wojnicz, and A. Matkowski. (2021). "Phytochemical Profile and Antioxidant Activities of Coleus amboinicus Lour. Cultivated in Indonesia and Poland". *Molecules.* **26** (10). 10.3390/molecules26102915.

[21] K. Gurning, S. Suratno, E. Astuti, and W. Haryadi. (2024). "Untargeted LC/HRMS Metabolomics Analysis and Anticancer Activity Assay on MCF-7 and A549 Cells

from Coleus amboinicus Lour Leaf Extract". *Iranian Journal of Pharmaceutical Research.* **23** (1): e143494. 10.5812/ijpr-143494.

[22] J. M. Onyancha, N. K. Gikonyo, S. W. Wachira, P. G. Mwitari, and M. M. Gicheru. (2018). "Anticancer activities and safety evaluation of selected Kenyan plant extracts against breast cancer cell lines". *Journal of Pharmacognosy and Phytotherapy.* **10** (2): 21 -26. 10.5897/jpp2017.0465.

[23] N. Z. Ismail, Z. Md Toha, M. Muhamad, N. N. S. Nik Mohamed Kamal, N. N. Mohamad Zain, and H. Arsad. (2020). "Antioxidant Effects, Antiproliferative Effects, and Molecular Docking of Clinacanthus nutans Leaf Extracts". *Molecules.* **25** (9). 10.3390/molecules25092067.

[24] R. Islamie, I. Iksen, B. C. Buana, K. Gurning, H. D. Syahputra, and H. S. Winata. (2022). "Construction of network pharmacology-based approach and potential mechanism from major components of Coriander sativum L. against COVID-19". *Pharmacia.* **69** (3): 689-697. 10.3897/pharmacia.69.e84388.

[25] M. A. Basar, M. F. Hosen, B. Kumar Paul, M. R. Hasan, S. M. Shamim, and T. Bhuyian. (2023). "Identification of drug and protein-protein interaction network among stress and depression: A bioinformatics approach". *Informatics in Medicine Unlocked.* **37**. 10.1016/j.imu.2023.101174.

[26] Y. Wang, Y. Zhang, Y. Wang, X. Shu, C. Lu, S. Shao, X. Liu, C. Yang, J. Luo, and Q. Du. (2021). "Using Network Pharmacology and Molecular Docking to Explore the Mechanism of Shan Ci Gu (Cremastra appendiculata) Against Non-Small Cell Lung Cancer". *Frontiers in Chemistry.* **9** : 682862. 10.3389/fchem.2021.682862.

[27] Y. S. Kurniawan, K. Gurning, I. Iksen, and A. Bikharudin. (2024). "Fight for Cancer Diseases using Natural Compounds and Their Semisynthetic Derivatives". *Bioactivities.* **2** (2): 1-22. 10.47352/bioactivities.2963-654X.221.

[28] D. Adisty Ridha, S. Eti Nurwening, and Mustofa. (2020). "Cytotoxicity of ((E)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) on HeLa cell line". *Indonesian Journal of Pharmacology and Therapy.* **1** (2). 10.22146/ijpther.606.

[29] Y. S. Kurniawan, T. Indriani, H. Amrulloh, L. C. Adi, A. C. Imawan, K. T. A. Priyangga, and E. Yudha. (2023). "The Journey of Natural Products: From Isolation Stage to Drug's Approval in Clinical Trials". *Bioactivities.* **1** (2): 43-60. 10.47352/bioactivities.2963-654X.190.

[30] K. Ganesan, B. Du, and J. Chen. (2022). "Effects and mechanisms of dietary bioactive compounds on breast cancer prevention". *Pharmacological Research.* **178** : 105974. 10.1016/j.phrs.2021.105974.

[31] A. B. Shirode, D. J. Bharali, S. Nallanthighal, J. K. Coon, S. A. Mousa, and R. Reliene. (2015). "Nanoencapsulation of pomegranate bioactive compounds for breast cancer chemoprevention". *International Journal of Nanomedicine.* **10** : 475-84. 10.2147/IJN.S65145.

[32] S. S. Dahham, Y. M. Tabana, M. A. Iqbal, M. B. Ahamed, M. O. Ezzat, A. S. Majid, and A. M. Majid. (2015). "The Anticancer, Antioxidant and Antimicrobial Properties of the Sesquiterpene beta-Caryophyllene from the Essential Oil of Aquilaria crassna". *Molecules.* **20** (7): 11808-29. 10.3390/molecules200711808.

