RESEARCH ARTICLE

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The Potential Compounds in Lansium parasiticum Leaf Extract for Breast Cancer Therapy: Metabolite Profiling, Pharmacological Network Analysis and *In Silico* Validation

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Abstract

Objective: This study aims to identify the compounds found in Lansium parasiticum leaf extract (LPLE) and explain its activity in the context of breast cancer prevention and therapy using a pharmacological network approach and its validation in silico to understand the molecular mechanisms involved. **Methods:** Identification of compounds in LPLE is done using Liquid Chromatography Tandem Mass Spectrophotometry (LC-MS/MS). We also identified absorption and bioavailability profiles using ADMET software. Predictions about the molecular mechanisms of the anti-cancer compounds of LPLE were made through a network pharmacological approach involving devices such as Cytoscape 3.9.1, GeneCards, Disgenet, STRING 2.0.0, the Kyoto Encyclopedia of Genes and Genomes (KEGG) path, and SRplot. Interactions between potential compounds with TP53 receptors were analyzed using site-specific molecular docking, using PyRx Autodock Vina 9.0 and Biovia Discovery Studio. **Result:** A total of 24 active compounds were successfully identified through LC-MS/MS. The results of the pharmacological network analysis of these compounds showed that there are four substances that have potential against the potential target gene of breast cancer, namely dihydrotestosterone with 8 target genes, Oxoberberine with 8 targets, Pregnenolone with 1 target gene, and Quercetine with 16 targets. The results of in silico validation revealed that the four compounds showed strong affinity to TP53, even higher than their original ligaments. **Conclusion:** The study successfully identified the active compounds in Lansium parasiticum leaf extract (LPLE) that have potential in the prevention and treatment of breast cancer.

Keywords: Oxoberberin- LCMS/MS- quercetine- TP53- pregnenolone

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Introduction

Breast cancer is the most common cancer in the world and is the leading cause of cancer death in women. According to data from the International Agency for Research on Cancer (IARC) by 2020, there are about 2.3 million new cases of breast cancer and more than 685,000 deaths from breast cancer worldwide [1]. Conventional breast cancer therapies, such as chemotherapy, radiation, and hormone therapy, are not always effective and are not free of side effects [2]. The development of drug resistance is also a problem in the treatment of breast cancer [3]. Other targeted therapies that can target specific genetic mutations associated with breast cancer are still not widely available [4]. Therefore, to address the current problem, the development of new anti-cancer drugs is still needed.

Lansium parasiticum is an indigenous Indonesian plant that is very easy to find in various regions and has been generally trusted as a medicinal plant for the Indonesians. The leaves of this plant have been shown to contain positive alkaloids, saponins, flavonoids, and polyphenols tested as antidiabetic and antibacterial [5]. Studies that have been to this day are still a group of compounds, the composition of the active compound of the plant has never been explored. It is therefore important to identify the metabolites found in the leaf Lansium parasiticum.

LCMS/MS is a highly sensitive method of analysis and can provide accurate information about the compounds contained in plants [6]. Using this technique, research can identify the active compounds in Lansium parasiticum more accurately, including compound that may have potential as an anti-cancer drug.

To explain the progression of disease from the point of view of system biology, pharmacology, and biological tissue, tissue pharmacologia is a promising research approach that combines pharmacologie, molecular biology and bioinformatics to determine tissue relationships between effective pharmacological components, related

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targets, paths, and diseases [7, 8]. This method not only predicts the relationship between drugs and diseases from a tissue perspective but also visualizes and analyzes complex biological systems [9, 10].

The integration of compound identification using LCMS/MS with the pharmacological network on Lansium parasiticum has important advantages in identifying active compounds accurately, understanding the molecular mechanisms involved in the treatment of breast cancer, and opening up opportunities for the development of more targeted anti-cancer drugs based on natural compositions from these plants [11]. This can help in developing drugs that are more effective in dealing with breast cancer and provide a strong knowledge base for further research.

The main objective of this study was to perform metabolite profiles of compounds using the LCMS/MS method, then analyze the mechanisms of molecular action, target genes, and potential pathways of Lansium parasiticum leaf extract in the treatment of breast cancer using tissue pharmacology approaches and bioinformatic. This research is intended to provide valuable insight into future pharmacological research and potential clinical therapeutic applications.

Materials and Methods

Preparing Leaf Extract from Lansium parasicum (LPLE)

Using Lansium parasiticum leaf obtained under the number 067/566/102.20/2023 from an East Javan location at a 400-meter altitude, Lansium parasiticum leaf Extract (LPLE) was extracted. The area experienced 125,49 mm of annual rainfall and an average temperature of 25 degrees Celsius. The leaf powder was extracted at a ratio of 1:10 with 70% ethanol and the Ultra Assisted Extraction (UAE) method. This process took place for 20 minutes at 40°C. The ethanol extract was then ready for additional examination by being baked for five hours at 40°C [12].

LC-MS/MS Examination

UPLC-MS equipment outfitted with a QToF analyzer and positive ESI as the ionization source were utilized to perform the LC-MS/MS study. An Acquity C18 column (1.8 µm; 2.1 × 150 mm) was used. The eluent was made up of two different types of water (HPLC grade) and formic acid (Merck, Darmstadt, Germany) in a ratio of 99.9/0.1 [v/v] and 99.9/0.1 [v/v] for acetonitrile (Merck, Darmstadt, Germany), respectively, using a gradient elution system. The desolvation temperature was set to 350°C, while the source temperature was set to 100°C. Absolute methanol was used to dissolve a 10 mg extract in a 10 ml volumetric flask. Five microliters of this solution were then introduced into the UPLC-MS apparatus. Spectra were obtained over a mass range of m/z 120 to 1000, with the analytical parameters set in positive ion mode. For processing the chromatogram and compound identification, Mass Lynx version 4.1 software (Waters, Massachusetts, USA) and PubChem (https://pubchem.ncbi.nlm.nih.gov/) were used. Based on MS/MS fragment matching with an error threshold of less than 5 ppm, a compound's accuracy was confirmed [6].

