ORIGINAL ARTICLE

The Pro-apoptotic Potential of Sungkai Leaves (Peronema Canescens Jack) in Breast Cancer via Network Pharmacology and Molecular Docking Analysis

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ABSTRACT

Introduction: Breast cancer is a major global health burden with limited treatment options due to high costs and adverse effects. Medicinal plants, such as Peronema canescens (Sungkai), offer safer alternatives, with studies suggesting anticancer potential through apoptosis induction. However, the molecular mechanisms underlying Sungkai's effects on breast cancer remain unclear. This study applies network pharmacology and molecular docking to explore and validate Sungkai's bioactive compounds and their targets in breast cancer therapy. **Methods:** Ethanolic extract of Sungkai leaves was analyzed using LC-MS/MS. Network pharmacology involved predicting compound targets (SwissTargetPrediction), intersecting them with breast cancer-related genes (GeneCards), performing GO and KEGG enrichment analyses using DAVID, and visualizing PPI networks via STRING and Cytoscape. Drug-likeness was assessed using Lipinski's Rule of Five. Molecular docking of active compounds against BCL-2 (PDB ID: 8U27) was conducted using MOE. **Result:** LC-MS/MS identified 11 bioactive compounds, including wogonin, isorhamnetin, chryseriol, quercetin, stemocurtisinol, norlaudanosine, carbocrome, chlorogenic acid, 4-methylphenyl dodecanoate, l-valinol, and trichagmalin A. GO and KEGG analyses showed these targets are mainly involved in cancer-related signaling pathways, particularly PI3K–Akt, which regulates apoptosis. Network pharmacology indicated that these compounds target key breast cancer proteins: ESR1, AKT1, BCL2, SRC, and JUN. Ten compounds satisfied Lipinski's Rule of Five, while trichagmalin A did not. Molecular docking showed strong binding affinities with BCL-2, with carbocromen reaching –7.2805 kcal/mol, comparable to venetoclax (–7.4 kcal/mol), suggesting competitive inhibition potential. **Conclusion:** These results indicate that Sungkai leaf compounds have potential as therapeutic agents for breast cancer, warranting further experimental validation.
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INTRODUCTION

Breast cancer is a leading cause of cancer-related mortality among women, characterized by significant clinical and molecular heterogeneity [1]. In 2022, it accounted for approximately 2.3 million new cases and 666,100 deaths worldwide, with rising incidence and mortality projected in Asia by 2050 [2, 3]. Standard cancer therapies, including chemotherapy, radiotherapy, and surgery, often face limitations such as toxicity, high cost, and incomplete eradication of tumor cells, which can lead to residual or recurrent disease [4–7]. Therefore, identifying safer and more effective therapeutic strategies is a continuing priority in cancer research.

Apoptosis, a programmed cell death process, plays a central role in maintaining cellular homeostasis and eliminating damaged or malignant cells [8]. In cancer, dysregulation of apoptotic pathways allows tumor cells to evade cell death, contributing to therapy resistance and disease progression [9]. While induction of apoptosis is a common mechanism exploited by anticancer drugs,

suboptimal activation can paradoxically promote tumor survival through compensatory mechanisms such as autophagy [10]. Moreover, the regulation of apoptosis is highly complex, involving intrinsic and extrinsic pathways, cross-talk with growth factor signaling, and key modulators such as BCL-2 family proteins [11]. This complexity presents challenges for drug development, yet it also opens opportunities for precision-targeted therapies that can selectively manipulate key apoptotic regulators to overcome therapy resistance. Indeed, ongoing clinical investigations of apoptosis-targeted agents highlight their promising potential to improve cancer treatment outcomes, particularly when integrated with conventional therapies or used to target residual disease.

Natural products have long been explored for their anticancer properties due to their accessibility, structural diversity, and relatively low toxicity[12]. Among these, Sungkai (Peronema canescens Jack), a native plant of Indonesia that is also commonly found in Sumatra, Kalimantan, Malaysia, and Thailand, has been traditionally used in medicine and is known for its immunostimulatory, antioxidant, and antimicrobial activities [13, 14]. Recent studies have demonstrated that Sungkai leaves exhibit anticancer effects against various cancer models, including colon, cervical, and breast cancers, largely due to bioactive compounds such as alkaloids, terpenoids, flavonoids, steroids, polyphenols, and saponins, which have demonstrated significant cytotoxic and antiproliferative properties [15]. However, despite these promising findings, most studies have focused on general cytotoxicity or single-target evaluations, leaving the underlying molecular mechanisms largely unexplored and necessitating further investigation.

