Molecular Insights into Breast Cancer Treatment: An Integrated Approach of Network Pharmacology and Component Analysis for *Lansium parasiticum* Bark Extract

Roihatul Mutiah[1]*, Rahmi Annisa[1], Syayida Roisatus Zahiro[2]

[1]Department of Pharmacy, Faculty of Medicine and Health Sciences, UIN Maulana Malik Ibrahim Malang, East Java, Indonesia

[2]Department of Medicine, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

Abstract

Medicinal plants containing multi-components have the potential for multi-target genes, multi-pathways, and various effects on diverse diseases. With the increasing technological advancements, understanding the complex interactions between multi-component substances and biological systems is becoming more crucial. In this context, conventional experimental research might have limitations as it typically focuses on the impact of one component on one gene. The objective of this research is to identify compounds in the bark extract of *Lansium parasiticum* and elucidate the molecular mechanisms of these compounds in inhibiting the progression of breast cancer cells using a network pharmacology approach. Compound identification in *Lansium parasiticum* bark extract (LPBE) has been conducted using Liquid Chromatography Tandem Mass Spectrophotometry (LC-MS/MS) technique. ADMET predictions were utilized to determine the absorption and bioavailability profiles. A network pharmacology approach employing Cytoscape 3.9.1, GeneCards, Disgenet, STRING 2.0.0, SRplot, and Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to predict the anti-cancer molecular mechanisms of these compounds. Seventeen active compounds have been successfully identified via LC-MS/MS. Among these, the compounds Moronic Acid, 4-Morpholineacetic Acid, and Ursolic Aldehyde were found in the highest concentrations. The results of the network pharmacology analysis indicate that the compounds in LPBE are involved in three potential pathways for breast cancer treatment: the *NF-κβ* signaling pathway (hsa 04064), microRNA in cancer (hsa05206), and apoptosis (hsa04210). Target genes implicated in these pathways include *BAX, BCL2, TNF-α, PARP1, STAT3, NOTCH1, and NF-κβ1*. It can be concluded that LPBE contains compounds with potential for treating breast cancer, as they are predicted to interact with relevant target pathways and genes. Therefore, further research is highly recommended, particularly in the development of drugs for breast cancer.

Keywords: *apoptosis, microRNA, NF-κβ, TNF-α, STAT3.*

Submitted: September 13, 2024
Revised: October 29, 2024
Accepted: November 28, 2024
Published online: December 19, 2024

*Corresponding author: roiha@farmasi.uin-malang.ac.id

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 (Sung, *et al*., 2021). Conventional breast cancer therapies such as chemotherapy, radiation therapy, and hormone therapy have limitations in terms of effectiveness and side effects (Palmer, *et al.*, 1980). Besides, the development of drug resistance is also one of the problems in the treatment of breast cancer (Saeki, *et al*., 2005). On the other hand, targeted therapy that can target specific genetic mutations in breast cancer is still not widely available (Maeda and Khatami, 2018). So until now, it is still necessary to develop anti-cancer drugs to deal with the existing problem.

Lansium parasiticum is a species of medicinal plant found in many parts of Indonesia. It is common to use certain portions of *L. parasiticum* in traditional medicine to treat some diseases, among others, as worm drugs, antipyretics, antidiarrhea, and anti-cancer drugs (Fadhilah, *et al*., 2021) (Indrawati, *et al*., 2019). It is common among the local communities that the benefits of this fruit are mostly applied only to the part of the fruit and leaves, but in other parts of the plant such as the barks are still rarely used as a basic ingredient of medicine. Previous research has that the contents of *Lansium parasiticum* bark extract (LPBE) contain a group of flavonoid compounds(Ramadhani, *et al*., 2018). Information on the content of secondary metabolites in LPBE in previous studies is still very limited. Besides, there is no discovery of molecular mechanisms, target genes, and pathways involving potential compounds in LPBE that can inhibit the growth of cancer cells.

Network pharmacology is a promising research approach that combines pharmacology, molecular biology, and bioinformatics to generate network relationships between effective pharmacological components, related targets, pathways, and related diseases (Zhou, *et al*., 2020). Thus, this approach explains the development of diseases from the biological point of view of systems, pharmacologies, and biological networks. This method can not only predict the relationship between drugs and disease from the perspective of networks but also visualize and analyze complex biological systems (Kibble, *et al*., 2015).

The main objective of this study was to perform metabolite profiles of compounds using the Liquid Chromatography Tandem Mass Spectrophotometry (LCMS/MS) method, then analyze the mechanisms of molecular action, target genes, and potential pathways of LPBE in the treatment of breast cancer using network pharmacology approaches. This research is intended to provide valuable insight into future pharmacological research and potential clinical therapeutic applications.

MATERIALS AND METHODS

Preparation of *Lansium parasiticum* Bark Extract (LPBE)

The extraction of *Lansium parasiticum* bark (LPBE) was conducted using *Lansium parasiticum* bark collected under the number 067/566/102.20/2023 from an area in East Java, located at an altitude of 400 meters. The region had an average temperature of 25ºC and an average annual rainfall of 125.49 mm. The powdered roots were subjected to extraction at a ratio of 1:20 using 96% ethanol and the Ultra Assisted Extraction (UAE) method, carried out at a temperature of 25ºC for 30 minutes. Subsequently, the ethanol extract was prepared for further analysis by being placed in an oven set at 40°C for 5 h (Mutiah, *et al*., 2020) .