[33] N. To'Bungan, R. Pratiwi, S. Widyarini, and L. H. Nugroho. (2022). "Cytotoxicity extract and fraction of knobweed (Hyptis capitata) and its effect on migration and apoptosis of T47D cells". *Biodiversitas Journal of Biological Diversity.* **23** (1). 10.13057/biodiv/d230162.

[34] A. Flippen, I. A. Khasabova, D. A. Simone, and S. G. Khasabov. (2024). "Systemic administration of Resolvin D1 reduces cancer -induced bone pain in mice: Lack of sex dependency in pain development and analgesia". *Cancer Medicine.* **13** (15): e70077. 10.1002/cam4.70077.

[35] D. Mattoscio, E. Isopi, A. Lamolinara, S. Patruno, A. Medda, F. De Cecco, S. Chiocca, M. Iezzi, M. Romano, and A. Recchiuti. (2021). "Resolvin D1 reduces cancer growth stimulating a protective neutrophil-dependent recruitment of anti-tumor monocytes".

Journal of Experimental and Clinical Cancer Research. **40** (1): 129. 10.1186/s13046-021-01937-3.

[36] I. A. Khasabova, M. Y. Golovko, S. A. Golovko, D. A. Simone, and S. G. Khasabov. (2020). "Intrathecal administration of Resolvin D1 and E1 decreases hyperalgesia in mice with bone cancer pain: Involvement of endocannabinoid signaling". *Prostaglandins Other Lipid Mediat.* **151** : 106479. 10.1016/j.prostaglandins.2020.106479.

[37] L. Sun, Y. Wang, L. Wang, B. Yao, T. Chen, Q. Li, Z. Liu, R. Liu, Y. Niu, T. Song, Q. Liu, and K. Tu. (2019). "Resolvin D1 prevents epithelial-mesenchymal transition and reduces the stemness features of hepatocellular carcinoma by inhibiting paracrine of cancer-associated fibroblast-derived COMP". *Journal of Experimental and Clinical Cancer Research.* **38** (1): 170. 10.1186/s13046-019-1163-6.

[38] E. C. Dominguez, R. Phandthong, M. Nguyen, A. Ulu, S. Guardado, S. Sveiven, P. Talbot, and T. M. Nordgren. (2022). "Aspirin -Triggered Resolvin D1 Reduces Chronic Dust-Induced Lung Pathology without Altering Susceptibility to Dust-Enhanced Carcinogenesis". *Cancers (Basel).* **14** (8). 10.3390/cancers14081900.

[39] T. Toyama, M. Tahara, K. Nagamune, K. Arimitsu, Y. Hamashima, N. M. Palacpac, H. Kawaide, T. Horii, and K. Tanabe. (2012). "Gibberellin biosynthetic inhibitors make human malaria parasite Plasmodium falciparum cells swell and rupture to death". *PLoS One.* **7** (3): e32246. 10.1371/journal.pone.0032246.

[40] S.-y. Zhu, F.-z. Luo, and P.-H. Sun. (2020). "Synthesis and antitumor activity of novel gibberellin derivatives with tetracyclic diterpenoid skeletons". *Medicinal Chemistry Research.* **29** (8): 1341-1354. 10.1007/s00044-020-02551-2.

[41] J. M. Khaled, N. S. Alharbi, R. A. Mothana, S. Kadaikunnan, and A. S. Alobaidi. (2021). "Biochemical Profile by GC-MS of Fungal Biomass Produced from the Ascospores of Tirmania nivea as a Natural Renewable Resource". *Journal of Fungi (Basel).* **7** (12). 10.3390/jof7121083.

[42] C. Wolff, C. Zoschke, S. K. Kalangi, P. Reddanna, and M. Schafer-Korting. (2019). "Tumor microenvironment determines drug efficacy in vitro - apoptotic and anti-inflammatory effects of 15-lipoxygenase metabolite, 13-HpOTrE". *European Journal of Pharmaceutics and Biopharmaceutics.* **142** : 1-7. 10.1016/j.ejpb.2019.06.003.

[43] S. M. Clemente, O. H. Martinez-Costa, M. Monsalve, and A. K. Samhan-Arias. (2020). "Targeting Lipid Peroxidation for Cancer Treatment". *Molecules.* **25** (21). 10.3390/molecules25215144.

[44] D. Lv, Y. Zou, Z. Zeng, H. Yao, S. Ding, Y. Bian, L. Wen, and X. Xie. (2020). "Comprehensive metabolomic profiling of osteosarcoma based on UHPLC-HRMS". *Metabolomics.* **16** (12): 120. 10.1007/s11306-020-01745-4.

