JPPIPA 10(Special Issue) (2024)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Identification and Analysis of Potential Breast Anticancer Agents in Pogostemon Cablin through Network Pharmacology

Syarifah Fathimah Azzahra[1], Essy Harnelly[2*], Muhammad Subianto[1], Wisnu Ananta Kusuma[3], Widya Sari[2]

[1] Department of Informatics, Faculty of Mathematic and Natural Science, Universitas Syiah Kuala, Banda Aceh, Indonesia.
[2] Department of Biology, Faculty of Mathematic and Natural Science, Universitas Syiah Kuala, Banda Aceh, Indonesia.
[3] Department of Computer Science, IPB University, Bogor, Indonesia.

Received: January 24, 2024
Revised: May 5, 2024
Accepted: August 25, 2024
Published: August 31, 2024

Corresponding Author:
Essy Harnelly
essy.harnelly@usk.ac

DOI: 10.29303/jppipa.v10iSpecialIssue.7049

Abstract: Breast cancer is one of the highest known causes of death in Indonesia, especially for women. Pogostemon cablin Benth is one of the Aceh's endemic herbal plants that has been studied to have potential such as anti-inflammatory, antiproliferative, antioxidant, antimicrobial, and proapoptotic. Network pharmacology approach was conducted to explore and analyze the potential of P. cablin as an anti-breast cancer. P. cablin plant compounds were obtained from GCMS and breast cancer genes data were obtained from OMIM, GeneCard, and DisGeNet databases. Target proteins and pathways involved were identified using STRING-DB and Metascape. Network analysis was performed using Cytoscape. A total of 65 plant compounds with 554 target proteins and 1854 disease genes were obtained. Based on the results of combining protein targets using Venn diagrams, 138 overlapping proteins between drug compounds and breast cancer disease targets were identified. Based on KEGG and GO analysis, P. cablin is known to have potential in breast cancer treatment/therapeutic mechanisms. Based on the "compound-protein target-pathway" multi-target mechanism, pogostol; 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)]-; 3-Hexen-1-ol, 2,5-dimethyl-, acetate, (Z)-, 5β,7βH,10α-Eudesm-11-en-1α-ol; Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl 1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester; and Humulenol-II interacts with proteins that play a significant role in breast cancer, which are MAPK1, EGFR, TNF, AKT1, and JAK2. Hence, it can be concluded that P. cablin has good potential to be a source of therapeutic treatment against breast cancer. However, it needs to be tested clinically to further determine the effects of this P. cablin compound.

Keywords: Breast cancer; EGFR; Network pharmacology; Pogostemon cablin benth

Introduction

Since ancient times humans have used materials from nature as a source of treatment for diseases (Popović-Djordjević et al., 2022). Traditional medicine can be developed to be an alternative natural anti-cancer treatment. Many of the traditional treatments have not been clinically tested and scientifically evaluated so it is not enough to support the safety and effectiveness of traditional treatments. Based on the World Health Organization (WHO) report in 2013 related to traditional medicine, it is necessary to build a knowledge base for the management of traditional and complementary medicines, strengthen the quality assurance, safeness, and effectiveness of products, as well as integrate traditional medicine services into health services to support universal health coverage (World Health Organization, 2013). Traditional medicine can also have adverse effects and affect the

How to Cite:
Azzahra, S. F., Harnelly, E., Subianto, M., Kusuma, W. A., & Sari, W. (2024). Identification and Analysis of Potential Breast Anticancer Agents in Pogostemon Cablin through Network Pharmacology. *Jurnal Penelitian Pendidikan IPA*, *10*(SpecialIssue), 107–119. https://doi.org/10.29303/jppipa.v10iSpecialIssue.7049

effectiveness of other drugs, if they are not first scientifically researched (Ekor, 2014).

Cancer is a health disorder caused by abnormal, uncontrolled cell growth, which can spread and invade and eventually damage the tissues of the human body (Mayo et al., 2015; National Cancer Institute, 2023). According to the Global Burden of Cancer Study (Globocan) report from the International Agency for Research on Cancer in 2020, there are 19.3 million cancer cases with almost 10 million deaths in the world, with the number of deaths due to breast cancer were 2.3 million cases or equivalent to 11.7% (Sung et al., 2021). In Indonesia in 2020 there were 396,914 cases of cancer. Breast cancer is the cancer with the most cases, with 65,858 cases or around 16.6% of all types of cancer in Indonesia (World Health Organization, 2023a). As an estimated 685,00 people in the world die from breast cancer (World Health Organization, 2023b). This proves that breast cancer is one of the most killing diseases in the world. The advancements in medical treatment have not been fully able to overcome breast cancer cases in the community. Chemotherapy and radiotherapy are one of the conventional therapies that are effective in fighting cancer cells, but often provide side effects that include physical and non-physical (psychological) aspects. From a physical point of view, disorders of the spinal cord, digestive tract disorders such as anorexia, toxicity to other organs such as the heart, liver, and kidneys, and hair loss or alopecia due to the use of certain drugs (Sari et al., 2019). Therefore, further research is needed to obtain alternative treatments that are more effective, with low side effects, and can target more than one aspect of breast cancer.

Treatments that are now widely used to treat cancer patients are hormone therapy and target therapy (Breastcancerorg, 2023). Target therapy is a type of treatment using drugs that directly target the cancer cells (Daly, 1994). Although target therapy is carried out to treat cancer, it can cause side effects such as damage to the heart or cardiomyopathy, liver damage, and diarrhea (Wicki et al., 2012). However, this can be minimized by an alternative medicine that has less side effects such as those derived from traditional herbal plants.

Aceh Province is known for its many endemic plants that are rich in medicinal potency. One of them is patchouli or Pogostemon cablin. Pogostemon cablin belongs to the Lamiaceae family and grows in Indonesia, Korea, China, and Japan (Yun et al., 2015). This plant has conducted several studies which show that it has potential as analgesic, anti-inflammatory, antioxidant, antimicrobial, anti platelet, antithrombotic, antidepressant, and antiemetic activities (Chien et al., 2020; Li et al., 2013; Yun et al., 2015).

The development of technology towards biology or what is called bioinformatics allows us to analyze the potential for compounds from plants to play a role or interact with proteins in a disease. In this case, the anticancer potential of the Pogostemon cablin compound against breast cancer will be identified using the Network Pharmacology method. Network pharmacology is a field of science that combines systems biology and network information in a holistic manner. In recent years, its application has been widely used in the development of new drugs (Hopkins, 2008). Network pharmacology is a discipline that analyzes the synergistic relationships between "multi-component, multi-target, and multi-pathway" between drugs, diseases, and targets using network methods. The synergistic relationship between “multi-component, multi-target, and multi-pathway” builds a bridge for research on the relationship between modern pharmacology and traditional herbal medicine (Shang et al., 2023). Network analysis is a tool that makes it easy to explore the mechanism of action of bioactive compounds in the context of biological networks. The networks used are compound-target networks, protein-protein interaction networks, and disease pathways. By analyzing several of these tissues, it can help identify the anticancer gene targets of the active components, increase the healing effect, and reduce reactions to drugs that are considered to have little impact. Complex diseases such as cancer are certainly not caused by mutations in just one target gene, but rather are caused by mutations in several genes that disrupt the balance system of biological tissues in the body (Shang et al., 2023). In this study, the target proteins from the two components taken were the Pogostemon cablin plant and breast cancer in the human body, where it is possible that the medicinal compounds have an influence on the disease and the pathway that leads to breast cancer.