Screening for Oral Bioavailability

Screening for Oral Bioavailability Oral bioavailability is a critical pharmacokinetic parameter that measures the quantity of medicine taken orally that enters the bloodstream and has pharmacological effects. SwissADME (http://www.swissadme.ch/index.php) was used to evaluate the molecular weight, gastrointestinal absorption, lipophilicity, H-bond donors (HBD), and acceptors (HBA) of the detected compounds [13].

Identification of Potential Targets for Breast Cancer

Identification of Potential Targets for Breast Cancer The objective of this study is to identify potential targets for breast cancer. A core element of pharmaceutical research entails the anticipation of the interactions between chemical compounds and certain molecular targets. The gene targets linked to the active chemicals in the LPLE, as determined using LC-MS/MS analysis, were discovered by utilizing the Gene Cards database available at https://www.genecards.org/. Concurrently, the DisGeNET database (https://www.disgenet.org) was utilized to investigate gene targets associated with breast cancer. Following that, a network pharmacology study was performed using Cytoscape software version 3.9.1 in order to acquire a comprehensive understanding of the interactions between active drugs and gene targets, as well as the outcomes pertaining to disease-gene targets [14].

The construction of pharmacological networks and protein-protein interactions

The process of establishing pharmacological network linkages, which encompass active substances, target genes, and illnesses, was conducted with Cytoscape version 3.10. To conduct a more comprehensive analysis, we identified gene targets that exhibited overlap between active chemicals and illnesses. These selected gene targets were then subjected to processing using the STRING platform version 12.0, which can be accessed at https://string-db.org/. The establishment of the Protein-Protein Interaction (PPI) network involved the inclusion of shared target proteins, with a specified minimum interaction score threshold of 0.400. The objective of doing PPI network analysis was to explore biological phenomena by scrutinizing functional annotations associated with Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) [15].

The analysis of GO and KEGG

The targets that were discovered underwent an investigation utilizing Gene Ontology (GO) and functional pathway enrichment. This analysis was conducted using the R programming language. The screening parameters for evaluating functional enrichment data were established at a significance level of $p = 0.05$ and a false discovery rate of $q = 0.05$. The GO analysis was conducted to identify the most noteworthy discoveries pertaining to cellular component (CC), molecular function (MF), and biological process (BP). These findings were then visually represented using R programming language to generate a bubble chart. Furthermore, the bubble diagrams for visual representation were generated using SRPlot (http://www.

bioinformatics.com.cn/srplot), employing the top thirty KEGG pathway enrichments [16].

The process of molecular docking

AutoDock Vina is commonly employed in molecular docking to determine the optimal conformation and binding affinity of ligands and proteins. The docking procedure was performed to investigate the interactions between dihydrotestosterone, oxoberberine, pregnenolone, and quercetine chemicals and six specific target receptors, namely *TP53* (PDB ID: 3DCY). The ligand structures were acquired from the PubChem database, whilst the receptor structures were sourced from the Protein Data Bank. To conduct the initial validation, the process of re-docking was carried out between the receptors and their corresponding original ligands. This involved evaluating the root-mean-square deviation (RMSD) parameter, which was required to be below 2.0 Å. The rankings of the compound docking results were determined based on the values of affinity energy. The visualization of the interactions between the ligand and receptor was performed using Biovia Discovery Studio and PyMol 19 software [17].

Results

The analysis of metabolite profiling 1

The objective of this study is to conduct metabolite profiling in order to determine the chemicals present in the 70% ethanol extract obtained from Lansium parasiticum leaves (LPLE). The utilization of the UPLC-QtoF-MS/MS apparatus has yielded findings that validate the presence of a chromatogram displaying 24 chemicals (Figure 1).

The metabolite profiles presented in Table 1 indicate that *LPLE* is composed of a total of 24 chemicals. These compounds can be categorized into different classes, including 11 alkaloids, 3 flavonoids, 2 phenols, 3 terpenoids, 4 steroids, and 1 indol alkaloid. The primary chemical found in *LPLE* is Emindole Sb, which constitutes the biggest area proportion at 19.3%. Bolandiol (19-Nor-4-androstenediol) follows with an area percentage of 13.93%, while 4-Acetamidobutyric acid accounts for 13.57% of the total area.

The assessment of oral bioavailability screening is conducted to predict the physiochemical characteristics of LPLE, with the primary objective of ascertaining the absorption and permeability of these substances. This prediction is grounded in Lipinski's fifth law and incorporates various characteristics, such as Molecular Weight, Log P, Hydrogen Bond Acceptors (HBA), Hydrogen Bond Donors (HBD), Torsion, and Polar Surface Area (PSA). Based on the screening findings, it was found that 14 compounds satisfy the five Lipinski rule parameters, indicating that the medicine has achieved oral bioavailability. The prediction of physiochemical properties for each compound is presented in Table 2.

In an attempt to find the potential target gene of the compound in the *LPLE* for breast cancer treatment using GeneCards, it has been revealed that of the 24 compounds contained in LPLE, they are connected to 109 potential target genes. On the other hand, based on data from Disgene, the target genes associated with breast cancer include 1,209 genes, including estrogen receptor positive breast cancer (CUI:C2938924), Breast Cancer Familial (CUI:C0346153), Estrogen-receptor-negative breast cancer, and HER2 negative breast cancer. (CUI:C4733095). Analysis of Venn's diagram comparing the target gene of a phytochemical compound with the disease target gene, found that there were 40 potentially overlapping target genes(Figure 2, Table 3). Furthermore, of these 40 target genes, further analysis related to the pharmacological tissue was carried out using the Cytoscape device (Figure 3).