Liquid Chromatography Tandem Mass Spectrophotometry (LC-MS/MS) Analysis

The LC-MS/MS analysis was conducted using UPLC-MS systems equipped with a QToF analyzer and positive ESI as the ionization

source. It utilized an Acquity C18 column (1.8 µm; 2.1×150 mm). The eluent consisted of (A) water (HPLC grade) with formic acid (Merck, Darmstadt, Germany) in a ratio of 99.9/0.1 [v/v] and (B) acetonitrile (Merck) with formic acid in a ratio of 99.9/0.1 [v/v], employing a gradient elution system. The source temperature was set at 100°C, and the desolvation temperature at 350°C. A 10 mg extract was dissolved in a 10 ml volumetric flask using absolute methanol, and 5 µL of this solution was injected into the UPLC-MS system. The analysis parameters were configured in positive ion mode, with spectra acquired across a mass range from m/z 120 to 1000. Mass Lynx version 4.1 software (Waters, Massachusetts, USA) and PubChem (https://pubchem.ncbi.nlm.nih.gov/) were employed for processing the chromatogram and compound identification. The confirmation of a compound's accuracy was based on MS/MS fragment matching with an inaccuracy threshold of less than 5 ppm (Mutiah, *et al*., 2019a).

Oral Bioavailability Screening

Oral Bioavailability Screening A crucial pharmacokinetic parameter, known as oral bioavailability, is employed to quantify the amount of orally administered medication that enters the bloodstream and exerts pharmacological effects. Key characteristics of the identified compounds, such as molecular weight, gastrointestinal absorption, H-bond acceptors (HBA), H-bond donors (HBD), and lipophilicity, were assessed using SwissADME (http://www.swissadme.ch/index.php) (Mutiah, *et al*., 2019b).

Identification of Potential Targets for Breast Cancer

Identification of potential targets for breast cancer one fundamental aspect of pharmaceutical research involves predicting the interactions between chemicals and specific targets. Gene targets associated with the active compounds, as determined by LC-MS/MS analysis in the LPBE, were identified using the Gene Cards database (https://www.genecards.org/). Simultaneously, gene targets linked to breast cancer were explored using the DisGeNET database (https://www.disgenet.org). Subsequently, network pharmacology analysis was conducted using Cytoscape software version 3.9.1 to obtain an overview of the interactions between active compounds and gene targets, along with the results related to disease-gene targets (Zeng and Yang, 2017).

Construction of Pharmacological Networks and Protein-Protein Interactions

The creation of pharmacological network connections involving active compounds, target genes, and diseases was performed using Cytoscape version 3.10. For further analysis, gene targets that overlapped between active compounds and diseases were selected and processed using the STRING platform version 12.0 (https://string-db.org/). The construction of the Protein-Protein Interaction (PPI) network included common target proteins with a minimum required interaction score of 0.400. The goal of PPI network analysis was to investigate biological activities by examining functional annotations related to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (Liu, *et al*., 2018).

Gene Ontology (GO) Analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment

The identified targets underwent GO analysis and functional pathway enrichment, which were carried out using R. Screening criteria were set at p=0.05 and q=0.05 to evaluate functional enrichment results. The most significant findings in terms of cellular component (CC), molecular function (MF), and biological process (BP) were identified in the GO analysis and presented visually using R to create a bubble chart. Additionally, the top thirty KEGG pathway enrichments were utilized to generate bubble diagrams for visual representation, utilizing SRPlot (http://www.bioinformatics.com.cn/srplot) (Tian, *et al*., 2022).

Indonesian Journal of Cancer Chemoprevention, June 2024
ISSN: 2088–0197
e-ISSN: 2355-8989

RESULT

Metabolite Profiling

The 70% ethanol extract from the LPBE has been analyzed using the UPLC-QtoF-MS/MS instrument. Based on the results of the metabolite profile in Figure 1 with the analysis results in Table 1, it is known that LPBE contains 17 compounds, consisting of 7 alkaloids, 3 terpenoids, 2 kumarins, 4 steroids and 1 flavonoid.

The dominant compounds in this extract are moronic acid at 14.29% (peak 17), 4-Morpholineacetic Acid at 12.2% (peak 1), and Ursolic aldehyde at 8.37% (peak 16).