To address this gap, network pharmacology offers a systematic approach to predict interactions between bioactive compounds and multiple molecular targets, providing insights into complex disease mechanisms and synergistic therapeutic effects [16] In the context of breast cancer, network pharmacology can help identify key signaling pathways modulated by Sungkai-derived compounds, which can then be validated through molecular docking studies to assess binding affinity and interaction with critical cancer-related proteins. Therefore, this study aims to predict the molecular targets and pathways modulated by Sungkai leaves compounds in breast cancer using a network pharmacology approach, followed by molecular docking validation. This integrated approach provides a rational framework to explore natural compounds as potential apoptosis-modulating agents for cancer therapy.

MATERIAL AND METHODS

Materials

Ethanolic extract of Sungkai leaves (Peronema canescens Jack) was obtained from the Biomedical Laboratory, Faculty of Medicine, Universitas Andalas, where it had been prepared using standard maceration with ethanol. For the in silico analysis, the 3D structures of bioactive compounds identified by LC-MS/MS, together with the reference drug venetoclax, were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/), while the 3D structure of BCL-2 (PDB ID: 8U27) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb/) in PDB format.

Metabolite Profiling using LC-MS/MS

High-resolution mass spectrometry analysis of Sungai leaves extract was performed using an ACQUITY UPLC® H-Class system (Waters, USA) coupled with a Xevo G2-S QTof mass spectrometer (Waters, USA). Chromatographic separation was achieved on an ACQUITY UPLC BEH C18 column (2.1 Ч 50 mm, 1.7 µm; Waters, USA) maintained at 40 °C. The mobile phases consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid, with a linear gradient elution: 5% B (0–1 min), increased to 100% B (1–20 min), held at 100% B (20–24 min), then returned to 5% B (24–25 min). The flow rate was 0.2 mL/min, and the injection volume was 5 µL, with samples filtered through a 0.2 µm syringe filter prior to injection.

Mass spectrometry detection was carried out under positive electrospray ionization (ESI+) with a capillary voltage of 3.0 kV, source temperature 100 °C, desolvation temperature 350 °C, cone gas flow 50 L/h, and desolvation gas flow 800 L/h. Data were acquired in the range of m/z 50–1200 at a resolution of 30,000, with collision energies ramped from 4 to 60 eV for MS/MS fragmentation.

Data acquisition and processing were performed using MassLynx software (v4.1), and putative metabolite identification was achieved by comparison of chromatographic retention times and MS/MS spectra with reference databases, including ChemSpider, MassBank, HMDB, MONA, and PubChem [17, 18].

Network Pharmacology

The Swiss Target Prediction (http://www.swisstargetprediction.ch) with default settings was used to predict the target genes of compounds from Sungkai leaves (P. canescens). Breast cancer-related targets were identified using GeneCards (https://www.genecards.org/) with a score >50. The resulting target genes were then combined and analyzed for intersections using InteractiVenn (http://www.interactivenn.net/). Subsequently, DAVID (https://david.ncifcrf.gov/) was used to KEGG pathway analyses with a

significance threshold set at P < 0.05. The STRING (https://STRING-db.org/), was used to investigate protein-protein interactions (PPIs). focusing on Homo sapiens and an interaction score >0.4. Ultimately, the relationships between compounds, target genes, and pathways were visualized using Cytoscape to further elucidate their interactions [19–21].

Molecular Docking

The Molecular Operating Environment (MOE) was used to execute out molecular docking. We evaluated the components' drug-likeness as possible drug candidates using SCFBio IIT Delhi's Lipinski's Rule of Five approach (http://www.scfbioiitd.res.in/software/drugdesign/lipinski.jsp#anchor-tag) in order to find possible ligands for molecular docking. [22] . All ligands that passed the Lipinski test were copied into MOE and stored as a ligand database. Proteins were downloaded and pretreated by removing water and adding hydrogen. Next, molecular docking was performed. Data analysis was based on binding affinity (S/kcal/mol) and Root Mean Square Deviation (RMSD) values obtained from the docking results displayed in tabular form.