The results of the pharmacological tissue analysis of the compounds in the *LPLE* with the breast cancer target gene showed that out of 24 existing compound,

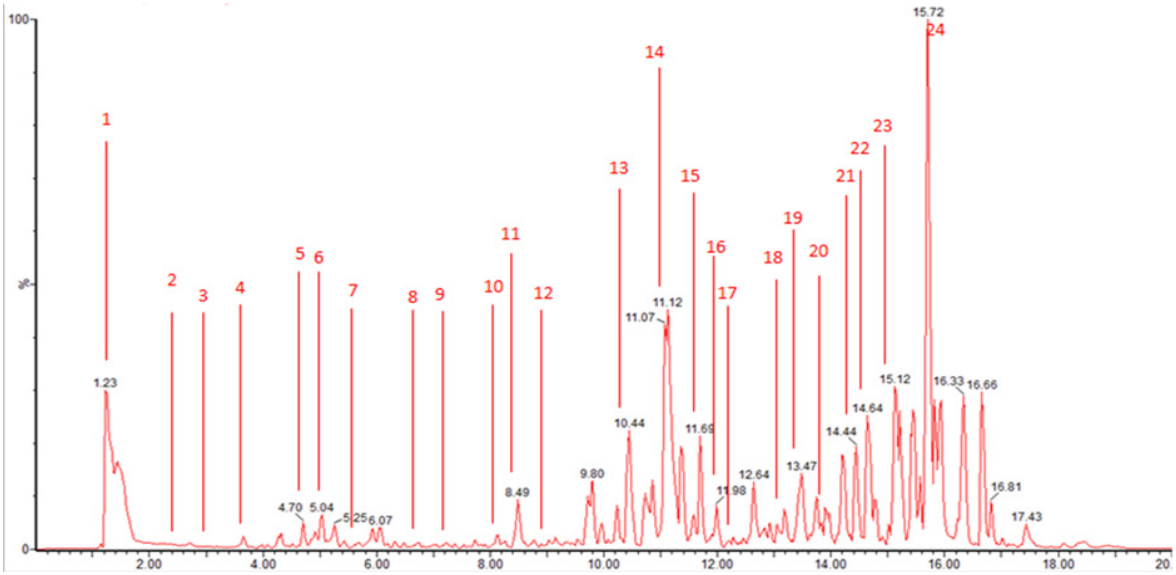

Figure 1. The Stationary Phase Utilized in This Experiment was a C18 Phase from the 18th Century. The mobile phase consisted of a mixture of water and formic acid in a ratio of 99.9:0.1 (v/v), as well as a combination of acetonitrile and formic acid in a ratio of 99.9:200. Each peak observed in the chromatogram represents a distinct chemical.

Table 1. Presents the Outcomes of Metabolite Identification for the Leaf Extract of Lansium parasiticum, Employing the UPLC Qtof MS/MS Technique.

No	Rt	%Area	Measured Mass	Calculated Mass	Rumus Formula	Compounds	Groups of compounds
1	1.303	13.57	1,460,818	1,460,817	$C_6H_{12}N0_3$	4-Acetamidobutyric acid	Alkaloid
2	2.709	0.35	1,660,867	1,660,868	$C_9H_{12}NO_2$	L-phenylalanine	Alkaloid
3	3.65	0.3	1,880,711	1,880,712	$C_{11}H_{10}N0_2$	methylquinoline-6-carboxylate	Alkaloid
4	4.311	0.36	4,081,764	4,081,804	$C_{15}H_{11}O_7$	Quercetine	Flavonoid
5	4.726	0.58	303,05	3,030,505	$C_{15}H_{11}O_6$	Kaemferol	Flavonoid
6	5.035	1.67	2,870,558	2,870,556	$C_{11}H_{17}O_3$	5-methoxyeugenol	Fenol
7	5.977	1.31	1,971,186	1,971,178	$C_{21}H_{22}N0_4$	oxoberberine	Alkaloid
8	7.237	0.17	3,521,541	3,521,549	$C_{31}H_{40}NO_{10}$	2-hydroxypropane-1,2,3-tricarboxylicacid;2-(2-piperidin-1-ylethoxy)ethyl2,2-diphenylcyclopropane-1-carboxylate	Alkaloid
9	7.735	0.13	2,752,024	2,752,011	$C_{29}H_{38}N_7O_9$	3-hydroxy-N-[3-[2-[(3-hydroxy-1-methyl-2-oxopyridine-4-carbonyl)amino]ethyl-[3-[(3-hydroxy-1-methyl-2-oxopyridine-4-carbonyl) amino]propyl]amino]propyl]-1-methyl-2-oxopyridine-4-carboxamide	Alkaloid
10	8.136	0.3	5,862,692	2,862,652	$C_{11}H_{17}O_2$	olivetol	Fenol
11	8.488	1.22	6,282,778	6,282,731	$C_{27}H_{33}O_{10}$	orbiculin D	Sesquiterpen (terpenoid)
12	9.12	0.16	1,811,229	1,811,229	$C_{25}H_{31}N_6O_7$	3-[[7-[3-[(2-methylpropan-2-yl) oxycarbonylamino]propoxy]-4-oxoquinolizine-2-carbonyl]amino]-2-(pyrimidin-2-ylamino) propanoic acid	Alkaloid
13	10.442	3.08	527,23	5,272,254	$C_{29}H_{35}O_9$	Territrem B	Terpenoid
14	11.124	13.93	5,272,283	5,272,281	$C_{18}H_{29}O_2$	Bolandiol (19-Nor-4-androstenediol)	Steroid
15	11.694	2.84	6,944,018	6,943,987	$C_{21}H_{35}O_3$	Tetrahydrodeoxycorticosterone	Diterpen (terpenoid)
16	11.982	0.79	2,772,155	2,772,168	$C_{27}H_{50}NO_9$	AAL Toxin TE2	Alkaloid
17	12.28	0.04	3,352,593	3,352,586	$C_{26}H_{46}N_3O_6$	8-phenyl-octanecarboxamie peptidomimetic	Alkaloid
18	13.163	0.95	4,963,403	4,963,387	$C_{19}H_{31}O_2$	dihydrotestosterone	Steroid
29	13.472	2.35	4,533,376	4,533,371	$C_{30}H_{45}O_3$	Moronate	Alkaloid
20	13.761	2.01	2,912,324	2,912,324	$C_{21}H_{33}O_2$	pregnenolone	Steroid
21	14.443	1.64	3,172,483	3,172,481	$C_{35}H_{60}NO_7$	CHEMBL4748940 (CID 162650677)	Steroid
22	14.661	4.45	6,344,494	6,364,449	$C_{31}H_{33}N_{10}O_4$	CHEMBL4855226 (CID 153347580)	Alkaloid
23	15.167	6.41	6,064,373	606,437	$C_{34}H_{41}O_9$	Scortechinone B	Xanthones (flavonoid)
24	15.715	19.3	5,932,731	5,932,751	$C_{28}H_{40}NO$	Emindole Sb	Terpenoid indol Alkaloid

there are 4 compounds that have the potential as potential target genes for breast cancer. The four compounds are Dihydrotestosterone with 8 target genes, Oxoberberine with 8 target genees, Pregnenolone with 1 target gene, and Quercetine with 16 target gene (Table 3).

