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Computational Driven Discovery of Anti-Breast Cancer Phytochemicals from Clove Leaf Waste (*Syzygium aromaticum*): An Integrated Molecular Docking Approach

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Abstract

Clove leaf (*Syzigium aromaticum*) waste represents a rich source of secondary metabolites that has been largely underutilized by the community, this study was conducted to identify compounds present in clove leaf waste extract and predict their binding affinity toward molecular targets relevant to breast cancer using a molecular docking approach. The extraction of clove leaf waste was performed using n-hexane as the solvent, followed by compound identification through GC-MS. The interactions between clove leaf waste compounds and a commercially used breast cancer drug were evaluated through docking simulations against the target protein using the VinaWizard plugin in PyRx 1.1 software. Docking were subsequently visualized using PyMOL software. SMILES-derived structures of clove leaf waste compounds. The results of molecular docking simulation showed that the control ligands, neratinib and tamoxifen, exhibited binding energies of -4.3 kcal/mol and -5.7 kcal/mol, α-humulene, and ß-caryophyllene -7.3 kcal/mol, caryophyllene oxide -6.7 kcal/mol and eugenol -5.9 kcal/mol. The reference drug tamoxifen demonstrated negative binding affinities (kcal/mol) against estrogen receptor alpha (ERα) and HER2, suggesting strong interactions with breast cancer-related molecular targets.

Keywords: *breast cancer; clove leaf waste; bioactive compounds; molecular docking.*

INTRODUCTION

The exploration of natural compounds as alternative or complementary anticancer therapies has gained considerable attention due to their multi-targeted mechanisms and relatively low toxicity profiles. Plant-derived secondary metabolites are well known for their diverse pharmacological activities, including antioxidant, anti-inflammatory, and anticancer effects (Nusaly, *et al*., 2023). Among these, clove (*Syzygium aromaticum*) has long been recognized as a rich source of bioactive compounds with significant therapeutic potential. Previous studies have demonstrated that major constituents of clove, particularly eugenol, exhibit cytotoxic and antiproliferative effects against various cancer cell

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types, including breast cancer, through mechanisms such as apoptosis induction, cell cycle arrest, and modulation of cellular signaling pathways (Prashar, *et al*., 2006). Despite these promising findings, most existing studies have primarily focused on plant parts with high economic value, especially clove flower buds, which are widely utilized in the spice and cigarette industries. This creates competition between medicinal research and commercial use, potentially limiting the sustainable development of clove-derived therapeutics. In contrast, clove leaves particularly those discarded as agricultural waste by farmers in Maluku, Indonesia remain largely underutilized, despite containing comparable phytochemical constituents such as eugenol, β-caryophyllene, α-humulene, and other terpenoid derivatives (Wael, *et al*., 2018; Fidyt, *et al*., 2016; El-Saber Batiha, *et al*., 2020).

The urgency of utilizing clove leaf waste therefore lies in its potential to serve as a sustainable, cost-effective, and non-competitive source of bioactive compounds, while simultaneously addressing environmental concerns associated with biomass waste accumulation.Furthermore, although the anticancer potential of clove-derived compounds has been widely reported, studies investigating their specific molecular interactions with key breast cancer targets remain limited. Proteins such as estrogen receptor alpha (ERα) and human epidermal growth factor receptor 2 (HER2) play crucial roles in tumor proliferation and progression, making them important targets for anticancer drug development. However, systematic studies integrating phytochemical identification from clove leaf waste with computational approaches, such as molecular docking, to evaluate ligand–receptor binding affinity toward these targets are still scarce. Therefore, this study aims to address this gap by identifying bioactive compounds present in clove leaf waste extract and predicting their binding affinity toward breast cancer- related molecular targets using a molecular docking approach (Saini, *et al*, 2024). By focusing on the valorization of agricultural waste, this research not only contributes to the development of natural product–based anticancer candidates but also supports the principle of sustainability through the utilization of underexploited biological resources. This study is novel in explicitly integrating the valorization of clove leaf waste with in silico molecular docking analysis to systematically elucidate the binding interactions of its phytochemical constituents against specific breast cancer molecular targets, namely ERα and HER2.

Breast cancer continues to be the primary cause of cancer-related deaths among women globally. The incidence and mortality rates are still increasing, especially in low- and middle-income countries, influenced by factors such as lifestyle changes, hormonal imbalances, genetic predisposition, and environmental exposures (Sung, *et al*., 2021; World Health Organization [WHO], 2023). Current treatment modalities for breast cancer include surgery, radiotherapy, chemotherapy, hormonal therapy, and targeted molecular therapy. Although considerable progress has been made in these therapeutic approaches, several limitations remain, including drug resistance, significant side effects, high treatment costs, and a marked reduction in patients quality of life (Harbeck, *et al*., 2019; Waks & Winer, 2019). Therefore, the development of new anticancer agents that are more effective, selective, and have fewer adverse effects remains a major focus in pharmaceutical and biomedical research. In this context, the exploration of natural compounds as alternative or complementary anticancer therapies has gained increasing attention. Plant-derived secondary metabolites have demonstrated diverse pharmacological activities, including anticancer, anti-inflammatory, and antioxidant effects (Nusaly, *et al*., 2023). Clove leaves, which are largely regarded as agricultural waste by farmers in Maluku, Indonesia, remain underutilized despite their promising phytochemical profile (Wael, *et al*., 2018).

