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In Silico Investigation of Phytochemical Inhibitors of STAT3 to Address Drug Resistance in Triple-Negative Breast Cancer

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Abstract: Breast cancer is the most common cancer among women and a major cause of cancer-related death worldwide, including Indonesia. Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer, characterized by high mortality and resistance to standard treatments. Cancer stem cells (CSCs) is known to be a key factor of drug resistance in TNBC, mediated by the signal tranducer and activator of transcription factor 3 (STAT3). This study aims to explore the bioactive compounds from Indonesian's natural product which potentially target CSCs by inhibiting STAT3. We used in silico methods, consist of literature review, followed by selection of promising compounds, ADMET analysis and selection using Lipinski's rules, and molecular docking simulation. Our results found curcumin, quercetin, α-mangostin, gingerol, and rocalglamide as the bioactive candidates which target CSCs. ADMET testing showed that only curcumin, quercetin, and gingerol meet the Lipinski's criteria. The molecular docking results showed that quercetin exhibited the strongest binding affinity (-8.3 kcal/mol) compared to curcumin (-6.9 kcal/mol) and gingerol (-5.9 kcal/mol). The results indicate that quercetin binds effectively at the active site of STAT3. The findings suggest that quercetin has potential as a STAT3 inhibitor. Further research is needed to determine the significant efficacy and potential as an anticancer.

Keywords: Bioactive compounds; STAT3 inhibition; Drug resistance TNBC; In silico screening

Introduction

Breast cancer is the most common cancer and major cause of cancer-related death in women, both globally and regionally in Indonesia (International Agency dor Research on Cancer WHO, 2022). Triple-negative breast cancer (TNBC) is a subtype of breast cancer, with the worst severity, highest morbidity and mortality rates compare to the other subtypes (Purwanto et al., 2020). The incidance rate of TNBC is 15-20%, and it predominantly occurs in productive-age women (Yao et al., 2017). Currently, there is no effective standard therapy for TNBC subtype, because of therapy resistance characteristics (McKenna et al., 2017). Therefore, TNBC requires more attention and further researches to support the development of effective therapies.

One of the stages in breast cancer therapy is chemotherapy using specific chemotheraputic agent. Doxorubicin is known as one of the most effective chemoterapeutic agent in the treatment of metastatic breast cancer, but it is not effective for TNBC therapy due to high resistance (Chiorean et al., 2013; Duan et al., 2003). Recent finding found that the presence of cancer stem cells (CSCs) mediated by a protein called signal transducer and activator of transcription 3 (STAT3) is one of the primary cause of chemoresistance in TNBC (Moreira et al., 2018). STAT3 increases the cells survival and finally prevents the death of cancer cells (Zhao et al., 2015).

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Indonesian natural producsts (plants) are known to have anticancer activity, for instances turmeric (*Curcuma domestica*), temulawak (*Curcuma xanthorrhiza*), sambiloto (*Andrographis paniculata*), mangosteen (*Garcinia mangostana L.*), and endemic plants such as kayu palado (*Aglaia ceramica*). Anticancer activity of the natural products is mediated by contains of phytochemicals compounds, such as curcumin, α-mangostin, rocalglamide, 6-gingerol, 8-gingerol, 10-gingerol, and andrographolide (Wanandi et al., 2020). However, there is still limited information about bioactive compounds from these plants that can address breast cancer resistance, particularly through STAT3 inhibition.

Recent advance in bioinformatics enhances the health research efficiency, including the development of natural products for therapy. Previous study reported the use of bioinformatics approach to identify alternative of doxorubicin by analyzing the anticancer activity of bioactive herbal compounds from Indonesian plants such as *Citrus sp., Curcuma sp., Caesalpinia sappan*, and *Alpinia galanga*, focusing on NF-kB inhibition (Amalina et al., 2020). In a subsequent study, the use of ester derivatives for inhibiting the STAT3 protein was investigated (Li et al., 2022). While, our study aims to identify bioactive compounds that can serve as additional therapy of doxorubicin, in order to enhance effectiveness of chemotherapy. This research not only focus on ester derivatives but also explore various compounds from Indonesian plants which has anticancer activity against STAT3.