RESULTS

Metabolite Profiling Results using LC-MS/MS

Based on data analysis, a total of 11 compounds were successfully identified. The compounds detected came from various groups, such as flavonoids, alkaloids, terpenoids, coumarins, esters, and amino acids. The components detected from the Sungkai leaves extract are summarized in Table I, and the LC-MS results can be seen in Fig. 1.

Table I: Compound detected in Sungkai leaves extract

Rt (min)	Area (%)	Measured Mass (m/z)	Compound name	Molecule formula	Fit Conf (%)	Group of compounds	CAS Number
1.26	2.35	104.1083	L-Valinol	C5H13NO	n/a	Amino acid	2026-48-4
3.87	0.88	355.1003	Chlorogenic acid	C16H18O9	100	Ester	327-97-9
5.32	3.18	285.0766	Wogonin	C16H12O5	91.12	Flavonoid	632-85-9
5.87	2.97	285.0722	Wogonin	C16H12O5	94.33	Flavonoid	632-85-9
6.25	3.37	317.0684	Isorhamnetin	C16H12O7	80.71	Flavonoid	480-19-3
6.81	1.95	301.0706	Chrysoeriol	C16H12O6	92.26	Flavonoid	491-71-4
7.10	0.08	344.1899	Norlaudanosine	C20H25NO4	98.10	Alkaloid	13074-31-2
7.34	1.32	301.0740	Chrysoeriol	C16H12O6	98.21	Flavonoid	491-71-4
8.07	2.37	331.0832	Quercetin 3,4′-dimethyl ether	C17H14O7	97.94	Flavonoid	33429-83-3
9.08	29.83	362.1975	Carbocromen	C20H27NO5	97.42	Coumarins	804-10-4
10.66	0.09	406.2221	Stemocurtisinol	C22H31NO6	27.09	Alkaloid	ChemSpider ID: 9525190
13.67	0.89	291.2356	4-Methylphenyl dodeca-noate	C19H30O2	97.24	Ester	10024-57-4
15.69	1.78	667.2747	Trichagmalin A	C36H42O12	84.33	Terpenoid	1266672-68-7

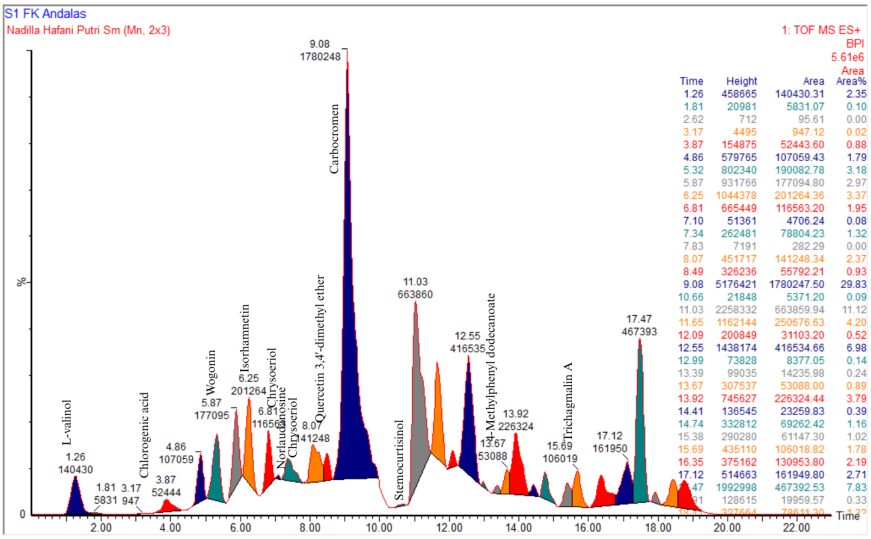

Fig 1: Total Ion Chromatogram of Sungkai Leaves (Peronema Canescens Jack) Extract using LC-MS/MS

The LC-MS/MS chromatogram analysis of the extract revealed several peaks representing different compounds. The most abundant compound was detected at a retention time of 9.08 minutes, with a relative peak area of 29.83%. Based on mass spectral data and database matching, this compound was identified as carbocromen, indicating it as the major constituent in the extract.