[45] H. Tallima and R. El Ridi. (2023). "Mechanisms of Arachidonic Acid In Vitro Tumoricidal Impact". *Molecules.* **28** (4). 10.3390/molecules28041727.

[46] L. Ouldamer, M. L. Jourdan, M. Pinault, F. Arbion, and C. Goupille. (2022). "Accumulation of Arachidonic Acid, Precursor of Pro-Inflammatory Eicosanoids, in Adipose Tissue of Obese Women: Association with Breast Cancer Aggressiveness Indicators". *Biomedicines.* **10** (5). 10.3390/biomedicines10050995.

[47] W. Li, X. Guo, C. Chen, and J. Li. (2022). "The prognostic value of arachidonic acid metabolism in breast cancer by integrated bioinformatics". *Lipids in Health and Disease.* **21** (1): 103. 10.1186/s12944-022-01713-y.

[48] C. Cantonero, J. Sanchez-Collado, J. J. Lopez, G. M. Salido, J. A. Rosado, and P. C. Redondo. (2020). "Arachidonic Acid Attenuates Cell Proliferation, Migration and Viability by a Mechanism Independent on Calcium Entry". *International Journal of Molecular Sciences.* **21** (9). 10.3390/ijms21093315.

[49] M. L. Luo, W. Huang, H. P. Zhu, C. Peng, Q. Zhao, and B. Han. (2022). "Advances in

indole-containing alkaloids as potential anticancer agents by regulating autophagy". *Biomedicine and Pharmacotherapy.* **149** : 112827. 10.1016/j.biopha.2022.112827.

[50] T. D. Hubbard, I. A. Murray, and G. H. Perdew. (2015). "Indole and Tryptophan Metabolism: Endogenous and Dietary Routes to Ah Receptor Activation". *Drug Metabolism and Disposition.* **43** (10): 1522-35. 10.1124/dmd.115.064246.

[51] D. Xu and Z. Xu. (2020). "Indole Alkaloids with Potential Anticancer Activity". *Current Topics in Medicinal Chemistry.* **20** (21): 1938 -1949. 10.2174/1568026620666200622150325.

[52] A. Dhiman, R. Sharma, and R. K. Singh. (2022). "Target-based anticancer indole derivatives and insight into structure–activity relationship: A mechanistic review update (2018-2021)". *Acta Pharmaceutica Sinica B.* **12** (7): 3006-3027. 10.1016/j.apsb.2022.03.021.

[53] M. Wicinski, A. Fajkiel-Madajczyk, Z. Kurant, S. Gajewska, D. Kurant, M. Kurant, and M. Sousak. (2024). "Can Asiatic Acid from Centella asiatica Be a Potential Remedy in Cancer Therapy?-A Review". *Cancers (Basel).* **16** (7). 10.3390/cancers16071317.

[54] S. Dutta, P. Chakraborty, S. Basak, S. Ghosh, N. Ghosh, S. Chatterjee, S. Dewanjee, and P. C. Sil. (2022). "Synthesis, characterization, and evaluation of in vitro cytotoxicity and in vivo antitumor activity of asiatic acid-loaded poly lactic-co-glycolic acid nanoparticles: A strategy of treating breast cancer". *Life Sciences.* **307** : 120876. 10.1016/j.lfs.2022.120876.

[55] Z. Zhu, L. Cui, J. Yang, C. T. Vong, Y. Hu, J. Xiao, G. Chan, Z. He, and Z. Zhong. (2021). "Anticancer effects of asiatic acid against doxorubicin-resistant breast cancer cells via an AMPK-dependent pathway in vitro". *Phytomedicine.* **92** : 153737. 10.1016/j.phymed.2021.153737.

[56] J. M. Grantz, V. P. Thirumalaikumar, A. H. Jannasch, C. Andolino, N. Taechachokevivat, L. M. Avila-Granados, and R. C. Neves. (2024). "The platelet and plasma proteome and targeted lipidome in postpartum dairy cows with elevated systemic inflammation". *Scientific Reports.* **14** (1): 31240. 10.1038/s41598-024-82553-x.

[57] A. Kabala-Dzik, A. Rzepecka-Stojko, R. Kubina, R. D. Wojtyczka, E. Buszman, and J. Stojko. (2018). "Caffeic Acid Versus Caffeic Acid Phenethyl Ester in the Treatment of Breast Cancer MCF-7 Cells: Migration Rate Inhibition". *Integrative Cancer Therapies.* **17** (4): 1247-1259. 10.1177/1534735418801521.