Method

Building a Pogostemon Cablin Database

Data compounds of Pogostemon cablin or Nilam from Aceh. In this study, it was obtained from GC-MS Atsiri Research Center (ARC) USK. Gas Chromatography-Mass Spectrometry (GC-MS) is used for the analysis samples which can enable the comparison of unknown compounds withreferences compounds. This helps identify the compounds present in the sample (Kelly et al., 2018). The data extracted from GC-MS pertains to the Neelam Light Fraction.

Three hits in the GC-MS data were taken, then combined and duplicates removed. A SMILES data search was carried out (He et al., 2022) for all these

compounds in the PubChem database (https://pubmed.ncbi.nlm.nih.gov/) Wu et al. (2021) with the keyword compound name from GC-MS data valid for synonymous names (Shang et al., 2023). The SMILES data taken is in the form of Canonical SMILES. Data on the Pogostemon cablin compound were analyzed for potential toxicity, predictive ability of the drug, absorption to the gastrointestinal tract, and violations of Lipinski's five rules (He et al., 2022; Zuhri et al., 2022). In general, according to these rules, molecules that violate more than one rule may have poor oral bioavailability values, following 5 rules: molecular weight (MW) < 500, calculated octane-water partition coefficient (LogP) not > 5, bond donor hydrogen bond (HBD) is not > 5, and hydrogen bond acceptor (HBA) is not > 10. This was done using swissADME http://www.swissadme.ch/ (Daina et al., 2017).

Building a Database of Target Proteins against Breast Cancer

Retrieval of target protein data from breast cancer was carried out via OMIM (https://www.omim.org/) (Amberger et al., 2018), GeneCards (https://www.genecards.org/) (Fishilevich et al., 2016; Stelzer et al., 2016), and DisGeNet (https://www.disgenet.org/) (Bauer-Mehren et al., 2010; Piñero et al., 2019). In OMIM, GeneCards, and DisGeNet a search was carried out with the keyword "Breast Cancer" (Li et al., 2013). For OMIM data, scraping was carried out (https://www.omim.org/search?index=entry&search=breast+cancer&start=1&limit=100&retrieve=geneMap&genemap_exists=true&phenotype_exists=true) to get all the genes related to breast cancer. Gene data were combined and deduplicated. The disease gene data is processed first by dividing the data into the gene/locus column, comparing the Approved symbols column which also contains gene data. All breast cancer gene data were verified using UniProt (https://www.UniProt.org/) (Shang et al., 2023).

Target Protein Prediction

The target protein of Pogostemon cablin was obtained from Swiss Target Prediction (http://www.swisstargetprediction.ch/) (Shang et al., 2023; Zuhri et al., 2022) with the selected species homosapiens (Daina et al., 2019). All target protein data for each compound is combined and removes unnecessary columns. The probability of each target protein is considered as a reference where the target protein data used is with a probability above zero (Shang et al., 2023).

The gene data that has been verified by UniProt is then built into a Protein-Protein Interaction (PPI) network using the String database (https://string-db.org/) (Szklarczyk et al., 2021) with the target organism, namely Homo sapiens, and the interaction score is set to high confidence (0.900) (Szklarczyk et al., 2019). PPI data that has appeared in the DB string is further analyzed using cytoscape.

The Intersection Between Pogostemon Cablin Target Proteins and Breast Cancer

The PPI tissue that was obtained at the previous stage was analyzed using cytoscape. The two PPI networks were combined to obtain a new network in the form of target protein slices from both networks (Qasim et al., 2023; Zuhri et al., 2022), namely the target protein network from Pogostemon cablin which is targeted for breast cancer.

Network Analysis

String-DB (https://string-db.org/) was used for protein-protein interaction analysis (Shang et al., 2023; Szklarczyk et al., 2019, 2021). The data of 138 proteins were entered in the multiple proteins column by setting the species “Homo sapiens” with the interaction score set to the highest accuracy (0.900) to obtain the Protein-Protein Interaction (PPI) Network. Network results data were further analyzed using cytoscape 3.9.1 (Wang et al., 2021).

Cytoscape is used to perform analysis, data integration, and visualize complex networks of molecular interactions and biological pathways related to biological data (cytoscape.org, 2023). In the cytoscape, a network was formed to analyze the mechanism of Pogostemon cablin in targeting breast cancer. The networks formed are (1) target compound-protein network, (2) target-pathway protein, and (3) target-pathway compound-protein (Qasim et al., 2023; Umar et al., 2023). Target protein, compound and pathway data are depicted as nodes and interactions between nodes are depicted as edges. Analysis of characteristic parameters obtained from the cytoscape in the form of degree, betweenness centrality, closeness centrality, and stress. In a network context, degree is a topological parameter that indicates how important a component, target, or path is in a network (Wu et al., 2021). In other words, the degree shows how many connections or interactions an element in the network has, and the higher the degree, the more important the element's role in the network (Wang et al., 2021). Nodes that are above the average degree value can become key nodes of the network (Tian et al., 2023).

Enrichment Analysis (KEGG Pathway and Gene Ontology Data from Sliced Target Proteins)

In this study, metascape version 3.5.20230501 (https://metascape.org/) and Enrichr

(https://maayanlab.cloud/Enrichr/) were used as tools to analyze and provide information regarding the function or role of genes in various biological processes, analyzing interactions between genes and pathways that play a role in genes (Zhou et al., 2019). The target protein data will be included in the metascape gene list with the analysis of the species "H. sapiens." The enrichment analysis is set with standard parameters, namely min overlap 3, P value cut off 0.01, min enrichment 1.5 (He et al., 2022) and for the analysis required are GO Molecular function, GO Biological Processes, GO Cellular Components, and KEGG Pathway. p-values were calculated based on the cumulative hypergeometric distribution (Metascape.org, 2023) and q-values were calculated using the Benjamini-Hochberg procedure to account for multiple testing (Hochberg et al., 1990). At Enrichr Mayaanlab, from the input of 138 target proteins, "Pathways and KEGG 2021 Human" was selected. KEGG (Kyoto Encyclopedia of Genes and Genomes) (https://www.genome.jp/kegg/) was used to obtain the most common disease pathways associated with target genes (Syahruni et al., 2023).

The data entered in the metascape represents 138 target proteins. The results of the enrichment analysis will be visualized based on a p-value < 0.05 (Qasim et al., 2023) and the 20 best using (https://www.bioinformatics.com.cn/) in the form of bars and bubble plots based on the p-value.