Based on this background, we designed a study focusing on discovering bioactive compounds from Indonesian natural products that can address resistance in TNBC by inhibiting STAT3 protein. Therefore, this research investigates the ability of various bioactive compounds to inhibit STAT3.

Method

Tools and Materials

In this work, we used a personal computer (PC) with a Windows 10 64-bit operating system. The bioactive compounds were firstly selected by reviewing the literatures from ScienceDirect (https://www.sciencedirect.com/) and PubMed (https://pubmed.ncbi.nlm.nih.gov/) databases. For the analysis, we utilized several softwares, including molecular docking simulaltion using PyRx, viasualization and analysis of docking results using BIOVIA Discovery Studio 2021, and evaluation of drug-likeliness properties using SwissADME (http://www.swissadme.ch/). The 3D structures of bioactive compounds and STAT3 were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and RCSB PDB (https://www.rcsb.org/) databases, respectively.

Literature Review

We conducted a comprehensive literature review by systematically gathering and analyzing various references from Sciencedirect and PubMed databases related to bioactive compounds from Indonesian natural materials with anticancer activity against CSCs. This stage aim to identify and compile a list of relevant bioactive compounds for subsequent analysis.

Candidates Drug Criteria Testing

The candidates drug criteria were analyzed using Lipinski's Rule of Five. This analysis was conducted using SwissADME (http://www.swissadme.ch/) to evaluate the ADMET properties of bioactive compounds to ensure their suitability as drug candidates. According to the Lipinski's rule, a good drug candidate should meet the following criteria: Molecular weight not exceeding 500 Da; Lipophilicity (LogP) less than 5; Number of hydrogen bond donors less than 5; Number of hydrogen bond acceptors less than 10; and Good bioavailability. The compounds meeting these criteria were further considered for molecular docking studies.

Preparation of Substrates and Proteins

Before conducting molecular docking tests, the three-dimensional (3D) structures of each bioactive compound (substrates) were collected from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) and downloaded in SDF format. The 3D crystal structure of the STAT3 protein was obtained from the RCSB (Research Collaboratory for Structural Bioinformatics PDB) at https://www.rcsb.org/structure/1BG1. The structure used was the transcription factor STAT3B/DNA complex with PDB ID 1BG1. The preparation process for the protein included removing water molecules, heatatm, and native ligands.

Molecular Docking Analysis

Molecular docking were conducted to analyze the potential of bioactive compounds in overcoming chemoresistance by inhibiting the STAT3 protein. Docking simulations and visualization were performed using PyRx and BIOVIA Discovery Studio 2021 software, respectively. The procedures consist of importing the protein and ligand structures into PyRx, where the ligands were optimized by energy minimization and converted into pdbqt format. The docking simulations were then executed to assess the binding affinity and interaction patterns of the ligands with the STAT3 protein, aiming to identify the most promising inhibitors. The method is shown in figure 1.