[58] M. Alam, S. Ahmed, A. M. Elasbali, M. Adnan, S. Alam, M. I. Hassan, and V. R. Pasupuleti. (2022). "Therapeutic Implications of Caffeic Acid in Cancer and Neurological Diseases". *Frontiers in Oncology.* **12** : 860508. 10.3389/fonc.2022.860508.

[59] M. Alam, G. M. Ashraf, K. Sheikh, A. Khan, S. Ali, M. M. Ansari, M. Adnan, V. R. Pasupuleti, and M. I. Hassan. (2022). "Potential Therapeutic Implications of Caffeic Acid in Cancer Signaling: Past, Present, and Future". *Frontiers in Pharmacology.* **13** : 845871. 10.3389/fphar.2022.845871.

[60] S. Mirzaei, M. H. Gholami, A. Zabolian, H. Saleki, M. V. Farahani, S. Hamzehlou, F. B. Far, S. O. Sharifzadeh, S. Samarghandian, H. Khan, A. R. Aref, M. Ashrafizadeh, A. Zarrabi, and G. Sethi. (2021). "Caffeic acid and its derivatives as potential modulators of oncogenic molecular pathways: New hope in the fight against cancer". *Pharmacological Research.* **171** : 105759. 10.1016/j.phrs.2021.105759.

[61] H. Rezaei-Seresht, H. Cheshomi, F. Falanji, F. Movahedi-Motlagh, M. Hashemian, and E. Mireskandari. (2019). "Cytotoxic activity of caffeic acid and gallic acid against MCF-7 human breast cancer cells: An in silico and in vitro study". *Avicenna Journal of Phytomedicine.* **9** (6): 574-586. 10.22038/AJP.2019.13475.

[62] G. E. Robertovna, K. D. Alexeevich, S. A. Alexeevich, G. M. Petrovna, and O. K. Kenzhebaevna. (2019). "A Traditional Medicine Plant, Onopordum acanthium L. (Asteraceae): Chemical Composition and Pharmacological Research". *Plants (Basel).* **8** (2). 10.3390/plants8020040.

[63] D. Wang, A. S. Badarau, M. K. Swamy, S. Shaw, F. Maggi, L. E. da Silva, V. Lopez, A. W. K. Yeung, A. Mocan, and A. G. Atanasov. (2019). "Arctium Species Secondary Metabolites Chemodiversity and Bioactivities". *Frontiers in Plant Science.* **10** : 834. 10.3389/fpls.2019.00834.

[64] X. Wang, Y. Yang, Y. An, and G. Fang. (2019). "The mechanism of anticancer action and potential clinical use of kaempferol in the treatment of breast cancer". *Biomedicine and Pharmacotherapy.* **117** : 109086. 10.1016/j.biopha.2019.109086.

[65] P. D. Thomas. (2017). "The Gene Ontology and the Meaning of Biological Function". *Methods in Molecular Biology.* **1446** : 15-24. 10.1007/978-1-4939-3743-1_2.

[66] M. Kanehisa and Y. Sato. (2020). "KEGG Mapper for inferring cellular functions from protein sequences". *Protein Science.* **29** (1): 28-35. 10.1002/pro.3711.

[67] I. Iksen, W. Witayateeraporn, T. Wirojwongchai, C. Suraphan, N. Pornputtapong, N. Singharajkomron, H. M. Nguyen, and V. Pongrakhananon. (2023). "Identifying molecular targets of Aspiletrein-derived steroidal saponins in lung cancer using network pharmacology and molecular docking-based assessments". *Scientific Reports.* **13** (1): 1545. 10.1038/s41598-023-28821-8.

[68] K. Banerjee and H. Resat. (2016). "Constitutive activation of STAT3 in breast cancer cells: A review". *International Journal of Cancer.* **138** (11): 2570-8. 10.1002/ijc.29923.

[69] J. Sgrignani, M. Garofalo, M. Matkovic, J. Merulla, C. V. Catapano, and A. Cavalli. (2018). "Structural Biology of STAT3 and Its Implications for Anticancer Therapies Development". *International Journal of Molecular Sciences.* **19** (6). 10.3390/ijms19061591.

[70] R. Bansal and P. C. Acharya. (2014). "Man-made cytotoxic steroids: exemplary agents for cancer therapy". *Chemical Reviews.* **114** (14): 6986-7005. 10.1021/cr4002935.