Prediction of Target Compounds and Genes

Eleven compounds from Sungkai leaves were analyzed using the Swiss Target Prediction database, yielding 517 target proteins. Using the keyword "breast cancer" on the GeneCards website, 278 target proteins were identified. A Venn diagram comparison revealed 34 overlapping proteins, including KIT, CYP19A1, ESR2, ESR1, EGFR, TERT, AR, PIK3R1, MET, SRC, ALK, AKT1, CDK2, MDM2, AURKA, ERBB2, PIK3CA, JUN, CDK4, VHL, BCL2, STAT3, FGFR1, RET, PDGFRA, FGFR3, FGFR4, FGFR2, CASP8, MAPK1, IDH1, HIF1A, CYP17A1, BRAF. The Venn diagram results can be seen in Fig. 2A.

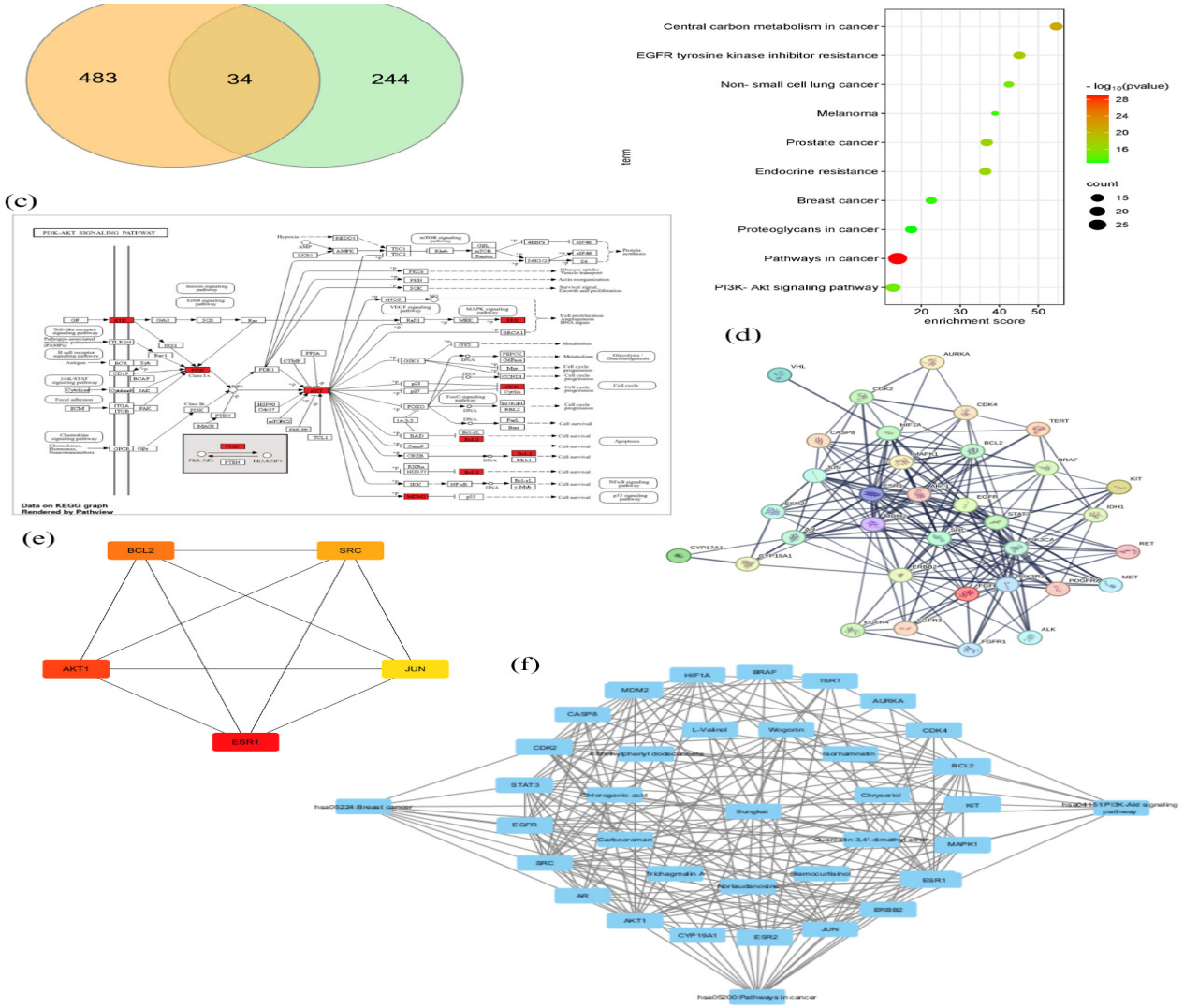

Fig 2: Network pharmacology-based analysis of Sungkai leaves (Peronema canescens Jack) in breast cancer treatment.(a) Venn diagram showing overlap between 517 predicted targets of Sungkai leaves and 278 breast cancer-related targets. A total of 34 intersecting targets were identified as candidate targets for Sungkai leaves in breast cancer therapy. (b) KEGG pathway enrichment analysis conducted using DAVID. Dot size indicates the number of genes involved in each pathway, while color represents statistical significance (p-value), with red denoting highly significant pathways. (c) KEGG pathway enrichment map showing Sungkai leaves candidate targets within the PI3K/AKT signaling pathway. Highlighted nodes (red) represent key molecules including PI3K, AKT, ERK, CDK, MDM2, and BCL2, which are associated with apoptosis and cell survival regulation. (d) STRING-based protein–protein interaction (PPI) network of the 34 intersecting targets. Line thickness represents the strength of data support. (e) Identification of five hub target proteins (AKT1, BCL2, ESR1, JUN, and SRC) through Cytoscape analysis using betweenness centrality, degree, and closeness. Darker red indicates higher centrality.(f) Network visualization in Cytoscape linking Sungkai leaves, their bioactive metabolites, targets, and enriched pathways in breast cancer, shown from center to outer layers.

Gene Ontology Analysis, Kyoto Encyclopedia of Genes and Genomes Pathway

TA total of 34 target proteins associated with the interaction between Sungkai leaves compounds and breast cancer were analyzed for pathway enrichment using KEGG via the DAVID platform (https://david.ncifcrf.gov/). The analysis identified 120 enriched pathways, with the top 10 KEGG terms highlighted in Fig. 2B ($P < 0.05$).