Several studies have reported that clove leaf waste contains bioactive compounds such as eugenol, β-caryophyllene, α-humulene, and other terpenoid derivatives, which exhibit potential anticancer activities through multiple molecular mechanisms (Fidyt, *et al*., 2016; El-Saber Batiha, *et al*., 2020). Natural products, particularly medicinal plants, continue to play a vital role in modern drug discovery, with a significant proportion of anticancer drugs originating from natural sources. Indonesia, as one of the world's megabiodiversity countries, possesses substantial potential for the development of pharmaceuticals derived from biological resources. Clove (*Syzygium aromaticum*), a plant widely used in traditional medicine and possessing high economic value, has primarily been exploited for its flower buds and essential oil (Letlora, *et al*., 2024). In contrast, clove leaves are often neglected and treated merely as agricultural waste. Recent evidence indicates that clove leaves are rich in secondary metabolites, including eugenol, β-caryophyllene, caryophyllene oxide, and other terpenoids (Wael, *et al*., 2018), which may exert anticancer effects by modulating cell proliferation, apoptosis, and oxidative stress pathways (Watuguly, *et al*., 2012). Clove contains various bioactive compounds, particularly eugenol, eugenol acetate, and β-caryophyllene, which are known to possess important pharmacological activities. Studies have shown that these compounds have the potential to inhibit the growth of breast cancer cells through multiple molecular mechanisms (Wang, *et al*., 2025). Eugenol, as the component, is capable of inducing apoptosis in breast cancer cells, such as MCF-7 cells, through the activation of caspase-3 and caspase-9, increased levels of reactive oxygen species (ROS) within cancer cells, and disruption of the mitochondrial membrane. These mechanisms ultimately trigger programmed cell death (apoptosis) (Wang, *et al*., 2025). The valorization of clove leaf waste as a source of bioactive compounds not only offers opportunities for discovering novel anticancer agents but also aligns with sustainable waste management and circular economy principles, providing environmental and socioeconomic benefits (Kiralan, *et al*., 2022; Saini, *et al*., 2024).

Recent progress in computational biology has facilitated the rapid and economical screening of bioactive compounds through *in silico* techniques, particularly molecular docking. This method enables the prediction of ligand–protein interactions, binding affinities, and binding conformations, thereby offering important insights into the potential biological activity of compounds before experimental testing is conducted (Pinzi & Rastelli, 2019). In breast cancer research, various molecular targets have been extensively identified and utilized in drug discovery studies, particularly those involved in the regulation of cancer cell proliferation, differentiation, and survival. Among these targets, ERα plays a central role in hormone receptor–positive breast cancer, where its activation can trigger gene transcription that promotes tumor cell growth and proliferation. In addition, HER2 is a key oncoprotein involved in highly aggressive proliferative signaling pathways, and its overexpression is associated with poor prognosis and a high rate of recurrence. Epidermal growth factor receptor (EGFR) also contributes to the regulation of signaling pathways such as MAPK and PI3K/AKT, which are implicated in enhanced proliferation, migration, and resistance to apoptosis in cancer cells (Loibl, *et al*., 2021). Based on this rationale, the present study was designed to identify potential anti-breast cancer compounds derived from clove leaf waste (*Syzygium aromaticum*) using a molecular docking approach. This research aims to evaluate the interaction profiles of bioactive compounds from clove leaves with key breast cancer-related proteins and to predict their potential as anticancer agents. Ultimately, the results of this study are expected to support the development of natural product–based anticancer therapies while also encouraging the valorization of agricultural waste into high-value pharmaceutical resources.

MATERIALS AND METHODS

Materials

Materials used study is dried clove leaf waste collected from clove plantations in Negeri Lima Village, Indonesia. Bioactive compounds from clove leaf waste were obtained through extraction and isolation procedures, followed by compound identification using GC-MS, a widely applied analytical technique for profiling volatile and semi-volatile phytochemicals (El-Saber Batiha, *et al.*, 2020). Chemical compound information and ligand structures were retrieved from the PubChem database, while the three-dimensional structures of breast cancer target proteins ERα and HER2 were obtained from the Protein Data Bank (PDB), a comprehensive repository for biomolecular structures (Kim, *et al*., 2023; Burley, *et al*., 2023).

Supporting *in silico* materials included protein structure files in PDB format and ligand structure files in SDF or MOL2 formats. Molecular docking analyses were performed using AutoDock Tools and AutoDock Vina integrated within PyRx software, which enables efficient virtual screening and binding affinity prediction (Trott & Olson, 2010; Dallakyan & Olson, 2015). BIOVIA Discovery Studio was employed for protein and ligand preparation as well as interaction analysis, PyMOL was used for three-dimensional visualization of docking results, ChemDraw was utilized for chemical structure depiction, and Open Babel was applied for file format conversion and ligand structure optimization (O'Boyle, *et al*., 2011).

Methods

Preparation of Clove Leaf Waste

Clove leaf waste was collected, thoroughly washed to remove impurities, and oven-dried at low temperature to preserve thermolabile compounds. The dried material was subsequently ground into a fine powder and extracted using n-hexane. The extract was then filtered and concentrated under reduced pressure using a rotary evaporator. The extract was injected into 0.5 µL syringe of GC-MS QP2010S SHIMADZU, the temperature of the column was set to 120-310°C with a 10°C increase per minute. GC–MS analysis was performed on the concentrated extract, with n-hexane serving as the extraction solvent; consequently, only compounds exhibiting solubility in n-hexane along with sufficient volatility and thermal stability were detected. The GC-MS results revealed several bioactive constituents, including eugenol, β-caryophyllene, α-humulene, and caryophyllene oxide (Wael, *et al*., 2018), which have been widely reported to possess potential anticancer activities (Kiralan *et al*., 2022; Fidyt, *et al*., 2016).