[71] T. Gritsko, A. Williams, J. Turkson, S. Kaneko, T. Bowman, M. Huang, S. Nam, I. Eweis, N. Diaz, D. Sullivan, S. Yoder, S. Enkemann, S. Eschrich, J. H. Lee, C. A. Beam, J. Cheng, S. Minton, C. A. Muro-Cacho, and R. Jove. (2006). "Persistent activation of stat3 signaling induces survivin gene expression and confers resistance to apoptosis in human breast cancer cells". *Clinical Cancer Research.* **12** (1): 11-9. 10.1158/1078-0432.CCR-04-1752.

[72] L. Wang, H. S. Choi, B. Lee, J. H. Choi, Y. S. Jang, and J. W. Seo. (2021). "13R,20-Dihydroxydocosahexaenoic Acid, a Novel Dihydroxy- DHA Derivative, Inhibits Breast Cancer Stemness through Regulation of the Stat3/IL-6 Signaling Pathway by Inducing ROS Production". *Antioxidants (Basel).* **10** (3). 10.3390/antiox10030457.

[73] P. A. Z. Hasibuan, R. A. Syahputra, E. Hey-Hawkins, M. F. Lubis, A. S. Rohani, and S. A. Pahlevi. (2024). "Phytochemical composition and safety of Vernonia Amygdalina ethanolic extract with anti-colon cancer properties". *Journal of Agriculture and Food Research.* **16**. 10.1016/j.jafr.2024.101205.

[74] H. Tang, Y. Kuang, W. Wu, B. Peng, and Q. Fu. (2023). "Quercetin inhibits the metabolism of arachidonic acid by inhibiting the activity of CYP3A4, thereby inhibiting the progression of breast cancer". *Molecular Medicine.* **29** (1): 127. 10.1186/s10020-023-00720-8.

[75] D. Satria, P. A. Z. Hasibuan, M. Muhammad, S. B. Waruwu, R. Y. Utomo, and S. H. Ghoran. (2024). "Cytotoxic and apoptotic effect of Vernonia amygdalina Delile. fractions against Hs578t triple-negative breast cancer cell lines". *Phytomedicine Plus.* **4** (4). 10.1016/j.phyplu.2024.100640.

[76] Y. Yin, Z. Wang, Y. Hu, J. Wang, Y. I. Wang, and Q. Lu. (2024). "Caffeic acid hinders the proliferation and migration through inhibition of IL-6 mediated JAK-STAT-3 signaling axis in human prostate cancer". *Oncology Research.* **32** (12): 1881-1890. 10.32604/or.2024.048007.

[77] M. Beytur and I. Avinca. (2021). "Molecular, Electronic, Nonlinear Optical and Spectroscopic Analysis of Heterocyclic 3-

Substituted-4-(3-methyl-2-thienylmethyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones: Experiment and DFT Calculations". *Heterocyclic Communications.* **27** (1): 1-16. 10.1515/hc-2020-0118.

[78] T. I. Adelusi, A.-Q. K. Oyedele, I. D. Boyenle, A. T. Ogunlana, R. O. Adeyemi, C. D. Ukachi, M. O. Idris, O. T. Olaoba, I. O. Adedotun, O. E. Kolawole, Y. Xiaoxing, and M. Abdul-Hammed. (2022). "Molecular modeling in drug discovery". *Informatics in Medicine Unlocked.* **29**. 10.1016/j.imu.2022.100880.

[79] L. Bai, H. Zhou, R. Xu, Y. Zhao, K. Chinnaswamy, D. McEachern, J. Chen, C. Y. Yang, Z. Liu, M. Wang, L. Liu, H. Jiang, B. Wen, P. Kumar, J. L. Meagher, D. Sun, J. A. Stuckey, and S. Wang. (2019). "A Potent and Selective Small-Molecule Degrader of STAT3 Achieves Complete Tumor Regression In Vivo". *Cancer Cell.* **36** (5): 498-511 e17. 10.1016/j.ccell.2019.10.002.

[80] S. Q. To, R. S. Dmello, A. K. Richards, M. Ernst, and A. L. Chand. (2022). "STAT3 Signaling in Breast Cancer: Multicellular Actions and Therapeutic Potential". *Cancers (Basel).* **14** (2). 10.3390/cancers14020429.

[81] J. J. Qin, L. Yan, J. Zhang, and W. D. Zhang. (2019). "STAT3 as a potential therapeutic target in triple negative breast cancer: a systematic review". *Journal of Experimental and Clinical Cancer Research.* **38** (1): 195. 10.1186/s13046-019-1206-z.

[82] Y. Dong, J. Chen, Y. Chen, and S. Liu. (2023). "Targeting the STAT3 oncogenic pathway: Cancer immunotherapy and drug repurposing". *Biomedicine and Pharmacotherapy.* **167** : 115513. 10.1016/j.biopha.2023.115513.