Construction of Pharmacological Networks and Protein-Protein Interactions

Construction of Pharmacological Networks and Protein-Protein Interactions (PPI) refers to the relationship between different types of proteins in *LPLE* leaf extracts that have a role in the biological pathway of breast cancer treatment. The results show that several compounds found in the extract, dihydrotestosterone, oxoberberine, pregnenolone, quercetine, have the potential to interact with specific proteins involved in biological processes relevant to breast cancer treatment. These interactions can provide important insights into how these compounds work in inhibiting or influencing the progression of breast cancer, which in turn can help in the development of more effective therapies for the disease.

GO Analysis and KEGG Pathway Enrichment

Genetic ontological analysis suggests that the compounds found in LPLE, especially dihydrotestosterone, oxoberberine, pregnenolone, quercetine, have an impact on biological processes, molecular functions, and cellular components. On the biological buble diagram, the process shows 10 potential biological processes that are affected by compounds in the *LPLE* (Figure 4A). The biological process Regulation of apoptotic signaling pathway has the highest value and involves the interaction of 13 genes in the cellular component. The highest cellular components are the nuclear envelopes, the transcription regulator complex and the nuclear membrane with eight target genes involved. On molecular functions (Figure 4C) there are

Table 2. Displays the Outcomes of Physical and Chemical Predictions.

No	Compound Name	Parameters of Lipinski's five laws						Lipinski's five laws
		BM	Log P	HBA	HBD	Torsion	PSA	
1	4-Acetamidobutyric acid	148.158	-0.0127	2	2	4	59.291	Yes
2	L-phenylalanine	165.192	0.641	2	2	3	70.822	Yes
3	Methyl quinoline-6-carboxylate	187.198	2.0214	3	0	1	81.338	Yes
4	Quercetine	302.238	1.988	7	5	1	122.108	Yes
5	Kaemferol (luteolin)	286.239	2.2824	6	4	1	117.313	Yes
6	5-methoxyeugenol	196.246	2.3236	3	1	4	84.335	Yes
7	Oxoberberine	351.402	4.019	5	0	4	152.596	Yes
10	Olivetol	180.247	2.8305	2	2	4	78.845	Yes
11	Orbiculin D	500.544	2.3311	9	0	0	209.29	Yes
14	Bolandiol (19-Nor-4-androstenediol)	276.42	4.801	2	0	3	122.827	Yes
15	Tetrahydrodeoxycorticosterone	334.5	3.5676	3	2	2	145.765	Yes
16	AAL Toxin TE2	531.687	3.5148	7	5	22	220.71	Yes

10 potential molecule functions that are influenced by these compounds. The molecular function that involves the most target genes and has the highest significance is DNA binding trancription factor binding.

In the KEGG enrichment analysis it was found that there are two potential pathways that have great potential in the treatment of breast cancer by LPLE. The potential pathway is Breast cancer that targets 10 genes and P53 sinaling pathway targeting 10 potential target genes. (Figure 5 and 6). All of this is predicted to have a correlation and influence on the biological process of breast cancer healing.

Molecular docking results revealed that dihydrotestosterone, oxoberberine, pregnenolone, quercetine showed strong affinities to *TP53*. All of these compounds have a higher affinity than native ligans with lower energy values. (Figure 6). Pregnenolone has the highest affinity to the *TP53* receptor followed by dehydrotestosterone quercetin and berberin. The higher the affinity, the more stable the bond between the drug and the receptor. In the context of drug interactions with receptors on this molecular docking, the compound in the prediction shows its influence on apoptosis and cell cycles.