Collection of Compound and Protein Data

The three-dimensional structures and SMILES notation of clove leaf waste compounds were downloaded from database of Pubchem. The identified compounds included eugenol (CID: 3314), β-caryophyllene (CID: 5281515), α-humulene (CID: 5281520), and caryophyllene oxide (CID: 1742210). Commercial drugs used as reference compounds were tamoxifen (CID: 2733526) and neratinib (CID: 9915743), which are clinically used homone receptor positive and HER2-positive breast cancer therapy, respectively (Waks & Winer, 2019). All ligand structures were energy-minimized and optimized using Open Babel integrated within PyRx software with the MMFF94 force field, followed by conversion into PDBQT format. The protein targets selected for this study were ERα and HER2. The crystal structures of ERα (PDB ID: 6CHZ) and HER2 (PDB ID: 7PCD) were retrieved from the PDB. These structures were selected based on the availability of high-resolution crystallographic data. Docking analysis was conducted only on HER2 and ERα. The co-crystallized ligands (4-OHT and lapatinib) were used exclusively for docking validation, not as part of the docking set. Protein preparation, including removal of water molecules and non-essential co-crystallized

ligands, was performed using PyMOL, followed by conversion into PDBQT format using PyRx, in accordance with standard molecular docking protocols (Pinzi & Rastelli, 2019).

Molecular Docking Analysis

The binding activity between clove leaf waste compounds and the reference drugs against the selected target proteins was evaluated through molecular docking simulations. Docking analyses were performed using the VinaWizard plugin within PyRx version 1.1, which is based on the AutoDock Vina algorithm. Binding affinity values (kcal/mol) were calculated and used to predict the strength and stability of ligand–protein interactions. The docking grid box was defined using the following coordinates: center_x = 6.20, center_y =−7.58, and center_z = −10.76, with grid dimensions of size_x = 14.90, size_y = 19.78, and size_z = 19.69 (Trott & Olson, 2010).

Ligand-Protein Interaction Analysis

The interactions between clove leaf waste compounds, commercial drugs, and target proteins obtained from docking simulations were further examined using BIOVIA Discovery Studio. This step aimed to explore the involvement of specific amino acid residues in ligand binding and to describe the types of molecular interactions at the active sites, including hydrogen bonds, hydrophobic forces, and other non-covalent interactions that are important for biological function (Meng, *et al*., 2011).

RESULTS

Profile of Bioactive Compounds in Clove Leaf Waste

Results og GC-MS clove leaf waste contains a diverse array of secondary metabolite, with eugenol, β-caryophyllene, α-humulene, and caryophyllene oxide identified as the predominant constituents. Based on the chromatographic profile, eugenol exhibited the highest relative peak area, followed sequentially by β-caryophyllene, α-humulene, and caryophyllene oxide. In addition to these major compounds, several other monoterpenoid and sesquiterpenoid derivatives were detected at lower abundances. The dominance of eugenol in clove leaf waste is consistent with previous studies which reported that eugenol remains the main bioactive component in various vegetative parts of Syzigium aromaticum (Wael, *et al*, 2018) including leaves, stems, and agricultural residues ((Jiang, *et al*., 2022; Waks, *et al*., 2019). These findings indicate that clove leaf waste retains a high content of pharmacologically relevant compounds despite being underutilized, highlighting its significant potential as a renewable source of natural anticancer agents.

GC–MS findings confirm that clove leaf waste represents a valuable and underexplored source of bioactive secondary metabolites with promising anticancer potential. The predominance of eugenol and biologically active sesquiterpenes supports the feasibility of utilizing clove leaf waste as a raw material for the development of natural chemopreventive agents or complementary therapies for breast cancer. These results underscore the importance of further investigations using integrated *in silico* and *in vivo* approaches to comprehensively evaluate the pharmacological efficacy, molecular mechanisms, and safety profiles of clove leaf–derived compound.

Ligand-Protein Interactions of Bioactive Compounds from Clove Leaf Waste

Docking simulations were employed to evaluate the interaction potential of bioactive compounds obtained from clove (*Syzygium aromaticum*) leaf waste with HER2 and ERα, two key molecular targets in breast cancer (Loibl, *et al*., 2021; Moasser, 2023). Docking was conducted at the active sites defined based on ligand-bound crystallographic structures obtained from the Protein Data Bank, particularly within the HER2 kinase domain