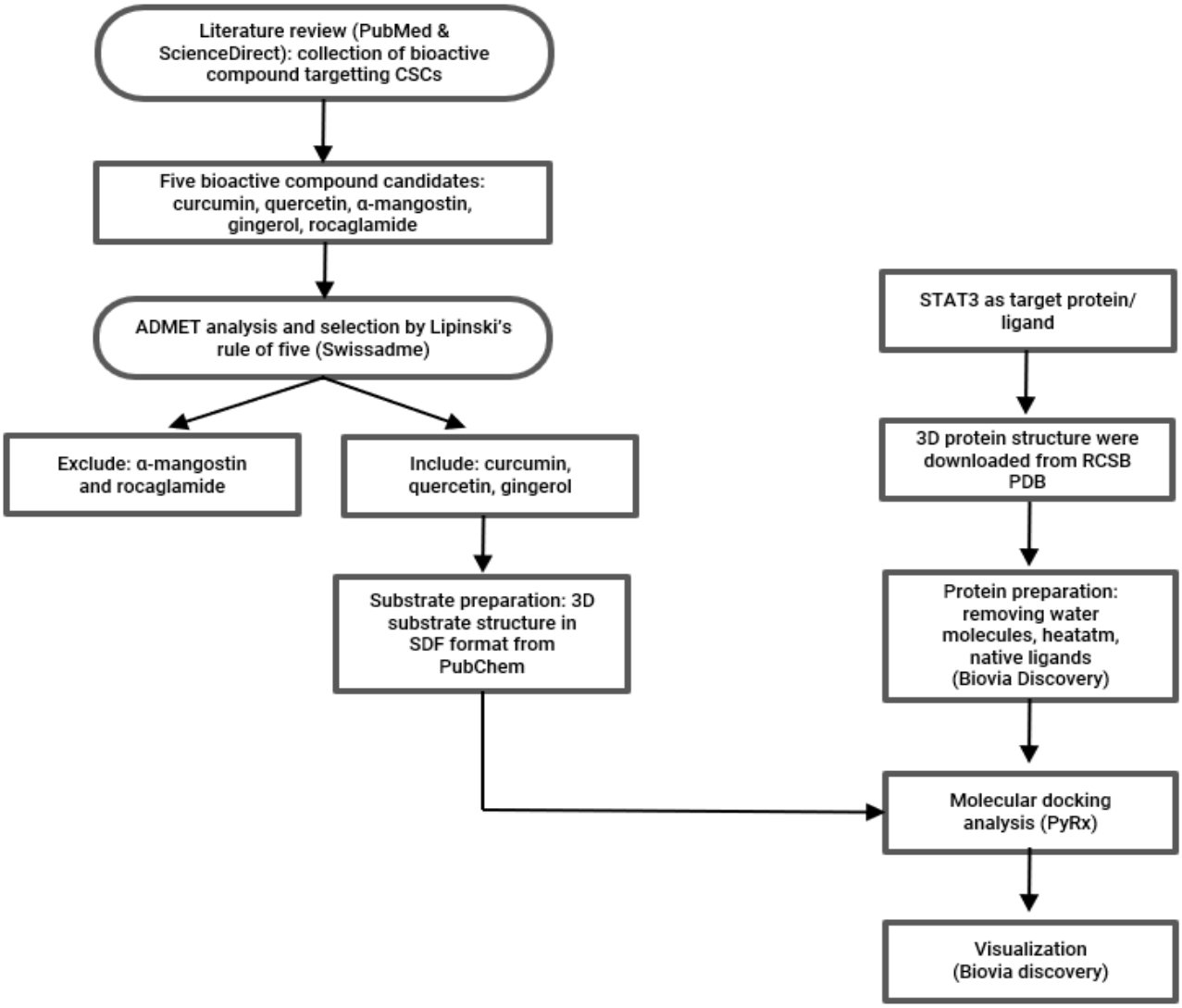


Figure 1. Methodology flowchart

Result and Discussion

The mechanisms of chemoresistance in breast cancer caused by CSCs consists of drug efflux, strong survival capabilities, and ability to evade the immune system (Khan et al., 2024). These resistance mechanisms are supported by specific proteins and markers of CSCs that can serve as therapeutic targets, among them is STAT3.

STAT3 is a transcription factor that involved in almost all of the cancer's hallmarks. These hallmarks consist of tumor growth, metastasis, angiogenesis, immune evasion, tumor-associated inflammation, metabolic reprogramming, drug resistance, and CSCs properties. Therefore, researches about the evaluation of STAT3 potential as a target therapy has emerged in various cancers (Hu et al., 2024). For example, in gastric cancer, STAT3 were known to be overexpressed in cancer stem-like cells (Hajimoradi et al., 2016). Other study found that STAT3 inhibits ferroptosis (a form of cell death), therefore inhibition of STAT3 can suppress tumor growth and alleviate chemoresistance (Ouyang et al., 2022). Researches about the potential of using natural products in repressing STAT3 has also attracted the researchers. A novel natural product derived from *Chaetomium globosum* has know to suppress tumor growth and metastasis by inhibiting STAT3 (Guan et al., 2023).

In TNBC, the important role of STAT3 has been explored in the initiation, progression, invasion, and immune evasion process (Qin et al., 2019). Previous study proved that quercetin inhibits proliferation, migration, and invasion of 4T1 cells via IL-6/JAK2/STAT3 signaling pathway (Liao et al., 2024). It suggested that inhibition of STAT3 protein or signaling pathway by quercetin serve as a potential way for TNBC therapeutic strategies.