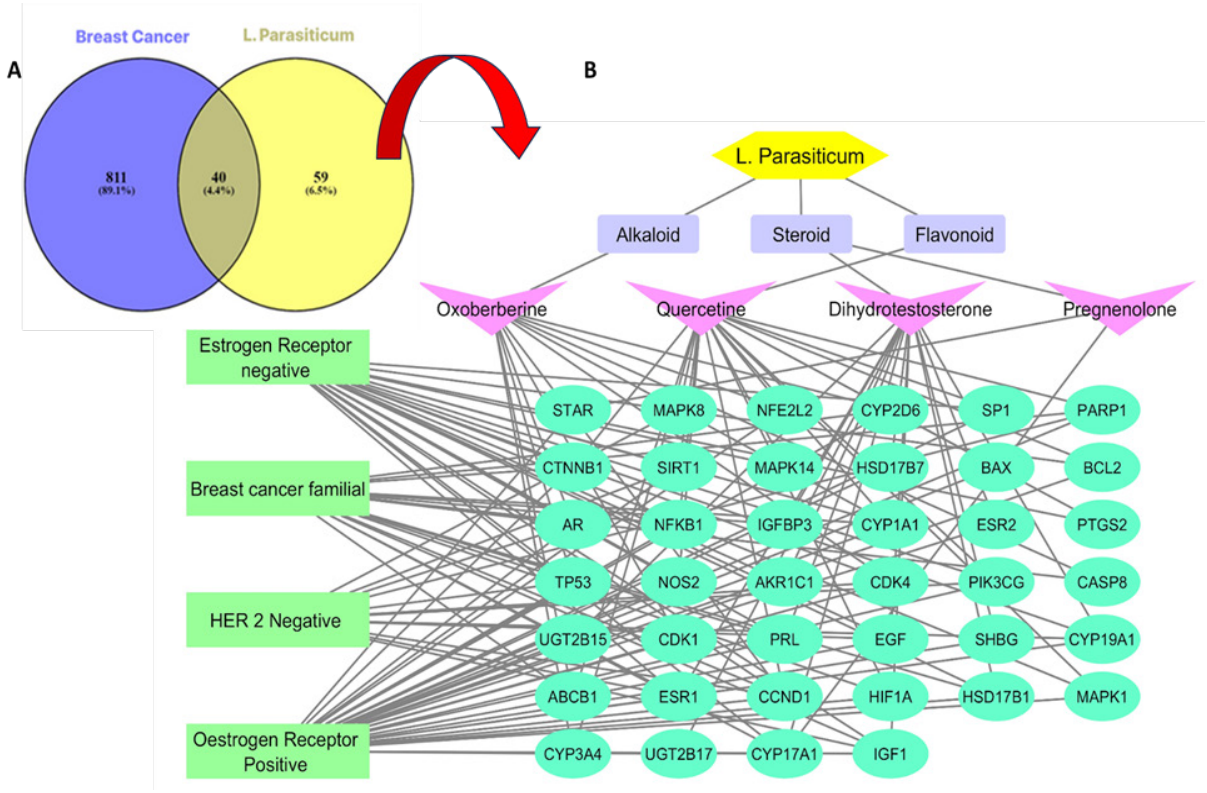

Figure 2. A) Venn diagram of gene targets for compounds in Lansium parasiticum leaf extract and breast cancer disease target genes, including four types: familial breast cancer, estrogen-negative breast cancers, oestrogen-positive breast cells, and HER2-negative bread cancers. (Number of nodes: 52, Number of edges: 138). Yellow: plant name, Old blue: compound group, purple: bioactive component, Green: Disease, Young blue: Target protein

Table 3. The Target Gene of the Compound in Lansium parasiticum and Relevance Score of Breast Cancer Target Gene (relevance score>7)

Compound	Gene symbol	Description	Gifts	Relevance score
Dihydrotestosterone	*ESR1*	Estrogen Receptor 1	58	8.640775
	IGF1	Insulin Like Growth Factor 1	52	7.461265
	EGF	Epidermal Growth Factor	55	7.802309
	PRL	Prolactin	48	7,393,603
	AKR1C1	Aldo-Keto Reductase Family 1 Member C1	47	8.600555
	ESR2	Estrogen Receptor 2	53	7.1124
	SHBG	Sex Hormone Binding Globulin	46	13.67667
	IGFBP3	Insulin Like Growth Factor Binding Protein 3	51	8.348991
Oxoberberine	*AR*	Androgen Receptor	55	10.68938
	CDK4	Cyclin Dependent Kinase 4	58	7.968866
	TP53	Tumor Protein P53	57	8.213263
	CCND1	Cyclin D1	57	9.082791
	CDK1	Cyclin Dependent Kinase 1	52	8.114895
	NFE2L2	NFE2 Like BZIP Transcription Factor 2	55	784,036
	ABCB1	ATP Binding Cassette Subfamily B Member 1	55	7.521132
	CASP8	Caspase 8	56	9.02974
Pregnenolone	*STAR*	Steroidogenic Acute Regulatory Protein	48	15.92391
Quercetine	*PIK3CG*	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Gamma	53	7.814326
	NOS2	Nitric Oxide Synthase 2	53	7.797181
	CTNNB1	Catenin Beta 1	57	7.093336
	MAPK1	Mitogen-Activated Protein Kinase 1	57	10.73088
	CCND1	Cyclin D1	57	7.307696
	PTGS2	Prostaglandin-Endoperoxide Synthase 2	53	7.04165
	BCL2	BCL2 Apoptosis Regulator	55	9.083662
	SP1	Sp1 Transcription Factor	50	7.159557
	SIRT1	Sirtuin 1	53	7.216932
	MAPK14	Mitogen-Activated Protein Kinase 14	54	8.378438
	PARP1	Poly(ADP-Ribose) Polymerase 1	54	7.956178
	MAPK8	Mitogen-Activated Protein Kinase 8	53	9.047739
	HIF1A	Hypoxia Inducible Factor 1 Subunit Alpha	52	7.232303
	NFE2L2	NFE2 Like BZIP Transcription Factor 2	55	8.494093
	BAX	BCL2 Associated X, Apoptosis Regulator	55	8.45645
	NFKB1	Nuclear Factor Kappa B Subunit 1	57	9.149029

Discussion

Lansium parasiticum is a traditional Indonesian medicinal plant that is still not much in exploration. Previous research has never any link between any compounds contained in Lansium parasiticum and the potential of the compound in suppressing the proliferation of cancer cells, especially breast cancer. In addition to target genes, pathways involved in the biological processes of cancer healing have also not been. The study aims to identify the metabolite profiles of the compounds using the LCMS/MS method and further dig molecular mechanisms, target genes, as well as potential pathways involved in the breast cancer treatment effects of Lansium parasiticum leaf extract (LPLE). In LCMSMS analysis, our research showed that *LPLE* contains 24 active substances, in the active compound there are 4 substances that have the potential as a potential target gene for breast cancer. The four compounds are dihydrotestosterone with 8 target genes, oxoberberine with 8 targets, Pregnenolone with 1 target gene, and Quercetine with 16 target gene. The P53 signal pathway plays an important role in breast cancer; mutations in p53 are often identified in human neoplasms and breast cancer, these mutations are associated with more aggressive diseases and worse survival [18].

The P53 signal path operates in standby mode under normal conditions, and is activated in response to various cellular stresses such as DNA damage and activated oncogen expression. This activation involves post-translation modifications such as phosphorylation, which activates proteins for DNA binding and transactivates the 'effector' gene that represents the p53 tumor suppressant