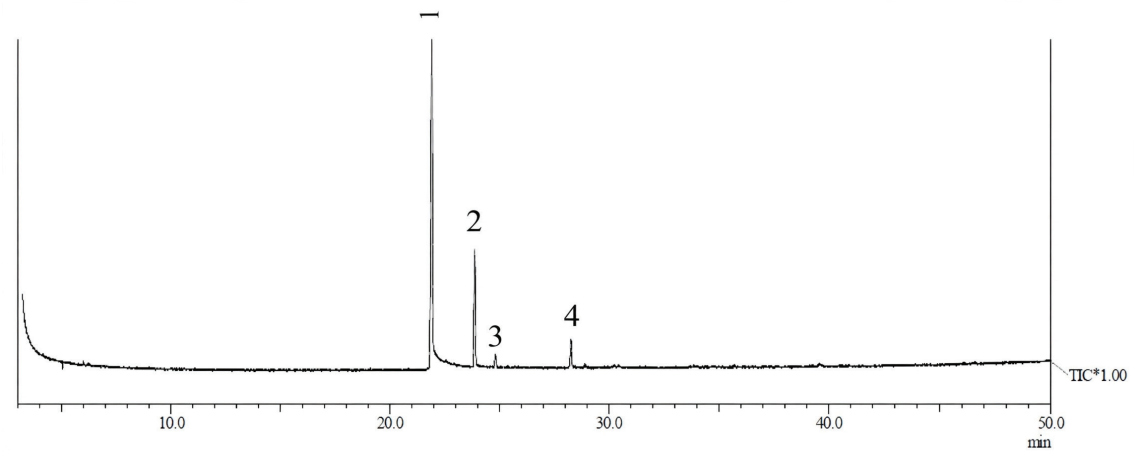


Figure 1. GC-MS chromatographic profile of clove leaf waste compounds.

Note: 1 eugenol, 2 ß-caryophillene, 3 α-humulene, 4 caryophyllene oxide

and the ligand-binding domain of ERα (Fuentes & Silveyra, 2019; Hanker, *et al*., 2020). The docking protocol was validated by redocking the native ligands into their respective binding sites to ensure accurate reproduction of binding poses, confirming the reliability of the docking parameters (Pagadala, *et al*., 2017; Pinzi & Rastelli, 2019). Following validation, eugenol, β-caryophyllene, α-humulene, and caryophyllene oxide were docked into HER2 and ERα. The interactions were analyzed based on binding affinity and key intermolecular interactions, including hydrogen bonding and hydrophobic contacts, to evaluate their potential inhibitory activity. Ligand structures were prepared from SMILES data retrieved from the PubChem database and optimized prior to docking.

Table 1. SMILES notation of bioactive compounds from clove leaf waste and commercial drugs.

No	Compouds	Pubchem ID	SMILES
1	Eugenol	3314	COC1=C(C=CC(=C1)CC=C)O
2	β-caryophyllene	5281515	C/C/1=C\CCC(=C)[C@H]2CC([C@@H]2CC1)(C)C
3	α-humulene	5281520	C/C/1=C\CC(/C=C/C/C(=C/CC1)/C)(C)C
4	Cariofilen okside	1742210	C[C@@]12CC[C@@H]3[C@H](CC3(C)C)C(=C)CC[C@H]1O2
5	Tamoxifen	2733526	CC/C(=C(\C1=CC=CC=C1)/C2=CC=C(C=C2)OCCN(C)C)/C3=CC=CC=C3
6	Neratinib	9915743	CCOC1=C(C=C2C(=C1)N=CC(=C2NC3=CC(=C(C=C3)OCC4=CC=CC=N4)Cl)C#N)NC(=O)/C=C/CN(C)C

The SMILES notations of the tested compounds and commercial drugs were retrieved from the PubChem database and are presented in Table 1. SMILES notation was employed to ensure accurate two-dimensional chemical structure representation, which could subsequently be converted into three-dimensional models for geometry optimization and molecular docking simulations. In this study, the test compounds derived from clove leaf waste included eugenol, β-caryophyllene, α-humulene, and caryophyllene oxide, while the reference drugs used for comparison were tamoxifen and neratinib. These two drugs were selected because they are clinically used in breast cancer management, although they target different molecular pathways. Tamoxifen is a selective estrogen receptor modulator (SERM) that primarily binds to ERα, whereas neratinib

Table 2. Molecular docking simulation results of clove leaf waste compounds and commercial drugs against Human Epidermal Growth Factor Receptor 2 (HER2).

Compounds	Binding Affinity Score Kcal/mol
Neratinib	-4.3
Tamoxifen	-5.7
β-caryophyllene	-7.3
α-humulene	-7.3
Caryophyllene oxide	-6.7
Eugenol	-5.9

is an irreversible tyrosine kinase inhibitor targeting HER2. The inclusion of these commercial drugs provides a relevant benchmark for evaluating the binding potential of natural compounds toward key molecular pathways involved in breast cancer progression (Burstein, 2020; Modi, *et al*., 2021).

Docking results demonstrated that both the test compounds and commercial drugs were capable of interacting with the target proteins ERα and HER2. The successful formation of a stable complex between tamoxifen and ERα further validated the docking protocol, as this interaction is consistent with the drug's established clinical mechanism of action. In our study, the assessment of binding stability was primarily based on docking scores (binding affinity) and the consistency of key intermolecular interactions, including hydrogen bonds and hydrophobic contacts within the active site. While these parameters are widely used in molecular docking studies, we acknowledge that they provide only a preliminary estimation of binding potential and do not fully represent the dynamic stability of the ligand–receptor complex under physiological conditions. Consistent with its known mechanism of action, neratinib demonstrated high binding affinity toward HER2, supporting its role as an irreversible tyrosine kinase inhibitor targeting HER2 signaling pathways (Guo, *et al*., 2023). Notably, compounds derived from clove leaf waste, exhibited comparable and, in some cases, more favorable binding energies than the reference drug. In addition to binding affinity, these compounds also maintained key interactions with critical amino acid residues within the binding pocket, suggesting a potentially stable binding mode.