KEGG pathway enrichment highlighted multiple cancer-related pathways, including general pathways in cancer, central carbon metabolism in cancer, EGFR tyrosine kinase inhibitor resistance, endocrine resistance, non-small cell lung cancer, PI3K–Akt signaling, breast cancer, melanoma, prostate cancer, and proteoglycans in cancer (Table II, Fig. 2B). Among these, the PI3K–Akt signaling pathway (hsa04151) was particularly significant, with 18 genes involved ($p = 1.3E^{-o}5$), reflecting its critical role in regulating cell proliferation, survival, apoptosis, and metabolism in breast cancer progression [23]. KEGG pathway analysis further showed that sungkai leaf extract affected several key proteins in this pathway, including PI3K, AKT, ERK, CDK, and members of the Bcl-2 family (Fig. 2C), which are directly associated with cell survival and anti-apoptotic mechanisms. These findings suggest that sungkai leaves extract can suppress growth signals while promoting apoptosis, supporting its potential as a natural therapeutic agent targeting breast cancer through modulation of the PI3K–Akt pathway.

Table II: KEGG pathway enrichment analysis of 34 protein targets of Sungkai (Peronema canescens) leaves correlated with breast cancer

No	Term	Count	P-value	Genes
1	hsa05200:Pathways in cancer	29	$1.0E^{-29}$	RET, ALK, PIK3R1, HIF1A, EGFR, CASP8, TERT, ERBB2, AKT1, MAPK1, VHL, PDGFRA, JUN, STAT3, BRAF, ESR1, ESR2, AR, PIK3CA, CDK4, KIT, CDK2, BCL2, MDM2, FGFR4, MET, FGFR3, FGFR2, FGFR1
2	hsa04151:PI3K-Akt signaling pathway	18	$1.3E^{-15}$	RET, PDGFRA, PIK3R1, EGFR, PIK3CA, CDK4, KIT, ERBB2, CDK2, MDM2, BCL2, AKT1, MAPK1, FGFR4, MET, FGFR3, FGFR2, FGFR1
3	hsa05230:Central carbon metabolism in cancer	15	$9.2E^{-22}$	RET, PDGFRA, IDH1, PIK3R1, HIF1A, EGFR, PIK3CA, KIT, ERBB2, AKT1, MAPK1, MET, FGFR3, FGFR2, FGFR1
4	hsa01521:EGFR tyrosine kinase inhibitor resistance	14	$5.3E^{-19}$	PDGFRA, SRC, STAT3, BRAF, PIK3R1, EGFR, PIK3CA, ERBB2, BCL2, AKT1, MAPK1, MET, FGFR3, FGFR2
5	hsa05215:Prostate cancer	14	$8.9E^{-18}$	PDGFRA, BRAF, PIK3R1, EGFR, AR, PIK3CA, ERBB2, CDK2, MDM2, BCL2, AKT1, MAPK1, FGFR2, FGFR1
6	hsa01522:Endocrine resistance	14	$1.0E^{-17}$	JUN, SRC, BRAF, PIK3R1, ESR1, EGFR, ESR2, PIK3CA, CDK4, ERBB2, MDM2, BCL2, AKT1, MAPK1
7	hsa05205:Proteoglycans in cancer	14	$1.9E^{-13}$	SRC, STAT3, BRAF, PIK3R1, HIF1A, ESR1, EGFR, PIK3CA, ERBB2, MDM2, AKT1, MAPK1, MET, FGFR1
8	hsa05224:Breast cancer	13	$9.5E^{-14}$	JUN, BRAF, PIK3R1, ESR1, EGFR, ESR2, PIK3CA, CDK4, KIT, ERBB2, AKT1, MAPK1, FGFR1
9	hsa05223:Non-small cell lung cancer	12	$9.8E^{-16}$	RET, ALK, PIK3CA, CDK4, ERBB2, STAT3, MAPK1, AKT1, BRAF, PIK3R1, MET, EGFR
10	hsa05218:Melanoma	11	$6.6E^{-14}$	PDGFRA, PIK3CA, CDK4, MDM2, MAPK1, AKT1, BRAF, PIK3R1, MET, EGFR, FGFR1