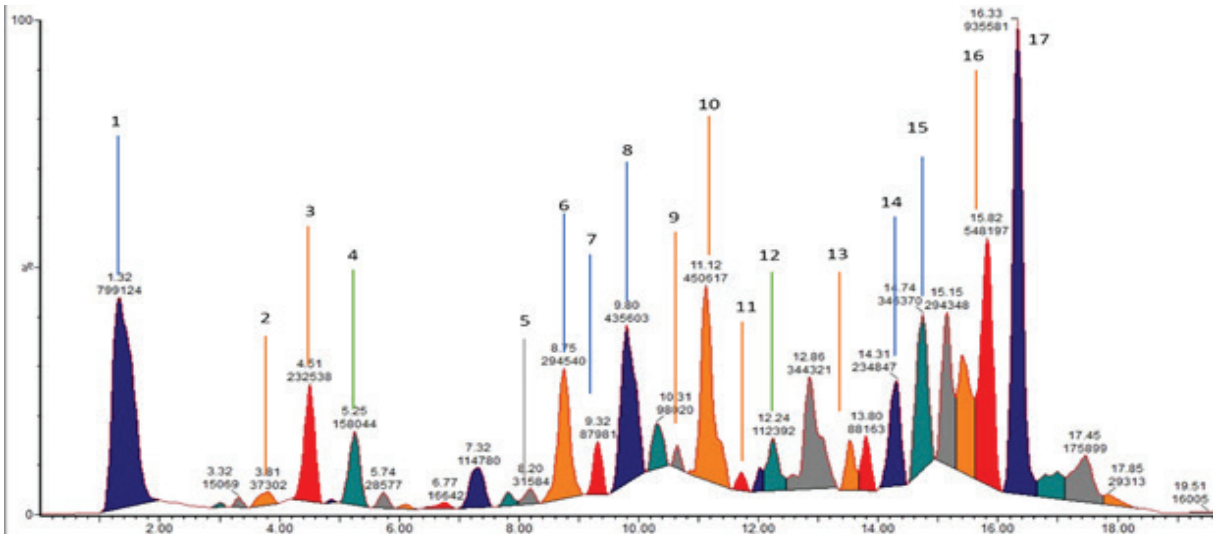

Figure 1. Chromatogram of *Lansium parasiticum* bark extract using UPLC-QToFMS/MS method. It was C18 stationary phase; the mobile phase was water/formic acid [99.9/0.1 (v/v)] and acetonitrile/formic acid 99.9/0.1 (v/v). Each chromatogram peak indicated one compound.

Table 1. The results of metabolite identification of *Lansium parasiticum* bark extract using UPLC Qtof MS/MS method.

No	Rt	%Area	Measured Mass	Calculated Mass	Formula	Name	Groups
1	1.324	12.2%	146.0821	146.0817	C6H12NO3	4-Morpholineacetic Acid	Alkaloid
2	3.826	0.57%	3 10.093	310.0927	C14H16 NO7	7-Deoxypancratistatin	Alkaloid
3	4.529	3 .5%	352.1613	352.1608	C14H26 NO9	Validoxylamine B	Alkaloid
4	5.253	2.41%	193.0498	193.0501	C10H9O4	Scopolet's	Coumarin
5	8.199	0.48%	175.1489	175.1487	C13H19	1,1,6- Trimethyltetral	Coumarin
6	8.747	4.50%	485.1828	485.1812	C26H29 O9	Dukunolide E	Terpenoids
7	9.296	1.34%	486.213	486.2128	C26H32 NO8	Saccharothriolide D	Alkaloid
8	9.802	6.65%	517.2096	517.2074	C27H33 O10	Ananolignan F	Alkaloid
9	10.639	0.44%	469.1882	469.1862	C26H29 O8	Dukunolide D	Triterpenoids
10	11.124	6.88%	439.2137	439.2121	C26H31 O6	3,6-Dimethylmangostin	Flavonoid
11	12.024	0.46%	618.2916	618.2888	C28H40 N7O9	Rotigaptide	Alkaloid
12	12.242	1.72%	627.2805	627.2779	C30H39 N6O9	Tripeptide-based inhibitor	Alkaloid
13	13.535	1.28%	453.3378	453.3402	C30H45O3	Ganoderic acid SZ	Steroid
14	14.309	3.59%	335.2578	335.2586	C21H35O3	Tetrahydrodeoxycorticosterone	Steroid
15	14.744	5.29%	471.3492	471.3474	C30H47 O4	Peaceful	Steroid
16	1 5.82	8.37%	441.3753	441.3733	C30H49 O2	Ursolic aldehyde	Steroid
17	16.326	14.29%	455.3551	455.3525	C30H47O3	Moronic acid	Triterpenoids

Predictions of the LPBE's physicochemical properties are made to determine the absorption and permeability of these compounds. This prediction is based on Lipinski's Rule of Five law and uses several parameters, including molecular weight, log *p*, hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), torsion, and polar surface area. (PSA). From the screening results obtained that 14 compounds meet the law parameter of five lipinski so have met bioavailability orally. Table 2 below is the result of the prediction of physical-chemical properties for each compound.

Table 2. Physicochemical prediction results.

No	Compound Name	Parameters of Lipinski's Rule of Five						Lipinski's five laws
		BM	Log *P*	HBA	HBD	Torsion	PSA	
1	4-Morpholineacetic Acid	145.158	-0.5968	3	1	2	59.387	Yes
2	7-Deoxypancratistatin	309.274	-1.9319	7	5	0	124.449	Yes
3	Validoxylamine B	351.352	-5.6061	10	10	4	137.480	Yes
4	Scopolet's	192.17	1.5072	4	1	1	79.352	Yes
5	1,1,6-Trimethyltetralin	174.287	3.60892	0	0	0	80.980	Yes
6	Dukunolide E	484.501	1.9084	9	2	1	200.944	Yes
7	Saccharothriolid e D	485.533	3.349	8	5	4	202.917	Yes
8	Ananolignan F	516.543	4.6109	10	0	6	215.182	No
9	Dukunolide D	468.502	2.6972	8	2	1	196.146	Yes
10	3,6-Dimethylmango stin	438.52	5.695	6	1	7	187.261	Yes
11	Rotigaptide	617.66	-3.3854	9	7	12	253.227	No
12	Tripeptide-based inhibitor	626.667	0.0385	9	3	12	258.813	No
13	Ganoderic acid SZ	452.679	7.528	2	1	5	200.348	Yes
14	Tetrahydrodeoxycorticosterone	334.5	3.5676	3	2	2	145.765	Yes
15	Peaceful	470.694	6.4126	3	2	1	205.515	Yes
16	Ursolic aldehyde	440.712	7.2038	2	1	1	196.560	Yes
17	Moronic acid	454.695	7.4418	2	1	1	200.721	Yes

Potential Target Genes for *Lansium parasiticum bark* Extract (LPBE) Compounds in Breast Cancer Treatment

In an attempt to find potential target genes of compounds in LDBE for breast cancer treatment using GeneCards, it has been revealed that of the 14 compounds contained in LPBE, they are connected to 301 potential target genes. On the other hand, the target genes associated with breast cancer included 1,209 genes, including Estrogen receptor-positive breast cancer (CUI: C2938924), Breast Cancer Familial (CUI: C0346153), Estrogen receptor-negative breast cancer, HER2-negative breast cancer, based on data from Disgenet. From the analysis of Venn's diagram comparing the phytochemical compound target gene with the disease target gene, it was found that there were 72 potentially overlapping target genes (Figure 2A). Furthermore, of these 72 target genes were further analyzed related to the pharmacological network using the cytoscape device (Figure 2B).