The docking results showed that several compounds from clove leaf waste exhibited favorable binding affinities and important intermolecular interactions with HER2 and ERα, particularly hydrophobic interactions and hydrogen bonding at the active sites. These findings suggest the potential of the identified compounds as candidate inhibitors of breast cancer–related targets and warrant further experimental validation. The docking simulation results against HER2 are presented in the following table.

The results of molecular docking simulation showed that the control ligands, neratinib and tamoxifen, exhibited binding energies of -4.3 kcal/mol and -5.7 kcal/mol, respectively. These compounds were used as positive controls because they have been experimentally proven to bind specifically to their respective molecular targets, namely the HER2 receptor (neratinib) and the ERα (tamoxifen). In this study, tamoxifen was selected as a reference ligand due to its widespread clinical use and its frequent application as a standard comparator in molecular docking studies targeting ERα. This choice was intended to maintain consistency with previous *in silico* studies and to provide a clinically relevant benchmark. 4-OHT may better represent the active binding interaction at the molecular level. Future studies will consider including 4-OHT to provide a more accurate representation of ERα binding interactions.

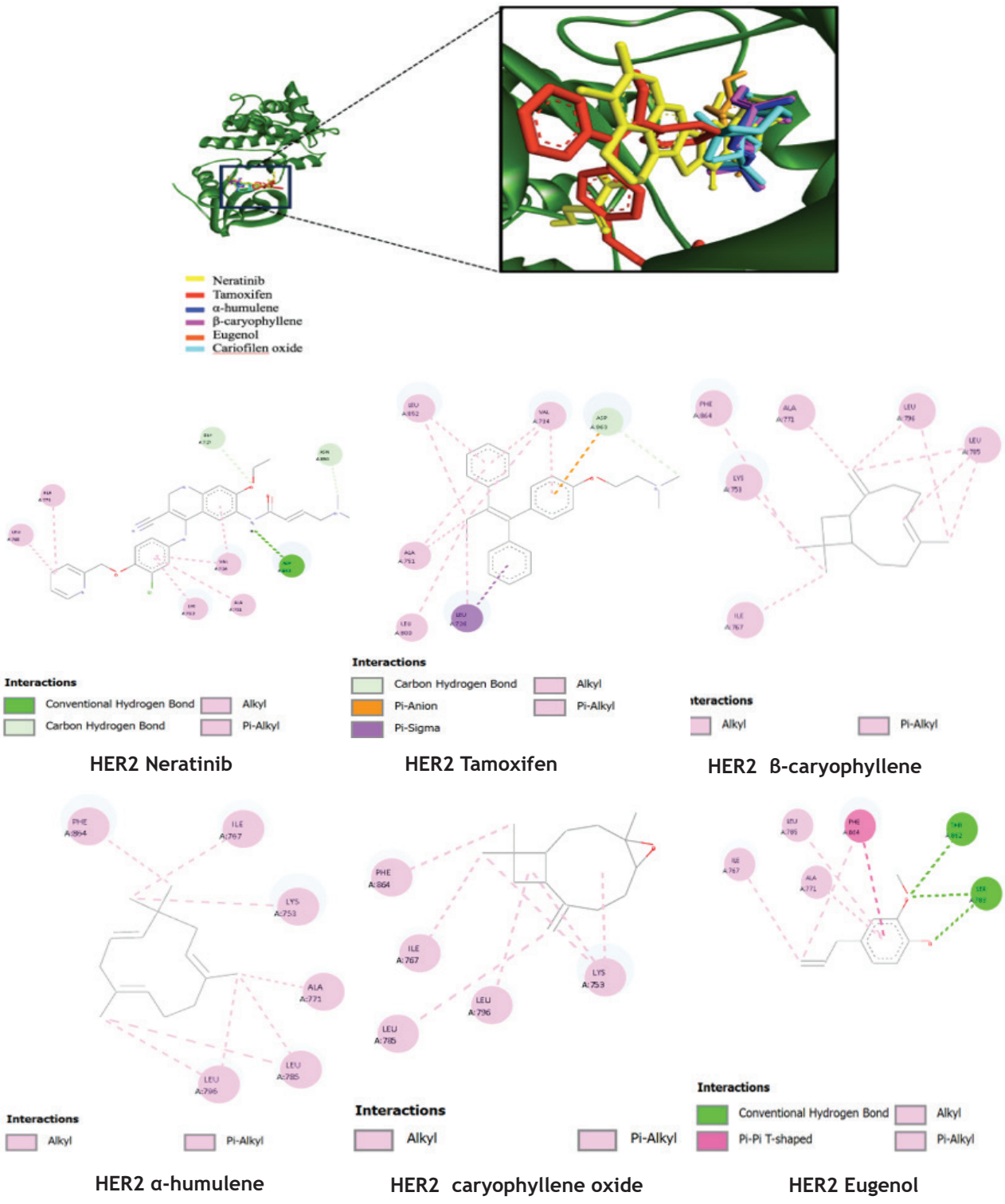


Figure 2. Molecular interactions between bioactive compounds from clove leaf waste and commercial drugs with the HER2 protein.