Construction of Protein-Protein Interactions (PPIs) and Compound-Targets-Pathway Network

The STRING database analysis of the 34 shared genes reveals a highly interconnected network with 34 nodes (proteins) and 181 edges (interactions), significantly surpassing the expected 35 interactions. Each node interacts with an average of 11 others. The high local clustering coefficient (0.662) shows that nodes form tight clusters, and the very low p-value (<1.0e-16) confirms that these interactions are biologically significant and not random, as shown in Fig. 2D. This suggests that the proteins are biologically connected as a group. The STRING data visualized with Cytoscape identified five key target proteins of Sungkai leaves compounds for breast cancer based on betweenness centrality, degree, and closeness centrality, as shown in Table III and Fig. 2E. These proteins are ESR1, AKT1, BCL2, SRC, and JUN. Analysis using STRING and Cytoscape indicated that BCL-2 serves as a central hub among five target proteins of Sungkai-derived compounds in breast cancer. This gene is associated with apoptosis-related biological processes according to GO analysis and is mapped to relevant KEGG pathways, including the PI3K–Akt signaling pathway (Fig. 2C). Other nodes, such as ESR1, AKT1, SRC, and JUN, also show topological significance in the network but are not directly linked to apoptosis. These findings suggest that Sungkai-derived compounds may influence breast cancer-related pathways, potentially including BCL-2-mediated regulation of apoptosis.

Table III: Characteristics of the Top 5 Hub Genes Targeted by Compounds from Sungkai Leaves in Breast Cancer.

Target	Name	Betweenness Centrality	Degree	Closeness Centrality
ESR1	Estrogen receptor 1	0.139069513	17	0.869565217
AKT1	AKT serine/threonine kinase 1	0.077765607	15	0.8
BCL2	B-cell lymphoma protein-2	0.062219053	15	0.8
SRC	Non-receptor tyrosine kinase	0.049025869	15	0.8
JUN	AP-1 transcription factor subunit	0.044677498	15	0.8

We constructed the Sungkai-common target genes-breast cancer network using Cytoscape to visualize and elucidate the pharmacological potential of Sungkai in treating breast cancer. There are 11 nodes representing Sungkai metabolites, 21 nodes representing common target proteins and breast cancer as shown in Fig. 2F

Molecular Docking

Before conducting molecular docking, the drug-likeness of each compound was assessed using Lipinski's Rule of Five (Table IV). According to this rule, a compound is likely to have good oral bioavailability if it has a molecular weight <500 Da, ≤5 hydrogen bond donors, ≤10 hydrogen bond acceptors, a log P value of less than 5, and a molar refractivity between 40 and 130. A compound can violate up to two of these criteria and still be considered drug-like. In this study, the compounds wogonin,isorhamnetin,chryseriol, quercetin, stemocurtisinol,norlaudanosine, carbocrome, chlorogenic acid, 4-methylphenyl dodecanoate, and l-valinol meet these criteria, making them viable drug candidates. However, Trichagmalin A does not meet the criteria. The results are detailed in Table IV.