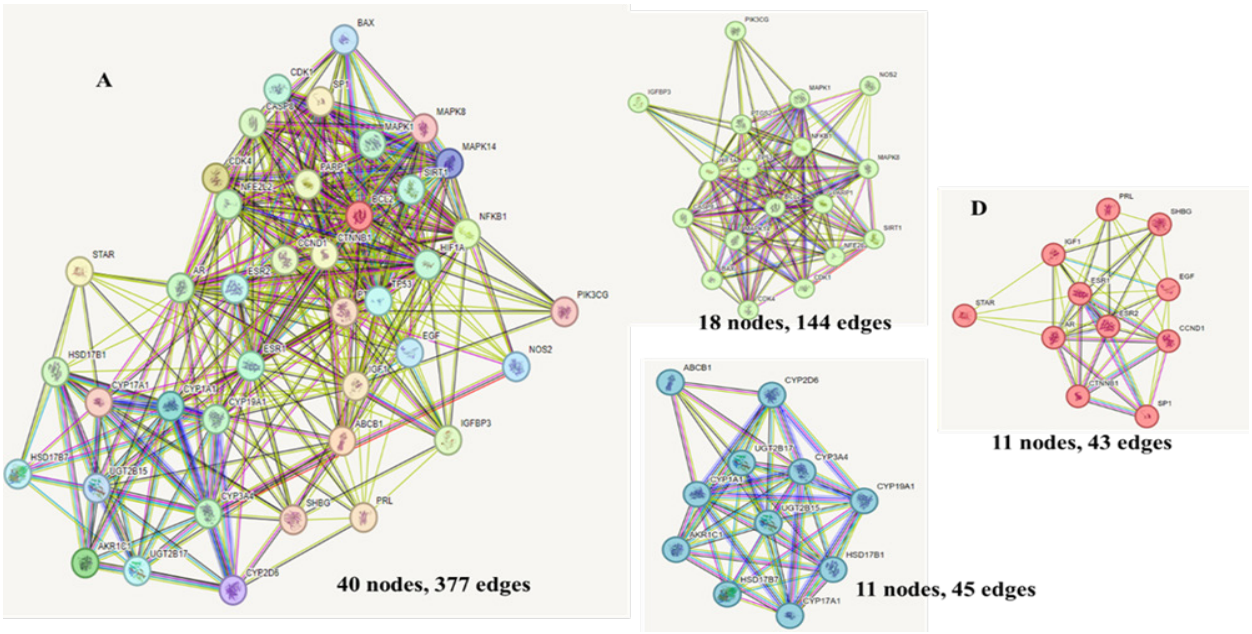

Figure 3. Protein-Protein Interaction (PPI) of Compounds in Lansium parasiticum leaf Extract Involved in the Biological Pathway of Breast Cancer Treatment. The compounds include Dihydrotestosterone, Oxoberberine, Pregnenolone, Quercetine. A) PPI interactions involving all target genes of the compounds, with 40 gene targets (nodes) and 377 interactions (edges). B) PPI interactions resulting from clustering PPI A, showing 18 gene targets (nodes) and 144 interactions (edges). C) PPI interactions resulting from clustering PPI B, yielding 11 gene targets (nodes) and 45 interactions (edges). D) PPI interactions resulting from clustering PPI C, yielding 11 gene targets (nodes) and 43 interactions (edges).

action [19].

The research also found that among the potential target genes involved in the P53 pathway are *TP53, Bax, BCl2*, *IGF-BP3, IGF*, *Cyclin D, CDK4/6, Cdc2*. The role of the target gene within the P53-path is crucial in the context [20] of breast cancer treatment. *TP53*, as a tumor suppressant, codes the p53 protein that functions to inhibit the growth of cancer cells; mutations in this gene are often associated with the development of more aggressive breast cancer . *Bax,* a pro-apoptotic protein, and *BCl2*, an

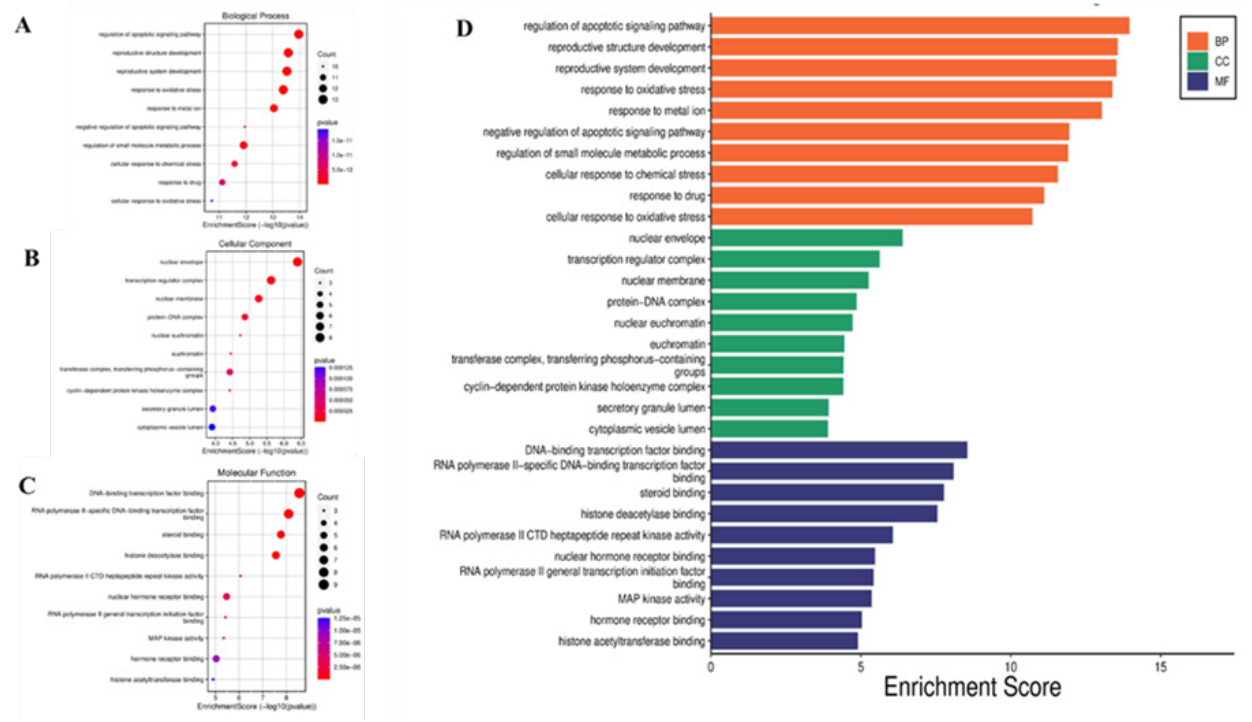

Figure 4. Analysis of Genetic Enrichment Ontology and KEGG Pathways; A) Bubble enrichment diagram of the 10 biological processes with the highest potential; B) bubble diagram for the 10 molecular functions with the most potential; C) bubbles diagram on the 10 cellular components with the greatest potential. D) An enrichment GO bar diagram for biological processes, cellular components, and molecular functions