Therefore, they were employed as reference compounds to evaluate the binding affinity and potential of the tested ligands toward the corresponding target receptors. (Guo, *et al*., 2023; Modi, *et al*., 2021). Neratinib and tamoxifen were used as positive controls ligands due to their well-established specificity toward their respective molecular targets, namely HER2 and ERα. These compounds were selected to validate the docking protocol and to provide reliable reference points for evaluating the binding affinity of the tested compounds against each receptor. Importantly, each control ligand was used exclusively for its corresponding target, with neratinib applied in HER2 docking simulations and tamoxifen in ERα docking simulations. Although previous studies have reported crosstalk between ERα and HER2 signaling pathways in breast cancer, this interaction was considered only as biological background information and did not serve as a basis for cross-target docking in this study. Therefore, no docking of tamoxifen against HER2 or neratinib against ERα was performed. This approach ensures a target-specific evaluation and avoids potential misinterpretation of ligand–receptor interactions (Harbeck, *et al*., 2019). Visualization of the interactions between clove leaf waste compounds, commercial drugs, ligand HER2 and protein is presented in Figure 2. Visualization of the ligand–HER2 protein interactions for clove leaf waste compounds is presented in the Figure 2.

The interactions between clove leaf waste–derived compounds and control drugs with the HER2 protein indicate that all ligands were able to recognize and bind to key residues within the active site. This is supported by the alignment of ligand binding poses with the co-crystallized ligand in the Protein Data Bank (PDB) structure, confirming that docking occurred within the biologically relevant binding pocket. Both the tested compounds and control drugs predominantly interacted within the tyrosine kinase domain of HER2, a critical region responsible for phosphorylation and downstream signaling involved in cancer cell proliferation and survival. Binding within this domain suggests a potential mechanism for inhibiting HER2 activity and disrupting oncogenic signaling pathways (Ariazi, *et al*., 2006; Modi, *et al*., 2021).

The observed ligand–receptor interactions, including hydrogen bonding, hydrophobic contacts, and electrostatic interactions, contribute to the stability of the complexes. The clove-derived compounds exhibited interaction patterns comparable to those of the control drugs, with involvement of conserved active-site residues, supporting their potential biological relevance (Waks, *et al*., 2019). Overall, the combination of favorable binding energies, consistent binding poses, and engagement with key residues indicates that these compounds may serve as promising candidates for HER2 inhibition. However, further validation through *in vitro* and *in vivo* studies is required to confirm their therapeutic potential.

The molecular docking analysis revealed variations in binding energy values between the test compounds derived from clove leaf waste and commercial drugs toward the target protein. Tamoxifen, which was used as a commercial

Table 3. Molecular docking results of bioactive compounds from clove leaf waste and commercial drugs targeting ERα.

Compounds	Binding Affinity Score Kcal/mol
Tamoxifen	-9.1
α-humulene	-7.9
Cariofilen oxide	-7.8
β-caryophyllene	-7.7
Neratinib	-7.1
Eugenol	-5.6