Bioactive Compounds Targetting STAT3

Our literature review process results in the finding of five bioactive compounds candidate which potentially addressing resistance in breast cancer. This review focus on the anticancer activity of natural products through the activity against cancer stem cells (CSCs). The literature review involved several articles obtained from PubMed and ScienceDirect. The selection criteria were that the compounds not only exhibit anticancer activity but also possess activity against CSCs.

Each bioactive compound candidate has activity against CSCs through various mechanisms (Table 1). Curcumin targets CSCs by inducing apoptosis and downregulating CSC markers. Quercetin which can be found in *Moringa oleifera* inhibits Notch1, enhances the PI3K/Akt pathway, and regulates ABC transporters. α-Mangostin, from *Garcinia mangostana,* suppresses Notch signaling and reduces the expression of key CSC markers like CD44, CD133, Oct-4, and SOX-2. Gingerol induces cell death by inhibiting iron metabolism, activating PTEN, suppressing VEGF and Bcl-2 expression. Last, rocaglamide were known to impairs CSC self-renewal, reduces Oct-4 and NANOG expression, and promotes apoptosis. These five compounds represent promising candidates for targeting CSCs in cancer therapy.

Table 1. Bioactive Compound Candidates and Their Activities Against CSCs

References	Bioactive compounds & PubMed CID	Originating Plants	Potential Activity Agains CSCs
(Gupta et al., 2021; C. Hu et al., 2019; A. Q. Khan et al., 2020; Wang et al., 2017; Zhou et al., 2015)	Curcumin (969516)	*Curcuma longa*	▪ Inducing apoptosis ▪ Reducing the expression of CSC markers ▪ Suppressing CSC resistance pathways, including Hedgehog and JAK/STAT3 pathways ▪ Reducing the expression of ABC transporters, ABCG2, and ABCC1, which are involved in drug efflux
(Biswas et al., 2022; Cao et al., 2018; Duo et al., 2012; Iriti et al., 2017; Shen et al., 2016; Srinivasan et al., 2016)	Quercetin (5280343)	*Moringa oleifera*	▪ Reducing the expression of Notch1 and inducing phosphorylation of PI3K/Akt ▪ Addressing drug efflux through ABC transporters ▪ Reversing multidrug resistance and improving chemosensitivity in human breast cancer cells ▪ Activating caspase-3, caspase-9, and cytochrome-c
(Chandra Boinpelly et al., 2020; Jo et al., 2022; Khaw et al., 2020; Liao et al., 2023)	α-Mangostin (5281650)	*Garcinia mangostana*	▪ Suppressing Notch signaling pathways ▪ Reducing the expression of CD44 and CD133 ▪ Reducing the expression of Oct-4, SOX-2, c-Myc, and Nanog
(Salari et al., 2023; Sp et al., 2021)	gingerol (442793)	*Zingiber officinale*	▪ Inducing cell death by inhibiting iron metabolism and inducing PTEN protein ▪ Inhibiting PD-L1 expression through the PI3K/Akt/p53 signaling pathway ▪ Suppressing the expression of VEGF and Bcl-2
(Luan et al., 2015; Nalli et al., 2018; Sridharan et al., 2019)	Rocaglamide (331783)	*Aglaia odorata*	▪ Reducing the self-renewal ability of cells ▪ Reducing the expression of Oct-4, NANOG, and drug transporters ▪ Inducing cell death (apoptosis) and increasing caspase-3 levels

Drug Candidate Criteria Compliance

The candidates drug criteria testing conducted using the SwissADME database (http://www.swissadme.ch/) and the results are presented in Table 2. The results were compared with Lipinski's criteria. Data analysis shows that three bioactive compounds meet the criteria for drug candidates: curcumin, quercetin, and gingerol. These findings are in accordance with previous researches which found that these compounds have potential as drug candidates because they meet all of Lipinski's criteria (Durojaye et al., 2019; Hasan et al., 2022; Moetlediwa et al., 2024).

In contrast, α-mangostin and rocaglamide violated one of the Lipinski's criteria, poor solubility and >500 kDa molecular weight, respectively. Therefore, these compounds were not subjected to molecular docking analysis in the next stage. These results are consistent with previous studies, which demonstrated that rocaglamide and α-mangostin each violated one of Lipinski's rules. However, α-mangostin's lipophilicity leads to a high plasma protein binding, suggesting its

greater distribution to tissues and its ability to penetrate adipose tissue such as breast cancer (dos Santos et al., 2022; Naing et al., 2023).