Three of the 14 compounds that are now in use can potentially be target genes for breast cancer, according to the results of the pharmacological network study of the compounds in LPBE that have been linked to breast cancer genes (relevance score >7). Ursolic aldehyde (with five target genes), moronic acid (with six target genes), and gonoderic acid SZ (with three target genes) make up the third compound (Table 3).

Indonesian Journal of Cancer Chemoprevention, June 2024
ISSN: 2088–0197
e-ISSN: 2355-8989

Table 3. The target gene of the compound in *Lansium parasiticum* bark extract and relevance score of breast cancer target gene.

Compound	Gene symbol	Description	Gifts	Relevance score
Ganoderic acid SZ	*WITH*	Androgen Receptor	55	17.7348671
Ganoderic acid SZ	*NOTCH1*	Notch Receptor 1	55	10.34684944
Ganoderic acid SZ	*NF-κβ1*	Nuclear Factor Kappa B Subunit 1	57	13.06387711
Moronic acid	*PRNP*	Prion Protein	52	11.71735382
Moronic acid	*TNF*	Tumor Necrosis Factor	55	24.96082306
Moronic acid	*CETP*	Cholesteryl Ester Transfer Protein	50	22.06339836
Moronic acid	*LHCGR*	Luteinizing Hormone/Choriogonadotropin Receptor	51	7.547442436
Moronic acid	*HSP90AA1*	Heat Shock Protein 90 Alpha Family Class A Member 1	55	11.80369377
Moronic acid	*CRP*	C-Reactive Protein	50	18.24633026
Ursolic aldehyde	*STAT3*	Signal Transducer And Activator of Transcription 3	58	7.44463253
Ursolic aldehyde	*PTGS2*	Prostaglandin-Endoperoxide Synthase 2	53	7.15674305
Ursolic aldehyde	*BCL2*	BCL2 Apoptosis Regulator	55	9.889139175
Ursolic aldehyde	*PARP1*	Poly(ADP-Ribose) Polymerase 1	54	7.774953365
Ursolic aldehyde	*SEE*	BCL2 Associated X, Apoptosis Regulator	55	7.488071918

Construction of Pharmacological Networks and Protein-Protein Interactions

Protein-protein interaction (PPI) refers to the relationship between different types of protein in the LPBE bark extract that has a role in the biological pathway of breast cancer treatment. The results show that some of the compounds found in the extract, Genoderic acid SZ, moronic acid, and ursolic aldehyde have the potential to interact with certain proteins involved in biological processes relevant to breast cancer treatments. 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.

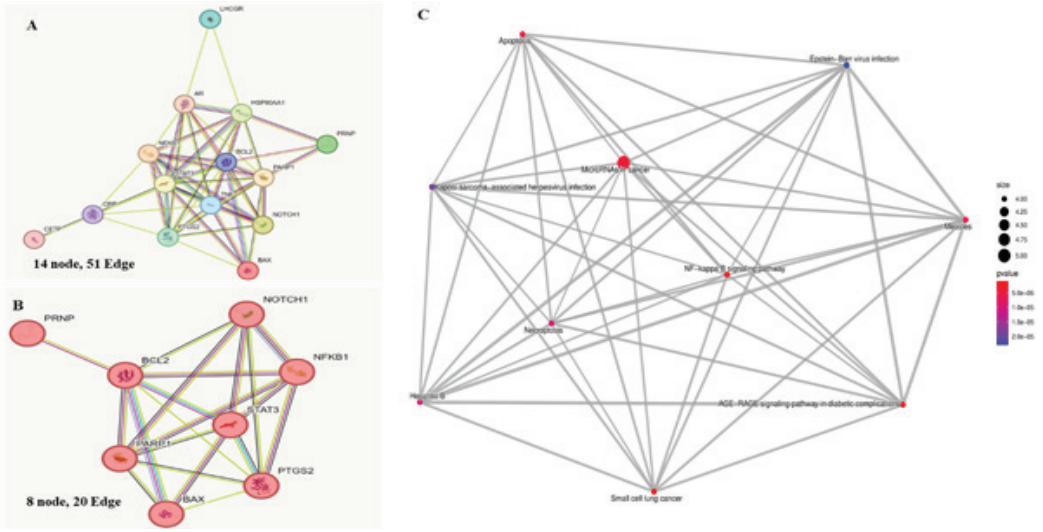

Figure 3. Protein-Protein Interaction (PPI) of compounds in *Lansium parasiticum* bark extract involved in the biological pathway of breast cancer treatment. The compounds include Genoderic acid SZ, moronic acid, and ursolic aldehyde. A. PPI interactions involving all target genes of the compounds with 14 nodes, 51 Edge B. Protein-protein interaction on the target cancer gene with 8 node 20 edge. C. Interaction pathway involved in breast cancer healing.

Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Analysis and Gene Ontology (GO) Enrichment

In the KEGG enrichment analysis, it was found that there are three routes that have great potential in the treatment of breast cancer affected by the compounds Genoderic acid SZ, moronic acid, and ursolic aldehyde. The three potential pathways are the NF-*κβ* signaling pathway (hsa 04064), microRNA in cancer, (hza05206) and apoptosis (hse04210) (Figure 4A). All of this is predicted to have a correlation and influence on the biological process of breast cancer healing. In addition, gene ontology analysis shows that the compounds found in LPBE impact biological processes, molecular functions, and cellular components (Figure 4B).

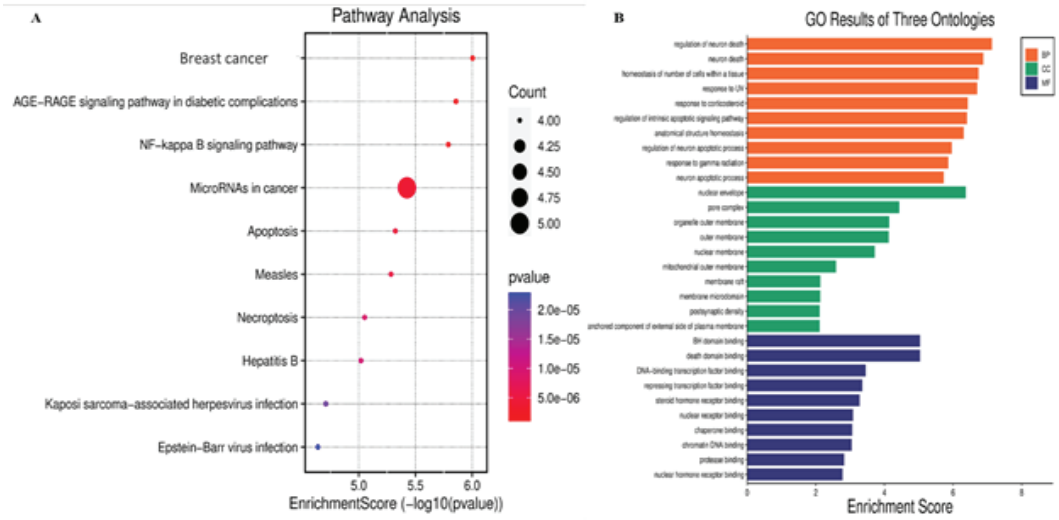

Figure 4. Gene Ontology Enrichment Analysis and KEGG Pathways; A) KEGG analysis bubble diagram of 10 potential pathways for compounds in inhibiting breast cancer B) Gene Ontology enrichment bar diagram of 10 potential processes in biological processes, molecular functions, and biological cellular components that have the highest potential.

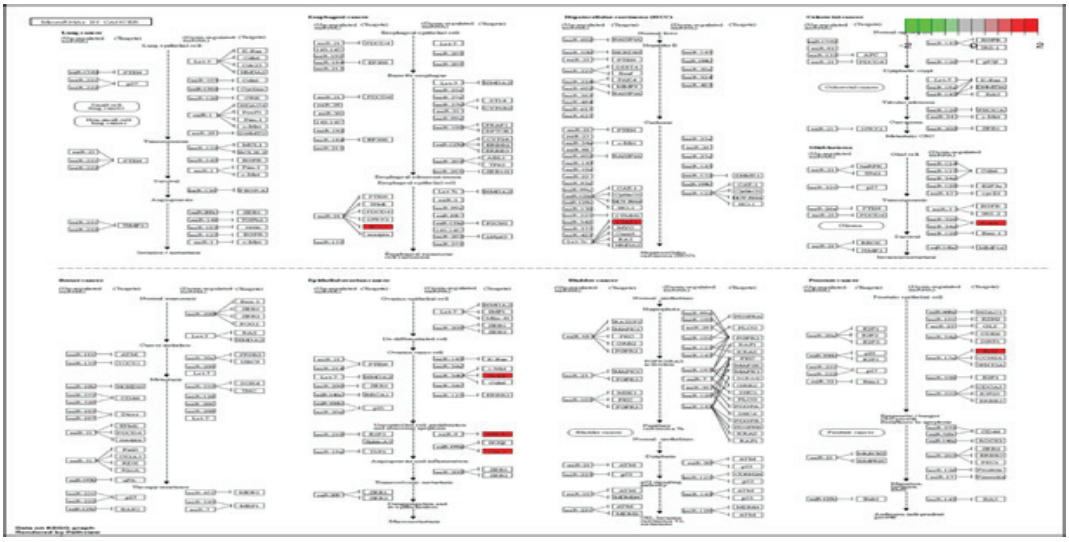

Figure 5. microRNA in cancer (hsa05206) signaling pathway involving 5 potential target genes (red marks), namely STAT3, BCL2, NOTCH1, NF-*κβ1*, COX2 in the treatment of breast cancer with *Lansium parasiticum* bark extract.

KEGG analysis also found 4-5 genes involved in each potential pathway due to compounds in LPBE, specifically the compound Genoderic acid SZ, moronic acid, and ursolic aldehyde. Each target gene in each pathway is presented in Table 4.

Table 4. Signaling pathways with KEGG analysis.