Table IV: Evaluating the Drug-Likeness of Sungkai Leaf Compounds via Lipinski's Rule of Five

No	Compound	Lipinski Rule of Five				
		Mass(Da)	Hydrogen Bond Donor	Hydrogen Bond Acceptors	LogP	Molar Refractivity
1	Wogonin	284	2	5	1.913670	66.516098
2	Isorhamnetin	316	4	7	1.049560	68.983696
3	Chryseriol	300	3	6	1.359860	68.049896
4	Quercetin 3,4'-dimethyl ether	330	3	7	1.536850	74.258392
5	Stemocurtisinol	405	1	6	3.991989	111.327286
6	Norlaudanosine	343	0	4	3.718539	94.455994
7	Trichagmalin A	666	1	12	5.289890	168.718307
8	Carbocromen	361	0	5	3.662199	100.065491
9	Chlorogenic acid	354	6	9	1.285940	78.631287
10	4-Methylphenyl dodecanoate	290	0	2	4.734410	96.074989
11	L-Valinol	103	2	5	1.323450	33.220196

Notes : *logP* = lipophilicity , *bold* **indicates** criteria which fail to meet the requirements

After determining the inclusion compound, molecular docking analysis was conducted to evaluate the binding affinity, which signifies the stability of the ligand-receptor interaction. A lower (more negative) binding energy indicates a stronger and more stable interaction, which is primarily influenced by hydrogen bonding, hydrophobic interactions, and electrostatic forces [24, 25].

Molecular docking analysis was performed to evaluate the binding affinity of selected compounds from Sungkai leaves (Peronema canescens Jack) leaves against the Bcl-2, a key regulator of apoptosis in breast cancer. As shown in Table V, the native ligand (8u27) exhibited the strongest binding affinity (–9.0568 kcal/mol). Due to the substantial difference between the binding affinity of the native ligand and those of the sungkai extract compounds, venetoclax

Table V: Binding Affinity and RMSD Molecular Docking.

No	Ligand	Bcl-2 Molecular Docking Results	
		Binding Affinity (kkal/mol)	RMSD (E)
1	Native ligand (8u27)	-9.0568	1.7339
2	Venetoclax	-7.4	1.759
3	Wogonin	-6.3596	1.7895
4	Isorhamnetin	-6.2319	0.9975
5	Chryseriol	-6.1688	1.4383
6	Quercetin 3,4′-dimeth-yl ether	-6.6503	1.9475
7	Stemocurtisinol	-6.7034	0.8799
8	Norlaudanosine	-6.7235	1.8061
9	Carbocromen	-7.2805	1.2900
10	Chlorogenic acid	-6.6631	1.6507
11	4-Methylphenyl do-decanoate	-6.7959	1.2855
12	L-Valinol	-4.9375	1.0921

Notes: Bold compounds with binding affinity values more negative than venetoclax

(–7.4 kcal/mol) was used as a more suitable reference. Carbocromen exhibited a docking score of -7.2805 kcal/mol, which was close to that of venetoclax, with a minimal difference of 0.1195 kcal/mol, suggesting a comparable binding affinity to the BCL-2 protein. Additionally, stermocurtisinol (-6.7034 kcal/mol), norlaudanosine (-6.7235 kcal/mol), chlorogenic acid

(-6.6631 kcal/mol), and 4-methylphenyl dodecanoate (-6.7959 kcal/mol) also showed favorable docking scores, indicating strong potential interactions with BCL-2. All compounds exhibited RMSD values below 2.0 E, confirming the reliability of the docking conformations.

DISCUSSION

The therapeutic potential of natural products in cancer treatment has gained considerable attention in recent years, particularly due to their structural diversity and multi-target mechanisms. Peronema canescens (Sungkai) has been traditionally used in various ethnomedicinal applications and has recently demonstrated promising anticancer activity, notably against colon and cervical cancers [15, 26]. While these findings have been encouraging, the possible role of Sungkai in breast cancer treatment remains relatively unexplored. To address this gap, the present study employed a combination of LC-MS-based metabolite profiling, network pharmacology, and molecular docking simulations to explore the anticancer relevance of Sungkai leaves compounds against breast cancer.