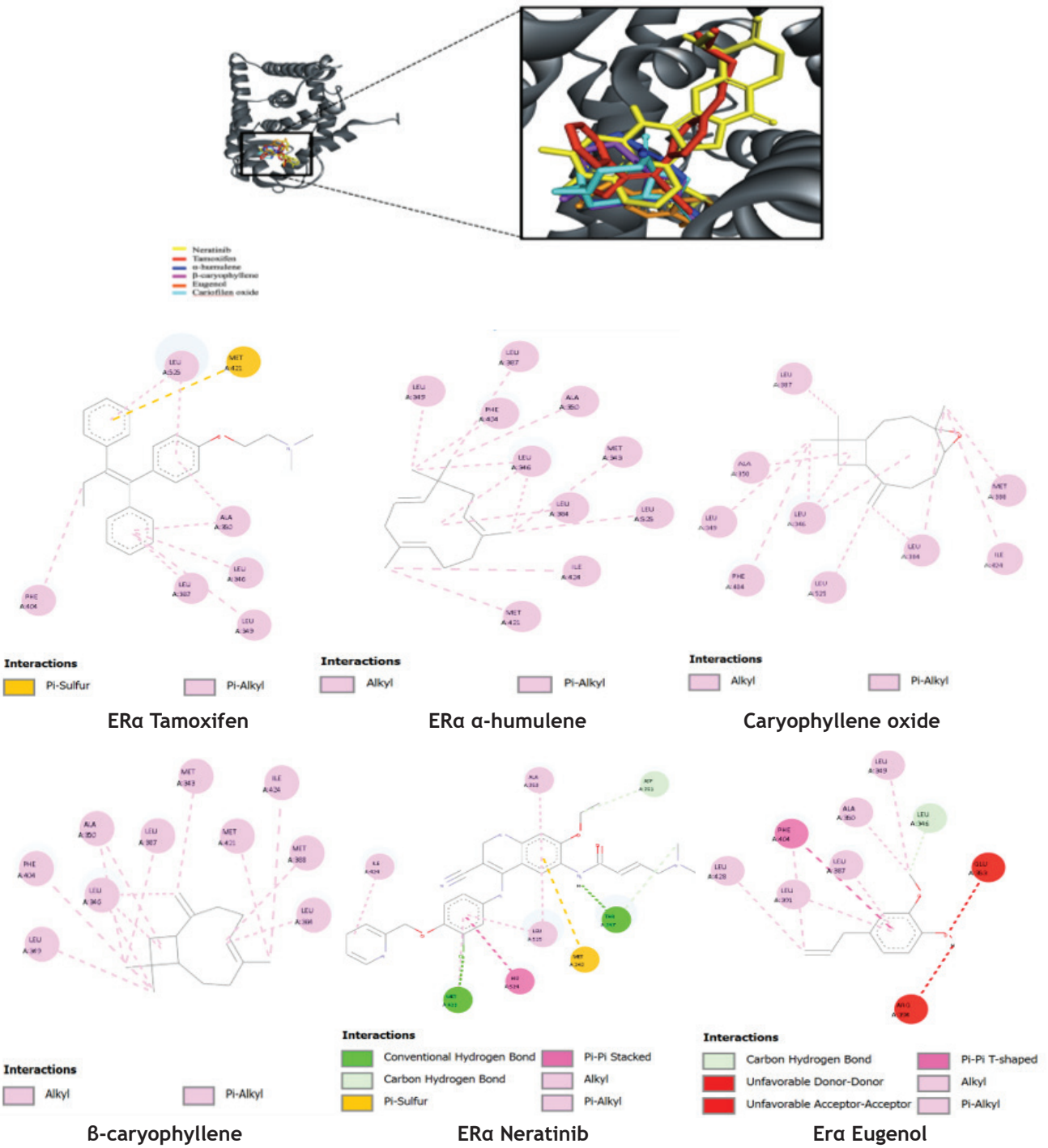


Figure 3. Interactions of compounds derived from clove leaf waste and commercial drugs with ERα.

control drug due to its established clinical application in estrogen receptor alpha (ERα) positive breast cancer therapy, exhibited the most negative binding energy value (-9.1 kcal/mol). This result indicates a very high binding affinity toward the ERα active site, which is consistent with its mechanism of action as a selective SERM. Strong binding affinity suggests that tamoxifen is capable of forming a stable complex with the target protein, thereby effectively inhibiting receptor function involved in breast cancer cell proliferation (Duan, 2022; Dallakyan, *et al*., 2015). The interaction of ERα with clove leaf waste–derived compounds is illustrated in Figure 3.

The Molecular docking results indicate that clove leaf waste–derived compounds and the control ligand effectively bind within the ERα ligand-binding pocket, as evidenced by the alignment of their docking poses with the co-crystallized ligand. This confirms that ligand binding occurs at a biologically relevant site and suggests a potential to interfere with endogenous ligand recognition and ERα-mediated transcriptional activity (Nilsson & Gustafsson, 2011; Hanker, *et al*., 2020). Ligand–receptor interactions were predominantly governed by hydrogen bonding, hydrophobic contacts, and electrostatic forces, which collectively determine binding affinity and stability. Hydrogen bonds contribute to binding specificity, whereas hydrophobic interactions play a dominant role within the largely nonpolar ERα binding cavity (Kavarthapu, *et al*., 2021). The interaction profile of tamoxifen is consistent with its established antagonistic activity, and several clove-derived compounds exhibited comparable interaction patterns, albeit with variations in binding energy. This similarity suggests that these compounds may partially emulate the molecular behavior of antiestrogenic agents (Jordan, 2021). The preferential localization of ligands within the ERα active site further supports the possibility of competitive binding with endogenous estrogen, which may lead to inhibition of estrogen-dependent signaling pathways implicated in breast cancer progression (Ariazi, *et al*., 2006). Overall, the observed binding characteristics provide preliminary *in silico* evidence of potential antiestrogenic activity; however, experimental validation is required to confirm these findings.

DISCUSSION

Profile of Bioactive Compounds in Clove Leaf Waste

The GC–MS analysis revealed that clove leaf waste contains a diverse profile of secondary metabolites, with eugenol identified as the predominant compound, followed by several sesquiterpenoids, including β-caryophyllene, α-humulene, and caryophyllene oxide. The most intense chromatographic peak corresponded to eugenol, confirming its relatively high abundance in the n-hexane extract of clove leaf waste. The presence of these bioactive constituents provides a rational basis for investigating the pharmacological potential of clove leaf waste, particularly its possible anti-breast cancer activity.

Eugenol is a phenylpropanoid compound that has been extensively investigated for its biological and pharmacological activities, including anticancer effects. Previous studies have reported that eugenol may exert anticancer activity through several mechanisms, including induction of apoptosis, cell-cycle arrest, inhibition of tumor cell proliferation, modulation of oxidative stress, and suppression of signaling pathways associated with cancer progression (Zang, *et al*., 2021; Shi, *et al*., 2026). In the context of breast cancer, these mechanisms are particularly relevant because dysregulation of ER signaling and HER2-associated pathways contributes substantially to tumor development and progression. Eugenol has also been reported to interfere with signaling mechanisms associated with estrogen receptors and HER2, suggesting that this compound may have relevance to molecular targets involved in breast cancer (Begum, *et al*., 2022).