ID	Pathway	Number of Genes	Genes
hsa04210	Apoptosis	4	TNF-α, PARP1, Bax, BCL2,
hsa05206	MicroRNAs in cancer	5	STAT3, BCL2, NOTCH1, NF-κβ1, COX2
hsa04064	NF-κβ signaling pathway	4	PARP1, BAX, BCL2, NF-κβ1

DISCUSSION

Medicinal plants that contain compounds of various components must have different target genes, pathways, and effects on various diseases (Nogales, *et al*., 2022). A comprehensive process is needed to explain the molecular mechanisms responsible for such interactions. Because experimental research usually tests only one part against one target gene, they can't explain the aspect (Yang, *et al*., 2023). Using big data, it is now possible to describe the interactions between proteins, target genes, pathways involved, and diseases affected by the multi-components present in herbal medicine (Li, *et al*., 2023). This approach is known as the Network of Pharmacology. Therefore, the study aims to identify compounds in the LPBE with LCMS/MS and continue to analyze target genes, biological processes, and potential pathways involved in breast cancer treatment with the Network pharmacology approach.

In this study, advanced techniques such as LC-MS/MS are used to identify compounds present in the LPBE. This technique has proven to be effective in determining the chemical composition of natural materials (Li, *et al*., 2012). Furthermore, using the ADMET program, the absorption profile and bioavailability of these compounds can be determined, an important step in developing effective drugs (Dong, *et al*., 2018).

The main approach of this research is the pharmacology network, which integrates information from various sources such as Cytoscape, GeneCards, and KEGG to predict the molecular anti-cancer mechanisms of the compounds found in LPBE. As explained by Wang, *et al*. 2018, this approach provides a holistic picture of how a drug or herbal extract can affect a complex biological network (Wang, *et al*., 2021).

Analysis using the KEGG found that LPBE shows potential as a therapeutic agent in the treatment of breast cancer. It is based on the presence of active compounds in the LPBE with several key pathways, in particular the microRNA pathway in cancer, NF-κB signaling pathway and apoptosis.

MicroRNA (miRNA) is a small group of non-coding RNA molecules with 21 to 23 nucleotides. They control post-transcription gene expression by blocking protein translation or by destroying target mRNA. MiRNA dysregulation has been widely observed in various stages of cancer with high-speed profiles. Upregulation (overexpression) of certain miRNAs can lead to suppression of expression of tumor-inhibiting genes, and downregulation of particular miRNAs can cause increased oncogene expression; both these conditions lead to subsequent malignant effects on cell proliferation, differentiation, and apoptosis, which in turn lead to tumor growth and development (Eastlack and Alahari, 2015) (Loh, *et al*., 2019) (Takahashi, *et al*., 2015).

Through KEGG analysis, it was discovered that the compounds in LPBE influence the miRNA pathway, identifying five potential target genes involved in cancer treatment. The compounds

are genoderic acid SZ, moronic acid, and ursolic aldehyde that target STAT3, BCL2, NOTCH1, NF-*κβ*1 and COX2. As a transcription factor, STAT3 plays an important role in cell proliferation, differentiation, and also resistance to apoptosis (Fatma, *et al*., 2021 and Merlin, *et al*., 2009). BCL2, known as the anti-apoptosis gene, overexpression of BCL2 can provide survival benefits for cancer cells and contribute to resistance to therapy (Radha, *et al*., 2017). Notch's pathway plays a key role in cell differentiation and proliferation (Mollen, *et al*., 2018). Mutation or dysregulation of this pathway can trigger the development of cancer. NF-κ*β*, encoded by the NF-*κβ*1 gene, is a transcription factor involved in inflammatory responses, cell differentiation, and proliferation (Wang, *et al*., 2015). COX2 is an enzyme involved in the synthesis of prostaglandins, which can trigger inflammation and cell proliferation (Davies, *et al*., 2002).

Thus, the involvement of the compounds genoderic acid SZ, moronic acid, and ursolic aldehyde in modulating the expression or activity of these genes is very promising in the context of developing cancer therapy. However, we have to note that the relationship between these compounds and the target genes can have many aspects, so a comprehensive understanding of how the compound works and the possible side effects are crucial before they can be applied in a clinical environment.

The NF-*κβ* pathway is one of the key signal pathways involved in inflammatory responses, cell differentiation, and proliferation. Dysfunction or hyperactivation of this pathway has been associated with a variety of diseases, including cancer (Kadam, *et al*., 2016). Therefore, the ability of LPBE to interact with the pathway shows the potential of the compounds in the extract to inhibit the proliferation of breast cancer cells. This is supported by previous research reporting the cytotoxic potential of *Lansium parasiticum* against T47D cells (Fadhillah, *et al*., 2020). In addition, polyphenol and flavonoid compounds in LPBE have also been reported to exhibit cytotoxic activity on MCF-7, T47D, and 4T1 cell lines (Febriani, *et al*., 2025).

Furthermore, the apoptosis pathway plays an important role in programmed cell death. Apoptosis is an important mechanism that prevents the growth of cancer cells by triggering the death of damaged or mutant cells (Kadam, *et al*., 2015, Nasimun, *et al*., 2020, Parveen, *et al*., 2020, and Jarret, *et al*., 2020).

Interestingly, genes like NF-*κβ*, TNF-*α*, PARP1, Bax, and BCL2 are involved in both pathways. For example, the NF-*κβ* and TNF-*α* genes play a role in the signal pathway of NF-κ*β*, while Bax and BCL2 play a part in apoptosis regulation. The interaction of LPBE with these genes confirms the therapeutic potential of this extract in the context of breast cancer.

However, although KEGG analysis provides early evidence of the potential of LPBE, further research is needed to validate these findings experimentally and clinically. It is important to understand how the specific interaction between the compounds in LDBE and these target genes affects the viability of breast cancer cells and whether there are potential side effects to be noticed.