Our LC-MS analysis identified eleven bioactive compounds from Sungkai leaves, expanding the phytochemical profile beyond what has previously been reported. Among these, several compounds such as L-valinol, isorhamnetin, and chrysoeriol had been previously reported, confirming the reproducibility of earlier findings [27]. However, novel identifications such as stemocurtisinol and trichagmalin A suggest that Sungkai harbors previously undocumented metabolites with potential pharmacological relevance. This expansion of the known chemical profile not only underscores the phytochemical richness of Sungkai, but also opens new avenues for exploring its bioactivity in the context of complex diseases such as breast cancer .

To better understand the chemical findings in a biological context, we applied a network pharmacology approach that identified 34 overlapping targets between Sungkai-derived compounds and breast cancer-related genes. Among these, ESR1, AKT1, BCL-2, SRC, and JUN emerged as highly connected nodes, indicating their central roles in tumor progression and therapy resistance. Although ESR1 showed the highest centrality values, we focused on BCL-2 for several reasons. First, ESR1 is already extensively targeted by existing endocrine therapies, leaving less room for novel intervention. Second, BCL-2 expression is enriched in breast cancer subtypes with ER and PR positivity, including luminal subtypes, where its anti-apoptotic function contributes to treatment resistance. This dual role in promoting cell survival while being associated with favorable prognosis underscores the importance of targeting BCL-2 as a therapeutic strategy [28, 29]

Enrichment analysis indicated that apoptotic signaling pathways were among the top biological processes influenced by Sungkai-derived compounds, including regulation of intrinsic and extrinsic apoptotic pathways, as well as PI3K–Akt signaling. These results suggest that Sungkai compounds may restore apoptotic sensitivity in tumor cells, particularly by modulating survival pathways that are commonly dysregulated in breast cancer. Molecular docking further supported this hypothesis, showing that compounds such as carbocromen

(-7.2805 kcal/mol), stermocurtisinol (-6.7034 kcal/mol), norlaudanosine (-6.7235 kcal/mol), chlorogenic acid (-6.6631 kcal/mol), and 4-methylphenyl dodecanoate (-6.7959 kcal/mol) exhibited strong predicted binding affinities for BCL-2, comparable to venetoclax, a clinically approved cancer drug chosen for comparison due to its highly specific inhibition of BCL-2 [30]. Notably, carbocromen's docking score closely approximated that of venetoclax (-7.4 kcal/mol), with only a minimal difference ($\Delta = 0.1195$ kcal/mol), suggesting competitive inhibition of BCL-2's anti-apoptotic function with similar thermodynamic stability. These findings highlight the potential for multiple bioactive compounds in Sungkai to synergistically target apoptosis-regulating proteins.

While these computational results are promising, they represent an early step in the drug discovery pipeline. Enrichment analysis and molecular docking suggest that Sungkai leaves-derived compounds may modulate apoptotic pathways, particularly through BCL-2 inhibition, with compounds such as carbocromen, stermocurtisinol, norlaudanosine, chlorogenic acid, and

4-methylphenyl dodecanoate showing binding affinities comparable to venetoclax, a clinically approved BCL-2-specific inhibitor [30]. Experimental validation, including apoptosis assays, BCL-2 knockdown in vitro, and in vivo breast cancer studies, is necessary to confirm their mechanisms, efficacy, and safety. Given BCL-2's dual role and regulation by ER signaling, careful patient stratification will be critical to maximize therapeutic benefit. By combining metabolite profiling, network pharmacology, and molecular docking, this study provides a robust framework for identifying and characterizing bioactive compounds from Sungkai, highlighting their potential as BCL-2-targeted anticancer agents.

CONCLUSION

This study provides compelling evidence that Sungkai (Peronema canescens) leaves-derived compounds hold significant promise as novel therapeutic agents for breast cancer, particularly through the modulation of apoptosis via BCL-2 inhibition. Through a comprehensive approach combining LC-MS-based metabolite profiling, network pharmacology, and molecular docking simulations, we identified several bioactive compounds with strong binding affinity to BCL-2, a key regulator of cell survival in breast cancer. The enrichment of apoptotic pathways further supports the hypothesis that Sungkai compounds may exert their anticancer effects by restoring programmed cell death in tumor cells. While these findings lay a solid groundwork, future in vitro and in vivo studies are essential to validate the predicted interactions, assess therapeutic efficacy, and ensure safety. Ultimately, this integrative strategy reinforces the potential of traditional medicinal plants as a valuable resource in the discovery of safer and more targeted cancer therapies.

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