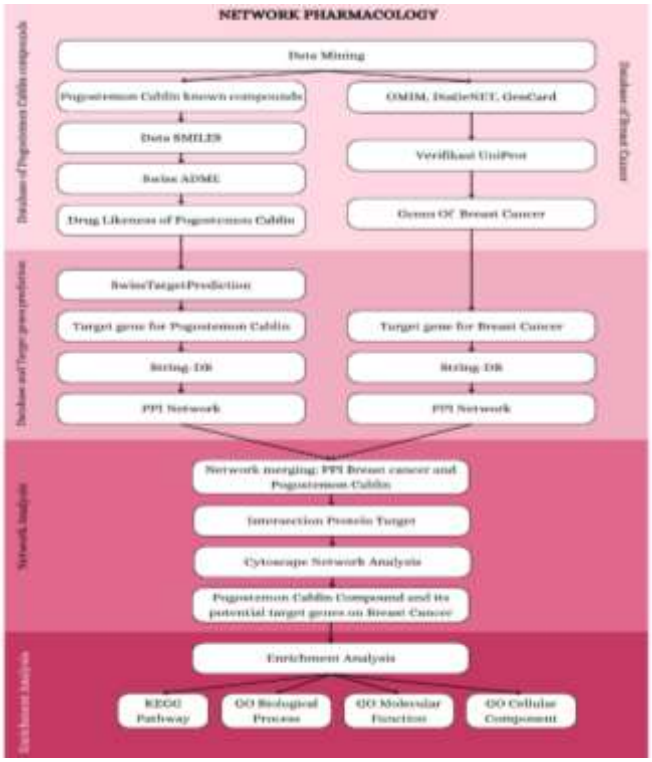

Figure 1. Flowchart of research methods using the network pharmacology approach

Result and Discussion

Database of Pogostemon Cablin

Based on data from GC MS patchouli light fraction (Hochberg et al., 1990), 65 Pogostemon cablin compounds were obtained. The results of druggability of compounds from Swiss ADME to drug compatibility based on Lipinski's five rules (Table 1). The molecular weight of the compounds was in the range of 98.11 - 278.39 with the smallest weight compound "2-Pentene, 4,4-dimethyl-" and the largest weight compound "Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl 1,2,3,5,6,7,8,8a-octahydronaphthalene-2-yl ester". Based on the prediction of the absorption of compounds in the gastrointestinal tract (gastrointestinal absorption), 34 out of 65 compounds have good absorption by the digestive tract which has the potential to reach systemic circulation and have a therapeutic effect. Based on Lipinski's 5 rules, 35 compounds did not violate all five rules and 30 compounds violated only one Lipinski rule. Of the 65 Pogostemon cablin compounds that were predicted using SWISS target prediction, 554 target proteins were found.

Breast Cancer Target Proteins

The OMIM scraping data contained 2675 lines, then the data cleaning process was performed so that the remaining clean data was 1514 lines or genes. GeneCards found 340 genes and DisGeNET found 183 genes. From the results of merging and deleting duplicates, the number of genes for breast cancer is 1854 target protein genes.

PPI Network Analysis

The target proteins from Pogostemon cablin (478 proteins, 2241 interactions) and breast cancer (1739 proteins, 13369 interactions) were combined to obtain overlapping proteins of 138 target proteins which were visualized using the Venn diagram in Figure 2. Data for 138 target proteins was entered into the database STRING to get a PPI Network with 138 nodes and 541 edges which can be seen in Figure 2. The results of the PPI network network analysis are visualized in Figure 2 based on degree values. Proteins with the highest to lowest levels are based on dark purple to white color gradations. The average value of degree in this network is 8.31, where there are 44 proteins that have a degree value above the average in table 2. In this study, the top 10 proteins based on degree were taken, so it was found that the main gene was most related to treatment. Pogostemon cablin against breast cancer is STAT3 (38 degree), AKT1(37 degree), EGFR(35 degree), ESR1(31 degree), HIF1A(29 degree), PIK3CA(28 degree),

PIK3R1(25 degree), JAK2(25 degree), and TNF(24 degree) with the highest degree.

Tabel 1. Results of Toxicity Analysis, Prediction of Druggability, Gastrointestinal Absorption and Violation of Lipinski's Five Rules of Pogostemon Cablin Compounds

Compound	Formula	MW	GI Absorption	Lipinsky #violations
(-)-Globulol	C15H26O	222.37	High	0
(1R,1aR,2aS,6R,6aS,7aS)-1,6,6a-Trimethyldecahydro-1,2amethanocyclopropa[b]naphthalene	C15H24	204.35	Low	1
(1R,4aS,6R,8aS)-8a,9,9-Trimethyl1,2,4a,5,6,7,8,8a-octahydro-1,6-methanonaphthalen-1-ol	C14H22O	206.32	High	0
(1S,1aS,1bR,4S,5S,5aS,6aR)-1a,1b,4,5aTetramethyldecahydro-1,5-methanocyclopropa[a]indene	C15H24	204.35	Low	1
…	…	…	…	…
Valerena-4,7(11)-diene	C15H24	204.35	Low	1

Table 2. Results of Network Analysis of 138 Target Proteins with Parameters Betweenness Centrality, Closeness Centrality, Degree and Stress. Data is Sorted Based on the Highest Level Value

Name	Betweenness Centrality	Closeness Centrality	Degree	Stress
STAT3	0.097617	0.467577	38	9830
AKT1	0.161100	0.482394	37	14294
EGFR	0.100119	0.461279	35	9748
ESR1	0.146666	0.462838	31	13510
HIF1A	0.156116	0.459732	29	12364
PIK3CA	0.027099	0.40176	28	4396
MAPK1	0.019284	0.412651	17	2322
PARP1	0.038133	0.40176	17	5930
…	…	…	…	…
CYP3A4	0.034413	0.29087	9	3056

Figure 2. Venn diagram of target protein of the plant Pogostemon cablin and breast cancer

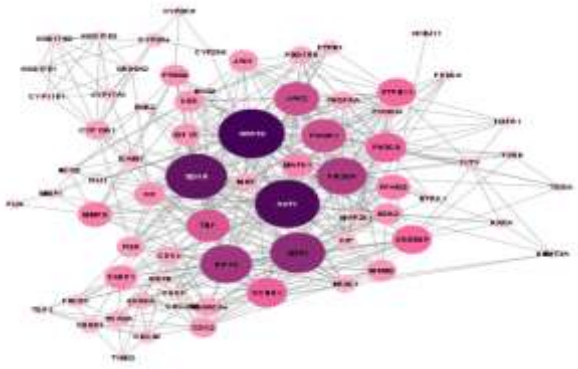

Figure 3. Protein Network Interaction of the medicinal plant Pogostemon cablin on breast cancer. The degree value from the highest is shown in the darkest to faded color and is based on the size of the node

Network Diagram between Drug Compound-target Proteins "Drug-Component-Target-Disease"

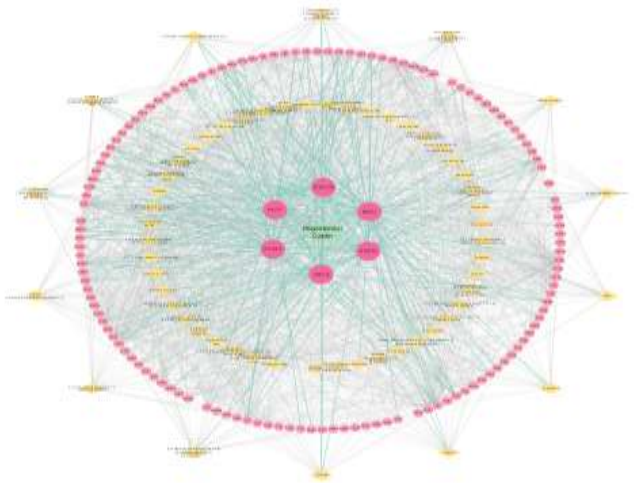

Figure 4. "Compound-Protein-protein" multitarget network. In this network there are connections between Pogostemon cablin proteins against breast cancer and these proteins are also connected to compounds that have relevance. Dark pink nodes on the inside of the circle indicate protein nodes that play an important role and yellow nodes on the outermost ring of the network represent compounds that have the most relevance to proteins