CONCLUSION

Using the LC-MS/MS method, 17 active compounds have been successfully identified in the Lansium parasiticum Barkextract (LPBE). Moronic acid, 4-Morpholineacetic acid, and Ursolic Aldehyde appeared as compounds with dominant concentrations. From network-based pharmacological analysis, it is indicated that the LPBE components participate in three significant pathways in breast cancer namely the NF-*κβ* signaling pathway (hsa 04064), microRNA in cancer (hsa05206), and apoptosis (hsa04210). Within these paths, genes such as BAX, BCL2, TNF-*α*, PARP1, STAT3, NOTCH1, and NFKB1 are identified as potential targets. Based on these results, in-depth research on LPBE is considered important, especially in the context of the therapeutic development of breast cancer.

ACKNOWLEDGMENT

We thank the Faculty of Medicine and Health Sciences of Universitas Islam Negeri (UIN) Maulana Malik Ibrahim Malang for the funding through the Competitive Grant 2024. This support is very valuable to our research.

REFERENCES

Davies, G., Martin, L.A., Sacks, N., and Dowsett, M., 2002, Cyclooxygenase-2 (COX-2), aromatase and breast cancer: a possible role for COX-2 inhibitors in breast cancer chemoprevention, *Annals of Oncology*, **13**(5), 669-678.

Dong, J., Wang, N.-N., Yao, Z.-J., Zhang, L., Cheng, Y., Ouyang, D., *et al.*, 2018, ADMETlab: a platform for systematic ADMET evaluation based on a comprehensively collected ADMET database, *J Cheminform.*, **10**, 29.

Eastlack, S.C., and Alahari, S.K., 2015, MicroRNA and breast cancer: understanding pathogenesis, improving management, *Non-Coding RNA*, **1**(1), 17–43.

Fadhilah, K., Wahyuono, S., and Astuti, P., 2021, Fractions and isolated compounds from Lansium domesticum fruit peel exhibited cytotoxic activity against T-47D and HepG2 cell lines, *Biodiversitas Journal of Biological Diversity,* **22**(9), 3743-3748.

Fadhilah, K., Wahyuono, S., and Astuti, P., 2021, A bioactive compound isolated from Duku (*Lansium domesticum* Corr) fruit peels exhibits cytotoxicity against T47D cell line, *F1000Research*, **9**, 3.

Febriani, H., Lubis, M.F., Sumaiyah, S., Hasibuan, P.A.Z., Syahputra, R.A., Astyka, R., and Juwita, N.A., 2025, Optimization of microwave-assisted extraction to obtain a polyphenol-rich crude extract from duku (*Lansium domesticum* Corr.) leaf and the correlation with antioxidant and cytotoxic activities, *Kuwait Journal of Science*, **52**(1), 100315.

Fatma, F., and Kumar, A., 2021, The Cell Cycle, Cyclins, Checkpoints and Cancer, *Asian Journal of Research in Pharmaceutical Sciences*, **11**(2), 175-183.

Indrawati, A., Hartih, N.A., and Muyassara, M., 2019, Isolasi dan uji potensi fungi endofit kulit batang langsat (*Lansium domesticum* Corr.) penghasil antibakteri terhadap Staphylococcus aureus dan Escherichia coli, *Media Farmasi*, **15**, 36–42.

Jarrett, N.M., Christobel, G.J.R., Abirami, M.P., Shyam, S.J., Radhakrishnan, S., Deepa, S., Abirami, S., and Shila, S., 2020, Cytotoxic and Apoptotic Effect of Citrus Flavonoid Naringin in Treating PA-1 Ovarian Cancer Cells, *Research J. Pharm. and Tech.*, **13**(9), 4051-4054.

Kadam, C.Y., and Abhang, S.A., 2016, Apoptosis Markers in Breast Cancer Therapy, *Advances in Clinical Chemistry*, **74**, 143-193.

Kibble, M., Saarinen, N., Tang, J., Wennerberg, K., Mäkelä, S., and Aittokallio, T., 2015, Network pharmacology applications to map the unexplored target space and therapeutic potential of natural products, *Nat Prod Rep.*, **32**, 1249–1266.

Li, H., Ortiz, R., Tran, L., Hall, M., Spahr, C., Walker, K., *et al.*, 2012, General LC-MS/MS Method Approach to Quantify Therapeutic Monoclonal Antibodies Using a Common Whole Antibody Internal Standard with Application to Preclinical Studies, *Anal. Chem.*, **84**, 1267–1273.

Li, J.-X., Han, Z.-X., Cheng, X., Zhang, F.-L., Zhang, J.-Y., Su, Z.-J., *et al.*, 2023, Combinational study with network pharmacology, molecular docking and preliminary experiments on exploring common mechanisms underlying the effects of weijing decoction on various pulmonary diseases, *Heliyon*, **9**.

Liu, X., Wu, J., Zhang, D., Wang, K., Duan, X., and Zhang, X., 2018, A Network Pharmacology Approach to Uncover the Multiple

Mechanisms of Hedyotis diffusa Willd. on Colorectal Cancer, *Evidence-Based Complementary and Alternative Medicine*, **2018**, e6517034.

Loh, H.-Y., Norman, B.P., Lai, K.-S., Rahman, N.M.A.N.A., Alitheen, N.B.M., and Osman, M.A., 2019, The regulatory role of microRNAs in breast cancer, *International Journal of Molecular Sciences*, **20**, 4940.