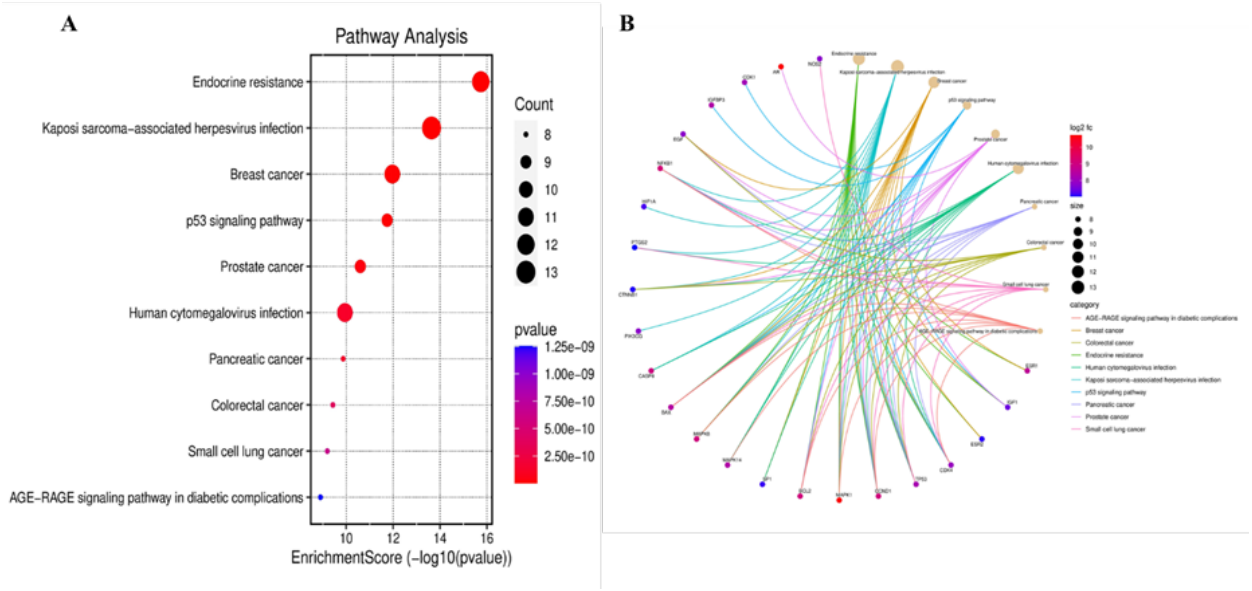

Figure 5. Diagram of Bubble Pathway Analysis and Target Genes Involved in each Pathway (A); Netwok Pharmacology pathway relationship and potential target gene (B).

anti-apoptotic protein, play a role in determining the fate of cancer cells through apoptosis regulation [20]. The balance of *Bax* and *BCl2* expression is the key to controlling the survival of cancer cells [21]. IGF-BP3 regulates growth factors like the Insulin-like Growth Factor (IGF), which affects the growth and survival of cancer cells. Increased IGF expression is often associated with uncontrolled cancer cell growth [22]. Cyclin D, working alongside CDK4/6, controls cell cycles and transitions, where their overexpression can lead to abnormal proliferation of cancer cells [23]. *Cdc2* or *CDK1*, which regulates the transition of the G2 phase to mitosis, is also important; disruptions in the function of *CdC2* can cause irregular tumor cell flows [24]. Treatment of breast cancer often involves therapies that target these pathways, with the aim of inhibiting the growth of cancer cells while minimizing damage to normal cells. This approach includes the use of drugs that induce apoptosis, inhibit cell growth pathways, and balance pro and anti-apoptotic factors.

Molecular docking is an important part of pharmacological network analysis in the treatment of breast cancer. Molecular docking is a method used to predict interactions between small molecules (such as drugs) with their target proteins. In this context, compounds such as dihydrotestosterone, oxoberberine, pregnenolone, and quercetine have been analyzed for