For the treatment of breast cancer using Pogostemon cablin, the regulatory network between drug compounds and disease targets is depicted using cytoscape. This network consists of 201 nodes and 2106 edges, as shown in Figure 3. The compounds found in the outer ring of the network are drug compounds with the most interactions with disease target proteins. The following is the drug compound, E)-2-((8R,8aS)-8,8a-Dimethyl-3,4,6,7,8,8a hexahydronaphthalen-2(1H)-ylidene)propan-1-ol (41 interactions), 2,3,3-Trimethyl-2-(3-methyl-buta-1,3- dienyl)-cyclohexanone (38 interactions), 2-Butenal, 2-methyl-4-(2,6,6-trimethyl -1-cyclohexen-1-yl)- (37 interactions), 1(2H)-Naphthalenone, octahydro-4a,8a-dimethyl-7-(1-methylethyl)-, [4aR-(4aa,7ß,8aa)] - (36 Interactions), Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl 1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester (35 interactions). It can be considered that these compounds are an important part of Pogostemon cablin in the treatment of breast cancer.

GO Enrichment and KEGG pathway analysis

From the results of GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) Pathway analysis of 138 target proteins. Based on KEGG (https://www.genome.jp/) pathways for the keyword "Breast Cancer" as seen in Figure 10 there are the PI3K-Akt signaling pathway, MAPK signaling pathway, and p53 signaling pathway. From the KEGG pathway metascape results, there is the PI3K-Akt signaling pathway in third place with a fairly small value, which indicates that this pathway has a high level of confidence in gene enrichment in a particular biological process or pathway. Based on the metascape results, it was also found that the breast cancer pathway was at number 21 with a value of -17.08 (Log10(p value)), the MAPK signaling pathway was at number 32 with a value of -15.75 (Log10(p value)), and the p53 signaling pathway was at value -4.61 (Log10(p value)).

GO term based on the smallest p value obtained, the top 5 biological processes are, Response to Hormones, Phosphorylation, Positive Regulation of the Phosphorus Metabolism Process, Positive Regulation of the Phosphate Metabolism Process, and Protein Phosphorylation. The top 5 cellular components are the Receptor Complex, Membrane Assembly, Membrane Microdomian, Neuron Cell Body, Cell Body, these are all closely related to the cell. The top 10 molecular functions are Phosphotransferase Activity, Alcohol Group as Recipient, Protein Kinase Activity, Kinase Activity, Protein Tyrosine Kinase Activity.

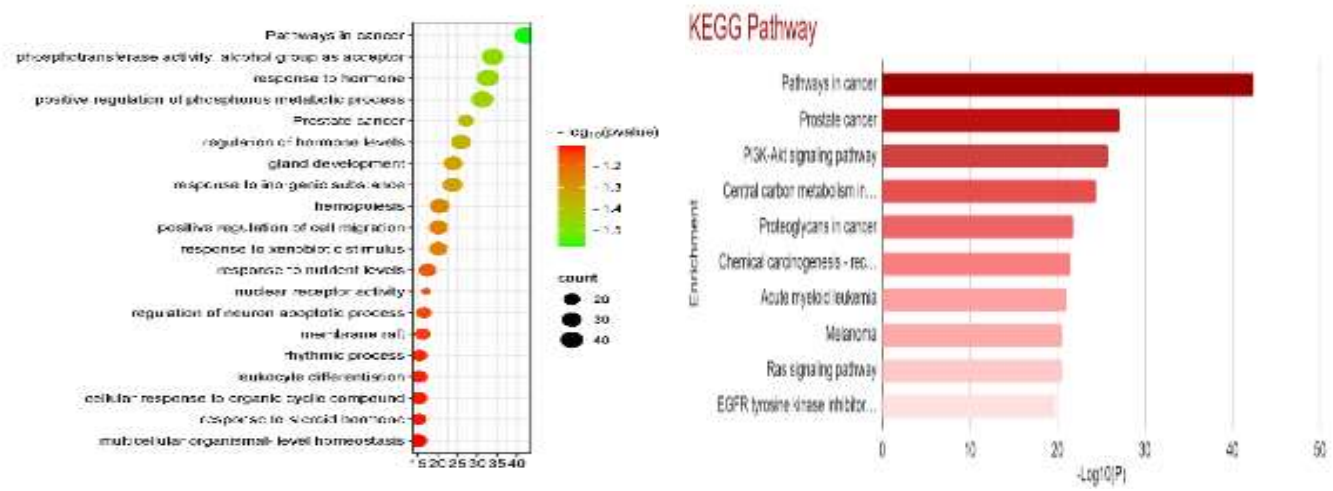

Figure 5. KEGG Pathway and Gene Ontology Enrichment analysis based on pvalue and count using Bubble plot

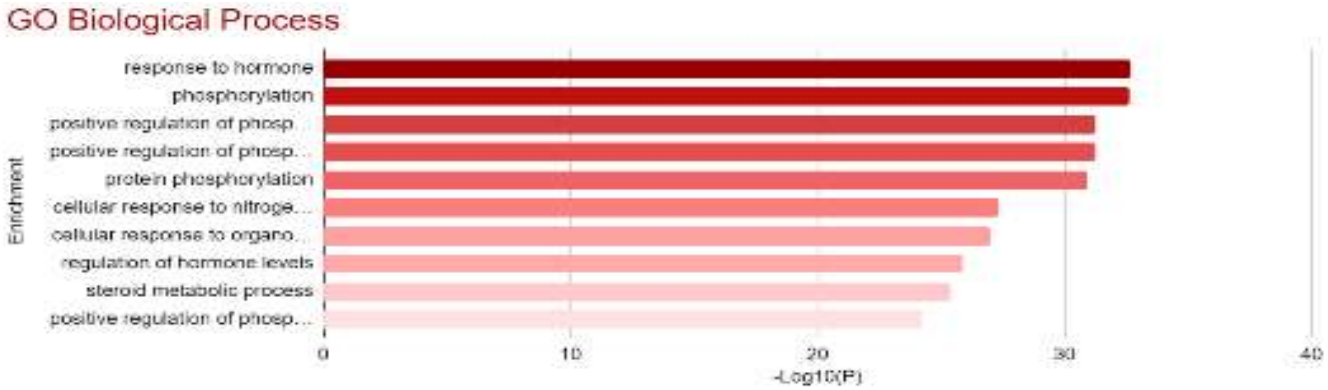

Figure 6. GO Biological process representation of pogostemon cablin proteins against breast cancer