Ma, J., Qin, L., and Li, X., 2020, Role of STAT3 signaling pathway in breast cancer, *Cell Commun Signal*, **18**, 33.

Maeda, H., and Khatami, M., 2018, Analyses of repeated failures in cancer therapy for solid tumors: poor tumor-selective drug delivery, low therapeutic efficacy and unsustainable costs, *Clin Trans Med.*, **7**(1), e11.

Merlin, N.J., Parthasarathy, V., Manavalan, R., Devi, P., and Meera, R., 2009, Apoptosis Significance and Molecular Mechanisms- A Review, *Asian J. Research Chem.*, **2**(4), 369-375.

Mollen, E.W.J., Ient, J., Tjan-Heijnen, V.C.G., Boersma, L.J., Miele, L., Smidt, M.L., and Vooijs, M.A.G.G., 2018, Moving Breast Cancer Therapy up a Notch, *Frontiers in Oncology*, **8**, 518.

Mutiah, R., Bhagawan, W.S., Ma'arif, B., and Rahmandika, J.M.S., 2019a, Metabolite Fingerprinting *Eleutherine palmifolia* (L.) Merr. Using UPLC-QTOF-MS/MS, *Majalah Obat Tradisional*, **24**, 139–159.

Mutiah, R., Firsyaradha, W.Y., Sari, R.A., Annisa, R., Kristanti, R.A., Indrawijaya, Y.Y.A., *et al.*, 2020, *Eleutherine palmifolia* (L.) Merr. extract increases the crypts and caspase-3 expression in colitis-associated colon cancer model, *Indonesian Journal of Pharmacy*, **31**, 1–9.

Mutiah, R., Hariz, M.F., Indrawijaya, Y.Y.A., and Ma'arif, B., 2019b, *In Silico* Prediction of Isoliquiritigenin and Oxyresveratrol Compounds to BCL-2 dan VEGF-2 Receptors, *Indonesian Journal of Cancer Chemoprevention*, **10**, 51–59.

Nasimun Islam, N., Jemimah Naine, S., Shaik Jameel, S.S. Khora, Venkat Kumar, Mohanasrinivasan V., C. Subathra Devi, and Panneerselvam, A., 2014, Cytotoxic Property of Cocos nucifera shell Extracts on HeLa Cells, *Research J. Pharm. and Tech.*, **7**(5), 521-525.

Nogales, C., Mamdouh, Z.M., List, M., Kiel, C., Casas, A.I., and Schmidt, H.H.H.W., 2022, Network pharmacology: curing causal mechanisms instead of treating symptoms, *Trends in Pharmacological Sciences*, **43**, 136–150.

Palmer, B.V., Walsh, G.A., McKinna, J.A., and Greening, W.P., 1980, Adjuvant chemotherapy for breast cancer: side effects and quality of life, *Br Med J.*, **281**, 1594–1597.

Parveen, S., and Nadumane, V.K., 2020, A Bioactive Fraction from Turbinaria ornata Inducing Apoptosis via Cell Cycle Arrest, *Research J. Pharm. and Tech.*, **13**(9), 4263-4268.

Radha, G., and Raghavan, S.C., 2017, BCL2: A promising cancer therapeutic target, *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, **1868**(1), 309-314.

Ramadhani, N., Yuliet, Y., and Khaerati, K., 2018, Aktivitas Ekstrak Kulit Batang Langsat (*Lansium parasiticum* (Osbeck) Sahni & Bennet) Terhadap Kelarutan Kalsium Batu Ginjal Secara *In vitro*, *Biocelebes*, **12**, 69-79.

Saeki, T., Tsuruo, T., Sato, W., and Nishikawsa, K., 2005, Drug resistance in chemotherapy for breast cancer, *Cancer Chemother Pharmacol*, **56**, 84–89.

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

Takahashi, R., Miyazaki, H., and Ochiya, T., 2015, The roles of microRNAs in breast cancer, *Cancers*, **7**, 598–616.

Tian, J., Xu, Y., Xiong, Y., Zuo, L., Zhou, M., Cao,

C., *et al.*, 2022, Metabolomics combined with network pharmacology to explore the mechanisms of modified Guishen pill to ameliorate polycystic ovary syndrome, *Computers in Biology and Medicine*, **148**, 105790.

Wang, W., Nag, S.A., and Zhang, R., 2015, Targeting the NFKB Signaling Pathways for Breast Cancer Prevention and Therapy, *Current Medicinal Chemistry*, **22**(2), 264-289.

Wang, X., Wang, Z.-Y., Zheng, J.-H., and Li, S., 2021, TCM network pharmacology: A new trend towards combining computational, experimental and clinical approaches, *Chinese Journal of Natural Medicines*, **19**, 1–11.

Yang, X., Tao, Y., Xu, R., Luo, W., Lin, T., Zhou, F., *et al.*, 2023, Analysis of active components and molecular mechanism of action of *Rubia cordifolia* L. in the treatment of nasopharyngeal carcinoma based on network pharmacology and experimental verification, *Heliyon*, **9**, e17078.

Zeng, L., and Yang, K., 2017, Exploring the pharmacological mechanism of Yanghe Decoction on HER2-positive breast cancer by a network pharmacology approach, *Journal of Ethnopharmacology*, **199**, 68–85.

Zhou, Z., Chen, B., Chen, S., Lin, M., Chen, Y., Jin, S., *et al.*, 2020, Applications of Network Pharmacology in Traditional Chinese Medicine Research, *Evidence-Based Complementary and Alternative Medicine*, 1–7.