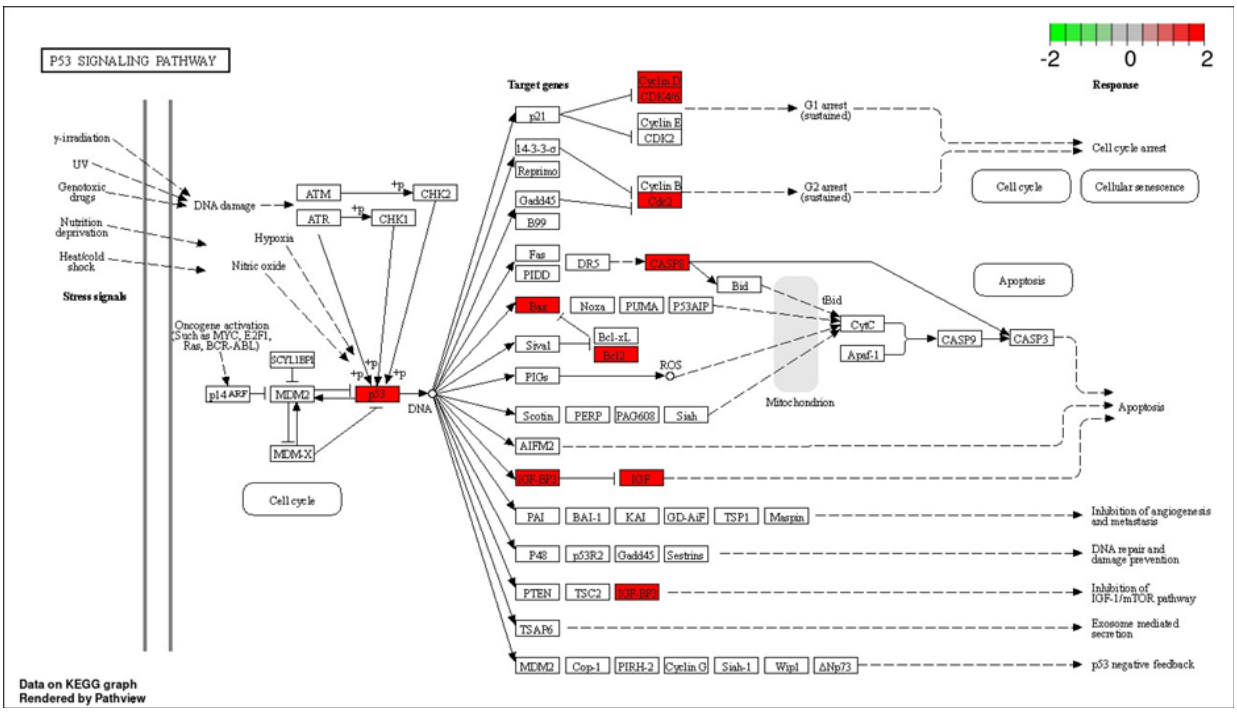

Figure 6. hsa04115 P53 Signaling Pathway Involving 10 Potential Target Genes (Red Mark) in the Treatment of Breast Cancer with Leaf Lansium parasiticum Extract

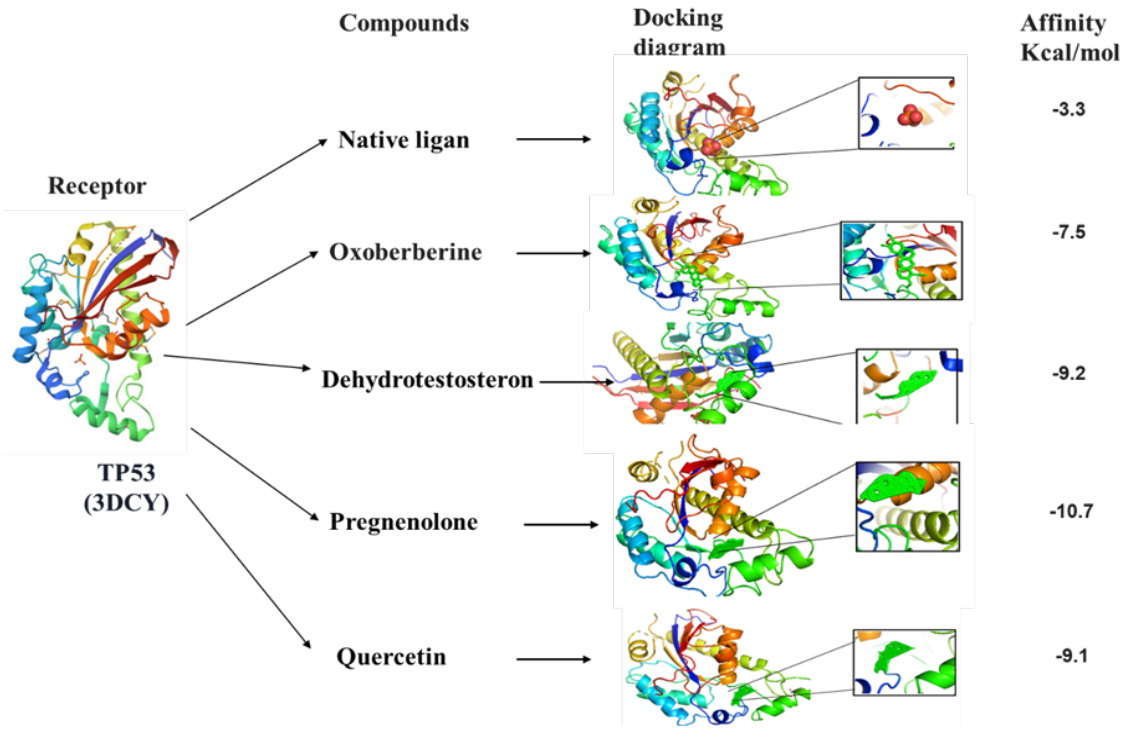

Figure 7. Docking Molecular Compounds Dehydrotestosterone, Oxoberberine, Pregnenolone, Quercetine with *TP53* Receptors

TP53, which is an important protein in the P53 pathway associated with the development of breast cancer [25].

The docking results showed that the four compounds had a stronger affinity to *TP53*, indicated by lower energy values compared to native *TP53* ligans. In terms of molecular docking, a lower energy value indicated stronger and more stable interactions between the ligans (in this case, the compound dihydrotestosterone, oxoberberine, pregnenolone, and quercetine) and the target protein (*TP53*) [26].

Overall, *LPLE* has potential as an anti-cancer drug candidate, especially in the treatment of breast cancer. The study has successfully identified 24 active compounds in Lansium parasiticum leaf extract (LPLE) and found that four of them, namely Dihydrotestosterone, Oxoberberine, Pregnenolone, and Quercetine, have potential as potential target genes in breast cancer treatment. Furthermore, KEGG analysis suggests that these compounds can affect the P53 signal pathway, which is an important pathway in breast cancer. Validation in silico mjuga has strengthened the discovery of pharmacological analysis on the route.

However, this research also has its limitations. One is that this study only uses bioinformatics and in silico methods to evaluate the potential of these compounds, so further research is needed involving in vitro and in vivo tests to confirm the anti-cancer effects of these Compounds. As a recommendation for further research, further studies need to be conducted to test the effects of the compound on breast cancer cells experimentally. Moreover, further research can also explore the more detailed molecular mechanisms of the compositions, including their influence on other proteins in the P53 path experimentally in the laboratory.

In conclusion, this study has successfully identified 24 active compounds in Lansium parasiticum leaf extract (LPLE) that have potential as anti-breast cancer agents. It was found that there are four compounds, namely dihydrotestosterone, oxoberberine, pregnenolone, and quercetine that have the potential for strong interactions with the breast cancer target genes. KEGG's pharmacological analysis showed that these compounds can interfere with the P53 signal pathway, which is an important pathway in breast cancer. This was reinforced by in silico validation which indicates that these four compound have a strong affinity to the *TP53* receptor, higher than its own ligation. This research opens up new opportunities in the development of breast cancer therapies based on natural compounds, by emphasizing the importance of exploration of Indonesian natural resources in the search for new therapeutic agents.

Author Contribution Statement

All authors contributed equally in this study.

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Approval

This study is part of a student's thesis that joins our research team.

Conflict of Interest

The authors declare that they have no conflict of interest.

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