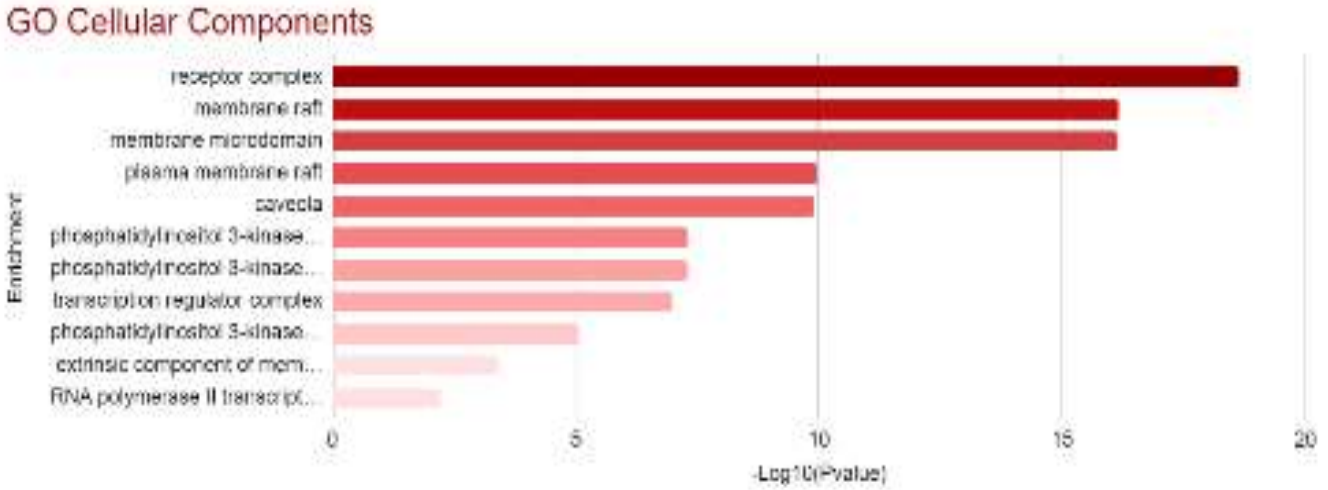

Figure 7. GO Biological process representation of pogostemon cablin proteins against breast cancer

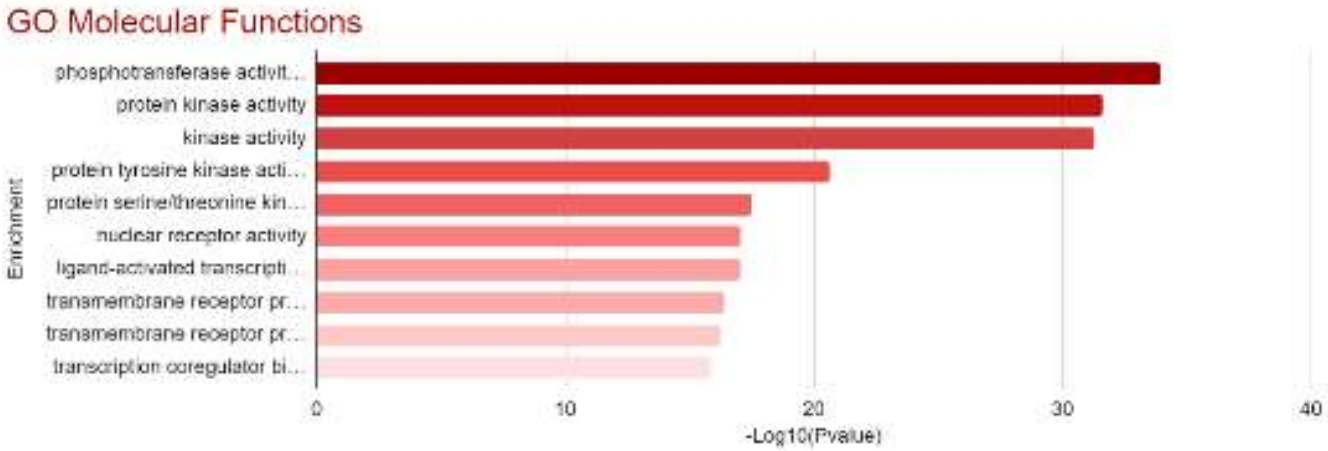

Figure 8. GO Cellular Components representation of Pogostemon cablin proteins against breast cancer

Network diagram between drug compound-target protein-pathway

To discover the core mechanism of the breast anticancer agent, the compound Pogostemon cablin, and its associated pathways. So a "Compound-target protein-pathway" network was built as shown in the figure….this network consists of 35 nodes (15 compounds, 15 target proteins, 5 pathways). Different shapes and colors indicate compounds, target proteins, and pathways. Each compound node interacts with several target proteins and each target protein can be connected to several pathways. This shows that the mechanism of anticancer treatment using Pogostemon cablin involves various compounds and targets. The compounds that play an important role here are 1,4-Dimethyl-7-(prop-1-en-2-yl)decahydroazulen-4-ol (Pogostol), 1H-Cycloprop[e]azulen-7-ol, decahydro-1, 1,7-trimethyl-4- methylene-, [1ar-(1aa,4aa,7ß,7aß,7ba)]-,

3-Hexen-1-ol, 2,5-dimethyl-, acetate, (Z)-, 5ß,7ßH,10a-Eudesm-11-en-1a-ol, Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl 1,2,3,5,6,7,8,8a-octahydronaphthalen- 2-yl ester, and Humulenol-II. This compound interacts with the target protein Pogostemon cablin which interacts with breast cancer which has the greatest influence on tissue, namely MAPK1, EGFR, TNF, AKT1, JAK2, CCND1, PIK3CD, PIK3R1, PPARG, PIK3CA, ESR1, CREBBPB, HIF1A, MMP9, STAT3. These proteins will be connected to the most relevant pathways related to breast cancer in the human body.

Discussion

Cancer is a disease characterized by the uncontrolled implantation and spread of body cells which can form tumors and disrupt normal organ function. Cancer is the second leading cause of death worldwide, causing millions of deaths each year and can begin in almost any organ or tissue (American Cancer Society, 2020; World Health Organization, 2023a). One of the types of cancer that is currently common is breast cancer. Breast cancer is one of the dominant cancers suffered by women. Breast cancer is the number one most common case in Indonesia with 58,256 cases in 2018 and it is predicted that there will be 89,512 cases in 2040 (World Health Organization, 2023). From previous research, various types of breast cancer have a significant relationship with different biological processes and genetic mutations such as hormone receptors, human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), cyclin-dependent kinase 4/6 (CDK4/6), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) involved in cell proliferation, PIK3CA mutation that is positive for the estrogen hormone receptor (ER) (Ju et al., 2018; Miricescu et al., 2020).

Conventional cancer treatments include surgery, radiation, hormone therapy, and chemotherapy (Shekar et al., 2020). This treatment still has many significant side effects. Apart from that, currently hormone therapy is one of the treatments that is currently popular, but because cancer is a very complex disease and contains several pathogenic mutations, hormone therapy or single targets are less than optimal. Multi-target therapy alternatives are needed which can target not just one target (García-Galindo et al., 2021).