Table 2. Drug-Likeness Criteria Based on Lipinski's Rule

Bioactive compound	GI absorption	Drug-likelines, Lipinski Violation	Bioavalaibility score	H-bond acceptors	H-bond donors	MW (g/mol)	Water solubility
Lipinski's role	High	Yes, 0	>0,5	<10	<5	<500	Moderate to high
Curcumin	High	Yes, 0	0,55	6	2	368,38	Soluble
Quercetin	High	Yes, 0	0,55	7	5	302,24	Soluble
α- Mangostin	High	Yes, 0	0,55	6	3	410,46	Poor
Gingerol	High	Yes, 0	0,55	4	2	294,39	Soluble
Rocaglamide	High	Yes, 0	0,55	7	2	505,56	Moderate

Molecular Docking Results

We used the 3D crystal structure of the STAT3 protein with PDB ID 1BG1. This structure contains inhibitor as a native ligand that can be used for predicting the binding site of STAT3 protein. The preparation of protein before molecular docking simulation consists of removal of water molecules, heatatm, and native ligands. The final STAT3 structure for molecular docking is shown in Figure 2. The binding site of the STAT3 protein with its inhibitor (native ligand) is located at Leu438, Asp369, Arg382, Gly442, Arg423, His457, Lys244, Glu444, Thr443, Thr456, and Lys244 (Khanam et al., 2019).

Based on the data analysis, gingerol binds only to one amino acid at the active site of the STAT3 protein, namely Arg382 (shown in red). Curcumin binds on two amino acids at the binding site (Arg382, Arg423), while other interactions occur outside the active site. Additionally, examining the amino acids outside the active site shows that the binding positions of gingerol and curcumin are close to each other. On the other hand, quercetin binds entirely on three amino acids at the active site of STAT3, specifically Thr456, His457, and Lys244. These results showed that quercetin bind more effectively to STAT3 compared to curcumin and gingerol, and the binding site were align well with the active site. Therefore, quercetin is a potential candidate as a STAT3 inhibitor.

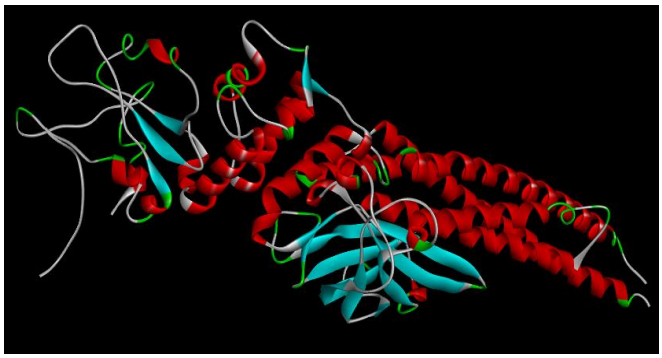


Figure 2. 3D Structure of the STAT3 Protein (PDB ID: 1BG1)