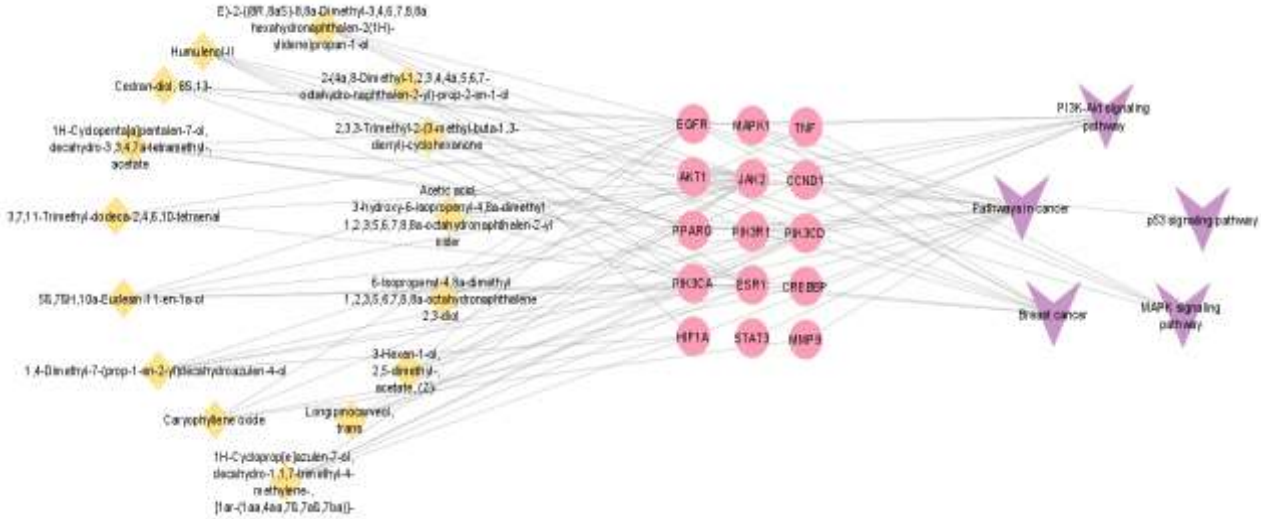

Figure 9. Drug network "active compound–target protein–pathway". Yellow diamonds represent active compounds, pink circles represent target proteins and purple arrows represent pathways

To minimize the side effects of excessive chemicals in conventional treatment. An analysis was carried out on one of the endemic plants of Aceh which has been widely used in traditional and herbal medicine, namely, Pogostemon cablin. This plant has been widely researched to contain various compounds with various biological activities that support digestion problems and have the potential to fight cancer. This plant exhibits anti-cancer, anti-inflammatory and antioxidant effects, so it is able to inhibit the growth of cancer cells, stop the cell cycle and function as an antioxidant (Chien et al., 2020).

The approach taken is through the Network Pharmacology method. This method is carried out through an approach of looking at the therapeutic effects of drug compounds on disease and gene interactions, but not just one gene but interactions with many proteins/genes. This method helps to understand how

chemical compounds in medicinal plants interact with disease genes in the human body (Sharma et al., 2022). This is a new technique for assessing drug capabilities that involves molecular interactions and is powerful in analyzing large biological data (Qasim et al., 2023). Plant compounds were tested for toxicity and violations of Lipinski's 5 rules. From the Swiss ADME results on the Pogostemon cablin compound, there are no compounds that violate more than 1 Lipinski rule and half of the total compounds have good absorption capacity in the human body. This proves that the compounds in medicinal plants still comply with the rules to become drug candidates.

From the results of this study which designed a multi-target protein therapy mechanism from the bioactive compound Pogostemon cablin for the treatment of breast cancer, it was found that the compounds pogostol, 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene -, [1ar-(1aa,4aa,7ß,7aß,7ba)]-, 3-Hexen-1-ol, 2,5-dimethyl-, acetate, (Z)-, 5ß,7ßH,10a-Eudesm-11 -en-1a-ol, Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl 1,2,3,5,6,7,8,8a-octahydronaphthalene-2-yl ester, and Humulenol-II plays an important role in breast cancer treatment targeting the MAPK1, EGFR, TNF, AKT1, JAK2 genes. In previous research, it was stated that AKT1 can inhibit the expression of a group of genes in breast cancer (George et al., 2022). EGFR/HER2 is part of the MAPK Signaling Pathway which signals breast cancer (Evans et al., 2018) and EGFR is found in ER-positive/HER2 breast cancer with high expression associated with increased infiltration by anti-cancer immune cells (Oshi et al., 2022).

This research also presents an analysis of gene-phenotype associations in the form of pathways, gene function and biological characteristics at the molecular level. Related to biological processes from several aspects, namely Cellular components, Molecular Function, and Biological Process. CC, which plays a lot of roles in genes, is related to cells. BP in this research gene is closely related to the response to hormones, phosphorylation and MAPK biological processes. This analysis revealed that the main pathways of Pogostemon cablin for the treatment of breast cancer include Pathway in cancer, breast cancer, PI3K-Akt signaling pathway, and MAPK signaling pathway, p53 signaling pathway. Based on previous studies, the PI3K/Akt/mTOR pathway is involved in growth, proliferation, is found in almost all human tumors, including breast cancer (Ortega et al., 2020) and is the main cause of cancer cell resistance to antitumor therapy (Martini et al., 2014). MAPK signaling pathway plays a role in various biological processes such as cell proliferation, cell differentiation, cell cycle regulation, cell apoptosis and is related to tumor formation. The MAPK signaling pathway can promote the transformation of normal cells into cancer cells, inhibiting this pathway can inhibit tumor growth in vivo and restore tumor cells to an unchanged state in vitro (Guo et al., 2020). Therefore, the target protein in the medicinal plant compound Pogostemon cablin has the potential and opportunity to become a drug for breast cancer. Because the proteins are interrelated between medicinal plants and disease as well as biological processes and pathways that are very relevant to breast cancer.

The use of network pharmacology is very useful for identifying new targets and identifying medicinal compounds in plants that reduce the level of side effects and also of course reduce the level of drug development costs (Zhang et al., 2019). However, this research has limitations because the database of genes involved and medicinal compounds may not be completely complete, such as therapeutic compounds from medicinal plants that can still be carried out further research. Analysis and examination of drug compound discovery using this method has not yet been carried out in animal models or clinical trials. As well as other side effects of medicinal plants, further research still needs to be done. However, this research is initial information that the medicinal plant Pogostemon cablin can be an alternative medicine for breast cancer.

Conclusion

In this study, network pharmacology was used to identify and analyze the mechanism of Pogostemon cablin against breast cancer. The Pogostemon cablin plant is used as one of the endemic plants of Aceh for traditional herbal medicine with the aim of minimizing the side effects of conventional treatment. It was found that the compound pogostol plays an important role in this mechanism. Pogostemon cablin induces the PI3K-Akt Signaling Pathway and MAPK Signaling Pathway as well as the biological processes that occur leading to phosphorylation and cells. So this is promising for further research as a treatment for breast cancer. Thus, network pharmacology is an interesting and promising approach to discover and analyze drug mechanisms in the future to treat cancer, especially breast cancer.

Acknowledgments
All author would like to thank to all parties who have helped this research.

Author Contributions
Syarifah Fathimah Azzahra conceptualized the research idea, designed of methodology, management and coordination responsibility; Essy Harnelly and Muhammad Subianto

analyzed data, conducted a research and investigation process; Wisnu Ananta Kusuma and Widya Sari conducted literature review and provided critical feedback on the manuscript.

Funding
This research is supported by funding through the Freedom of Learning Independence Campus (MBKM) research grant with contract number 359/UN11.2.1/PT.01.03/PNBP/2023.

Conflicts of Interest
The author declared no conflict of interest.

References

Amberger, J. S., & Hamosh, A. (2018). Knowledgebase of Human Genes and Genetic Phenotypes. *Curr Protoc Bioinformatics*, *58*, 1–20. https://doi.org/10.1002/cpbi.27.Searching

American Cancer Society. (2020). *The American Cancer Society medical and editorial content team*. Retrieved from https://www.cancer.org/cancer/diagnosis-staging/signs-and-symptoms-of-cancer.html

Bauer-Mehren, A., Rautschka, M., Sanz, F., & Furlong, L. I. (2010). DisGeNET: a Cytoscape plugin to visualize, integrate, search and analyze gene–disease networks. *Bioinformatics*, *26*(22), 2924–2926. https://doi.org/10.1093/bioinformatics/btq538

Breastcancerorg. (2023). *Target therapy," breastcancer.org*. Retrieved from https://www.breastcancer.org/treatment/targeted-therapy

Chien, J.-H., Lee, S.-C., Chang, K.-F., Huang, X.-F., Chen, Y.-T., & Tsai, N.-M. (2020). Extract of Pogostemon cablin Possesses Potent Anticancer Activity against Colorectal Cancer Cells In Vitro and In Vivo. *Evidence-Based Complementary and Alternative Medicine*, *2020*(1). https://doi.org/10.1155/2020/9758156

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*(1), 42717. https://doi.org/10.1038/srep42717

Daina, A., Michielin, O., & Zoete, V. (2019). SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Research*, *47*(W1), W357–W364. https://doi.org/10.1093/nar/gkz382

Daly, P. A. (1994). Drug therapy for breast cancer. *Irish Medical Journal*, *87*(5). Retrieved from https://www.cancer.org/cancer/types/breast-cancer/treatment/targeted-therapy-for-breast-cancer.html

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, *4*, 1–10. https://doi.org/10.3389/fphar.2013.00177

Evans, M. K., Brown, M. C., Geradts, J., Bao, X., Robinson, T. J., Jolly, M. K., Vermeulen, P. B., Palmer, G. M., Gromeier, M., Levine, H., Morse, M. A., Van Laere, S. J., & Devi, G. R. (2018). XIAP Regulation by MNK Links MAPK and NFκB Signaling to Determine an Aggressive Breast Cancer Phenotype. *Cancer Research*, *78*(7), 1726–1738. https://doi.org/10.1158/0008-5472.CAN-17-1667

Fishilevich, S., Zimmerman, S., Kohn, A., Iny Stein, T., Olender, T., Kolker, E., Safran, M., & Lancet, D. (2016). Genic insights from integrated human proteomics in GeneCards. *Database*, *2016*, baw030. https://doi.org/10.1093/database/baw030

García-Galindo, G., Castro, J., Matés, J., Bravo, M., Ribó, M., Vilanova, M., & Benito, A. (2021). The Selectivity for Tumor Cells of Nuclear-Directed Cytotoxic RNases Is Mediated by the Nuclear/Cytoplasmic Distribution of p27KIP1. *Molecules*, *26*(5), 1319. https://doi.org/10.3390/molecules26051319

George, B., Gui, B., Raguraman, R., Paul, A. M., Nakshatri, H., Pillai, M. R., & Kumar, R. (2022). AKT1 Transcriptomic Landscape in Breast Cancer Cells. *Cells*, *11*(15), 2290. https://doi.org/10.3390/cells11152290

Guo, Y., Pan, W., Liu, S., Shen, Z., Xu, Y., & Hu, L. (2020). ERK/MAPK signalling pathway and tumorigenesis (Review). *Experimental and Therapeutic Medicine*, 1997–2007,. https://doi.org/10.3892/etm.2020.8454

He, S., Wang, T., Shi, C., Wang, Z., & Fu, X. (2022). Network pharmacology-based approach to understand the effect and mechanism of Danshen against anemia. *Journal of Ethnopharmacology*, *282*, 114615. https://doi.org/10.1016/j.jep.2021.114615

Hochberg, Y., & Benjamini, Y. (1990). More powerful procedures for multiple significance testing. *Statistics in Medicine*, *9*(7), 811–818. https://doi.org/10.1002/sim.4780090710

Hopkins, A. L. (2008). Network pharmacology: the next paradigm in drug discovery. *Nature Chemical Biology*, *4*(11), 682–690. https://doi.org/10.1038/nchembio.118

Ju, J., Zhu, A., & Yuan, P. (2018). Progress in targeted therapy for breast cancer. *Chronic Diseases and Translational Medicine*, *4*(3), 164–175. https://doi.org/10.1016/j.cdtm.2018.04.002

Kelly, K., & Bell, S. (2018). Evaluation of the reproducibility and repeatability of GCMS retention indices and mass spectra of novel psychoactive substances. *Forensic Chemistry*, *7*, 10–

18. https://doi.org/10.1016/j.forc.2017.11.002

Li, C.-W., Wu, X.-L., Zhao, X.-N., Su, Z.-Q., Chen, H.-M., Wang, X.-F., Zhang, X.-J., Zeng, H.-F., Chen, J.-N., Li, Y.-C., & Su, Z.-R. (2013). Anti-Inflammatory Property of the Ethanol Extract of the Root and Rhizome of Pogostemon cablin (Blanco) Benth. *The Scientific World Journal*, *2013*(1). https://doi.org/10.1155/2013/434151

Martini, M., De Santis, M. C., Braccini, L., Gulluni, F., & Hirsch, E. (2014). PI3K/AKT signaling pathway and cancer: an updated review. *Annals of Medicine*, *46*(6), 372–383. https://doi.org/10.3109/07853890.2014.912836

Mayo, B., & Staff, C. (2015). Disease and condition Vaginitis. *Mayoclinic.Org.*, *24*, 1–7. Retrieved from https://www.mayoclinic.org/diseases-conditions/cancer/symptoms-causes/syc-20370588

Metascape.org. (2023). *Metascape Gene List Analysis Report*. Retrieved from https://metascape.org/gp/index.html#/reportfinal/tdcg18s8s

Miricescu, D., Totan, A., Stanescu-Spinu, I.-I., Badoiu, S. C., Stefani, C., & Greabu, M. (2020). PI3K/AKT/mTOR Signaling Pathway in Breast Cancer: From Molecular Landscape to Clinical Aspects. *International Journal of Molecular Sciences*, *22*(1), 173. https://doi.org/10.3390/ijms22010173

National Cancer Institute. (2023). *What Is Cancer? was originally published by the National Cancer Institute*. Retrieved from https://www.cancer.gov/about-cancer/understanding/what-is-cancer

Ortega, M. A., Fraile-Martínez, O., Asúnsolo, Á., Buján, J., García-Honduvilla, N., & Coca, S. (2020). Signal Transduction Pathways in Breast Cancer: The Important Role of PI3K/Akt/mTOR. *Journal of Oncology*, *2020*, 1–11. https://doi.org/10.1155/2020/9258396

Oshi, M., Gandhi, S., Tokumaru, Y., Wu, R., Yan, L., Yamada, A., Ishikawa, T., Endo, I., & Takabe, K. (2022). Abstract P5-03-01: Conflicting roles of EGFR expression by subtypes in breast cancer. *Cancer Research*, *82*. https://doi.org/10.1158/1538-7445.SABCS21-P5-03-01