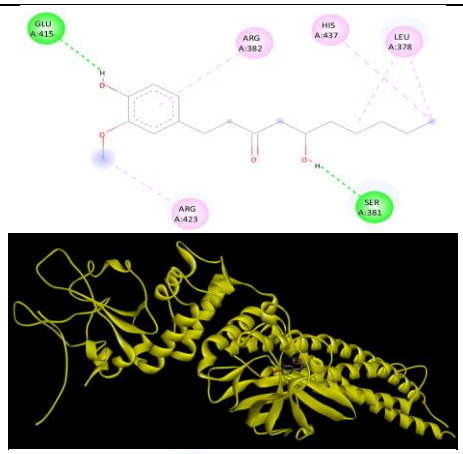
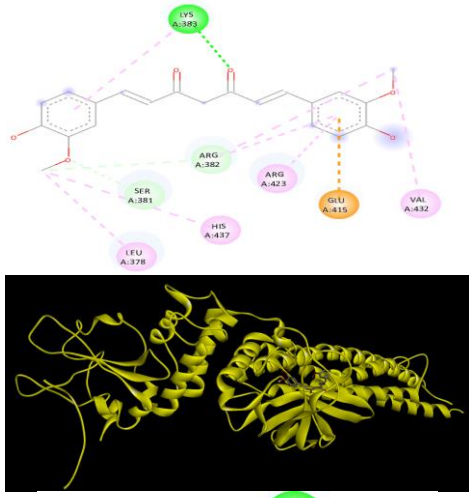
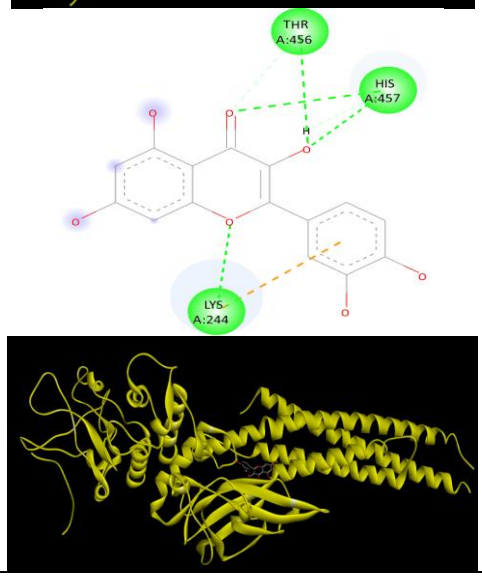
Binding Energy of STAT3 Protein and Bioactive Compounds

Evaluation of the binding energy values were conducted as the final confirmation to analyze the potential of bioactive compounds as STAT3 protein inhibitors. Binding energy represents the amount of energy which is required by a complex protein/ligand to bind each other. In this case, the protein in STAT3 and the ligands are curcumin, quercetin, and gingerol. The less energy required means that the binding between protein and ligands is easier and stronger, because it requires less energy to form the bond. Based on the results, the binding energy values for gingerol, curcumin, and quercetin are -5.9, -6.9, and -8.3 kcal/mol, respectively. These results indicate that STAT3 has the strongest binding with quercetin. Previous research in prostate cancer are accordance with this result, demonstrating that quercetin can bind STAT3 (Kalungi et al., 2023).

A study about the phytochemicals contain in Yiqi Huayu Decoction (YQHY), showed that quercetin is the most abundant component. It can induces ferroptosis via the JAK/STAT3 pathway (Song et al., 2022), leading to cancer cells death. Other studies report that quercetin enhances the degradation of STAT3 protein in liver cancer (Sethi et al., 2023).

The significant potential of quercetin as a candidate anti-cancer compound prompts further research. For example, nanoformulations of quercetin are found to improve the stability and cellular bioavailability of quercetin (Joshi et al., 2023). Encapsulation using acid-modified liposomes have known to inhibit proliferation and evade immune cells in osteosarcoma through the JAK2-STAT3-PD-L1 signaling pathway (Jing et al., 2022). Thus, the overall data support the conclusion that quercetin holds the potential to address the chemoresistance in TNBC by targeting CSCs thorugh STAT3 inhibition.

Table 3. Overview of Protein-Ligand Interactions

Bioactive Compound	Ligand-Protein Interaction	Binding Site
Gingerol		Glu415, Arg382, His437, Leu378, Arg423, Ser381
Curcumin		Lys383, Arg382, Ser381, Leu378, His437, Arg423, Glu415, Val432
Quercetin		Thr456, His457, Lys244

Conclusion

This study demonstrates that bioactive compounds curcumin, gingerol, quercetin, rocaglamide, and α-mangostin exhibit anticancer activity; however, only curcumin, gingerol, and quercetin meet the criteria as drug candidates. Among these, quercetin emerged as the most promising inhibitor of the STAT3 protein, with a binding energy of -8.3 kcal/mol and interactions at amino acids Thr456, His457, and Lys244. These results highlight the potential of quercetin to overcome drug resistance in TNBC by targeting CSCs thorugh STAT3 inhibition. Experimental researches are needed to validate these in silico findings, and exploration of innovative delivery methods are needed to optimize therapeutic impact of quercetin.

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Author Contributions
Conceptualization, methodology, formal analysis, and writing original draft preparation D.H; Validation, writing-review and editing, data curation, software operation, S.S and S.I. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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