Piñero, J., Ramírez-Anguita, J. M., Saüch-Pitarch, J., Ronzano, F., Centeno, E., Sanz, F., & Furlong, L. I. (2019). The DisGeNET knowledge platform for disease genomics: 2019 update. *Nucleic Acids Research*, *48*(D1), 845– 855,. https://doi.org/10.1093/nar/gkz1021

Popović-Djordjević, J., Quispe, C., Giordo, R., Kostić, A., Katanić Stanković, J. S., Tsouh Fokou, P. V., Carbone, K., Martorell, M., Kumar, M., Pintus, G., Sharifi-Rad, J., Docea, A. O., & Calina, D. (2022). Natural products and synthetic analogues against HIV: A perspective to develop new potential anti-HIV drugs. *European Journal of Medicinal Chemistry*, *233*(114217), 114217. https://doi.org/10.1016/j.ejmech.2022.114217

Qasim, M., Abdullah, M., Ali Ashfaq, U., Noor, F., Nahid, N., Alzamami, A., Alturki, N. A., & Khurshid, M. (2023). Molecular mechanism of Ferula asafoetida for the treatment of asthma: Network pharmacology and molecular docking approach. *Saudi Journal of Biological Sciences*, *30*(2), 103527. https://doi.org/10.1016/j.sjbs.2022.103527

Sari, S. L., Indra, R. L., & Lestari, R. F. (2019). Korelasi Persepsi Tentang Efek Samping Kemoterapi Dengan Kualitas Hidup Pasien Kanker Payudara. *Jurnal Cakrawala Promkes*, *1*(2), 40. https://doi.org/10.12928/promkes.v1i2.1771

Shang, L., Wang, Y., Li, J., Zhou, F., Xiao, K., Liu, Y., Zhang, M., Wang, S., & Yang, S. (2023). Mechanism of Sijunzi Decoction in the treatment of colorectal cancer based on network pharmacology and experimental validation. *Journal of Ethnopharmacology*, *302*, 115876. https://doi.org/10.1016/j.jep.2022.115876

Sharma, B., & Yadav, D. K. (2022). Metabolomics and Network Pharmacology in the Exploration of the Multi-Targeted Therapeutic Approach of Traditional Medicinal Plants. *Plants*, *11*(23), 3243. https://doi.org/10.3390/plants11233243

Shekar, N., Mallya, P., Gowda, D. V, & Jain, V. (2020). Triple-negative breast cancer: challenges and treatment options. *International Journal of Research in Pharmaceutical Sciences*, *11*(2), 1977–1986. https://doi.org/10.26452/ijrps.v11i2.2127

Stelzer, G., Rosen, N., Plaschkes, I., Zimmerman, S., Twik, M., Fishilevich, S., Stein, T. I., Nudel, R., Lieder, I., Mazor, Y., Kaplan, S., Dahary, D., Warshawsky, D., Guan-Golan, Y., Kohn, A., Rappaport, N., Safran, M., & Lancet, D. (2016). The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. *Current Protocols in Bioinformatics*, *54*(1). https://doi.org/10.1002/cpbi.5

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

Syahruni, R., Umar, A. H., Rahman, H. N., & Kusuma, W. A. (2023). Exploration of Annona muricata (Annonaceae) in the Treatment of Hyperlipidemia

through Network Pharmacology and Molecular Docking. *Sains Malaysiana*, *52*(3), 899–939. https://doi.org/10.17576/jsm-2023-5203-17

Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N. T., Morris, J. H., Bork, P., Jensen, L. J., & Mering, C. von. (2019). STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research*, *47*(D1), D607–D613. https://doi.org/10.1093/nar/gky1131

Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N. T., Legeay, M., Fang, T., Bork, P., Jensen, L. J., & von Mering, C. (2021). Correction to 'The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets.' *Nucleic Acids Research*, *49*(18), 10800–10800. https://doi.org/10.1093/nar/gkab835

Tian, Q., Hu, P., Wang, M., Hu, Y., Xie, L., Li, Z., Xu, Z., Liu, F., & Huang, C. (2023). Study on the mechanism of Gao-Liang-Huo decoction against dextran sulfate sodium-induced ulcerative colitis based on metabolomics and network pharmacology. *Pharmacological Research - Modern Chinese Medicine*, *9*, 100304. https://doi.org/10.1016/j.prmcm.2023.100304

Umar, A. H., Ratnadewi, D., Rafi, M., Sulistyaningsih, Y. C., Hamim, H., & Kusuma, W. A. (2023). Drug candidates and potential targets of Curculigo spp. compounds for treating diabetes mellitus based on network pharmacology, molecular docking and molecular dynamics simulation. *Journal of Biomolecular Structure and Dynamics*, *41*(17), 8544–8560. https://doi.org/10.1080/07391102.2022.2135597

Wang, X., Wang, Z.-Y., Zheng, J.-H., & Li, S. (2021). TCM network pharmacology: A new trend towards combining computational, experimental and clinical approaches. *Chinese Journal of Natural Medicines*, *19*(1), 1–11. https://doi.org/10.1016/S1875-5364(21)60001-8

Wicki, A., & Rochlitz, C. (2012). Targeted therapies in breast cancer. *Swiss Medical Weekly*, *142*(APRIL). https://doi.org/10.4414/smw.2012.13550

World Health Organization. (2013). *Traditional medicine*. Retrieved from https://apps.who.int/gb/ebwha/pdf_files/EB134/B134_24-en.pdf

World Health Organization. (2023a). *360 indonesia fact sheet*. Retrieved from https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf

World Health Organization. (2023b). *Breast cancer*. Retrieved from https://www.who.int/news-room/fact-sheets/detail/breast-cancer

Wu, T., Zhang, L., Li, D., Wu, T., Jiang, Y., & Zhao, L. (2021). Study on the Mechanism of Ginkgo Seeds in Treating Bronchitis by Network Pharmacology. *Sains Malaysiana*, *50*(5), 1433–1444. https://doi.org/10.17576/jsm-2021-5005-22

Yun, S. G., Jin, S., Jeong, H. Y., Yun, H. J., Do, M. young, Kim, B. W., & Kwon, H. J. (2015). Anti-oxidant, Anti-inflammatory and Anti-cancer Effect of Methanol Extract of Pogostemon cablin. *Journal of Life Science*, *25*(1), 44–52. https://doi.org/10.5352/JLS.2015.25.1.44

Zhang, R., Zhu, X., Bai, H., & Ning, K. (2019). Network Pharmacology Databases for Traditional Chinese Medicine: Review and Assessment. *Frontiers in Pharmacology*, *10*(February), 1–14,. https://doi.org/10.3389/fphar.2019.00123

Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., Benner, C., & Chanda, S. K. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nature Communications*, *10*(1), 1523. https://doi.org/10.1038/s41467-019-09234-6

Zuhri, U. M., Purwaningsih, E. H., Fadilah, F., & Yuliana, N. D. (2022). Network pharmacology integrated molecular dynamics reveals the bioactive compounds and potential targets of Tinospora crispa Linn. as insulin sensitizer. *PLOS ONE*, *17*(6), e0251837. https://doi.org/10.1371/journal.pone.0251837