In addition to eugenol, β-caryophyllene and α-humulene were detected as major sesquiterpenoid constituents. These compounds have attracted considerable attention because of their reported anticancer, anti-inflammatory, and chemosensitizing properties. β-Caryophyllene, a natural bicyclic sesquiterpene, has been reported to modulate cannabinoid receptor 2 (CB2) and influence signaling pathways such as PI3K/Akt and MAPK, thereby potentially increasing cancer cell sensitivity to conventional chemotherapeutic agents (Fidyt, *et al*., 2016; Saini, *et al*., 2024). Similarly, α-humulene has demonstrated antiproliferative activity in several cancer models, including effects associated with mitochondrial-mediated apoptosis and inhibition of angiogenic processes (Eghtedari, *et al*., 2021). Caryophyllene oxide, an oxygenated derivative of β-caryophyllene, has also demonstrated cytotoxic activity against several cancer cell types and has been suggested to act through mechanisms involving oxidative stress and disruption of cellular processes (Nahar, *et al*., 2022). Therefore, the simultaneous presence of these compounds suggests that the biological activity of clove leaf waste may not be attributable to a single constituent but may instead result from the combined contribution of several secondary metabolites.

The potential contribution of multiple compounds is consistent with the concept of phytochemical synergy, in which the biological effects of plant-derived extracts may arise from interactions among several constituents rather than from the activity of the most abundant compound alone (Caesar & Cech, 2019; Hemaiswarya, *et al*., 2013). Such interactions may involve simultaneous modulation of several molecular pathways associated with breast cancer, including ER signaling, HER2-mediated signaling, PI3K/Akt and MAPK pathways, oxidative stress, and apoptosis. Although the present study did not experimentally evaluate synergistic effects, the diverse chemical profile identified by GC–MS provides an important basis for further investigation of the collective pharmacological potential of clove leaf waste.

The identification of these compounds through GC–MS subsequently provided a rational basis for ligand selection in the molecular docking analysis. Eugenol, β-caryophyllene, α-humulene, and caryophyllene oxide were selected as representative ligands because of their relatively prominent occurrence in the GC–MS profile and their previously reported biological activities. ERα and HER2 were selected as molecular targets because both proteins play central roles in breast cancer biology and represent clinically relevant therapeutic targets, particularly in hormone receptor-positive and HER2-positive breast cancer, respectively (Loibl, *et al*., 2021; Waks & Winer, 2019). Molecular docking was therefore employed as a preliminary computational approach to evaluate the predicted binding affinity and interaction patterns of the identified compounds with these breast cancer-related proteins.

Interaction of Clove Leaf Waste Compounds with HER2

The molecular docking analysis against HER2 demonstrated favorable predicted binding affinities for all four clove leaf waste-derived compounds. α-Humulene and β-caryophyllene exhibited the lowest predicted binding energies, both at −7.3 kcal/mol, followed by caryophyllene oxide at −6.7 kcal/mol and eugenol at −5.9 kcal/mol. Under the same docking conditions, tamoxifen and neratinib exhibited binding energies of −5.7 and −4.3 kcal/mol, respectively. Thus, α-humulene, β-caryophyllene, caryophyllene oxide, and eugenol showed more negative predicted binding energies than the reference compounds in this particular docking simulation.

The favorable docking scores of α-humulene and β-caryophyllene may be related to their highly hydrophobic structures. As nonpolar sesquiterpenes, these compounds are capable of establishing extensive hydrophobic contacts with nonpolar regions of the HER2 binding pocket. Hydrophobic

interactions can contribute substantially to ligand recognition and binding by promoting favorable packing between the ligand and hydrophobic residues within the binding site (Pattil, *et al*., 2010). Van der Waals interactions may further contribute to the predicted binding affinity by reinforcing short-range non-covalent contacts. In contrast, caryophyllene oxide contains an epoxide functional group that introduces additional polarity into its otherwise hydrophobic structure, potentially allowing a combination of hydrophobic and polar interactions within the binding pocket. Eugenol, which contains hydroxyl and methoxy functional groups, may similarly establish polar interactions in addition to hydrophobic contacts, although its smaller molecular framework may limit the extent of hydrophobic contact compared with the larger sesquiterpenoids (Peluso, *et al*., 2022).

The predominance of hydrophobic interactions observed for α-humulene and β-caryophyllene is consistent with their chemical structures and may explain their relatively favorable docking scores (Takur, *et al*., 2025). However, docking affinity should not be interpreted solely on the basis of hydrophobic interactions or as direct evidence of biological inhibition. Binding energy represents a computational estimate under a defined set of simulation parameters and does not fully account for protein flexibility, solvent effects, entropic contributions, or the dynamic behavior of ligand–protein complexes (Du, *et al*., 2016). Therefore, the more negative docking scores observed for the natural compounds indicate favorable predicted interactions but do not establish that these compounds are biologically more effective HER2 inhibitors than clinically used drugs.

The predicted interactions nevertheless provide an important preliminary indication that clove leaf waste-derived compounds can occupy the HER2 binding region and may potentially interfere with receptor function. The molecular interaction patterns observed in the docking analysis are illustrated in Figure 2. These findings support further investigation of the identified compounds using complementary computational approaches, such as molecular dynamics simulations and binding free-energy calculations, followed by experimental validation.

Interaction of Clove Leaf Waste Compounds with ERα

The docking analysis against ERα revealed a similarly favorable interaction profile for several compounds derived from clove leaf waste. Among the natural compounds evaluated, α-humulene exhibited the most negative predicted binding energy at −7.9 kcal/mol, followed by caryophyllene oxide at −7.8 kcal/mol and β-caryophyllene at −7.7 kcal/mol. Eugenol exhibited a less negative predicted binding energy of approximately −5.6 kcal/mol. Tamoxifen showed the most negative binding energy among the tested ligands at −9.1 kcal/mol, whereas neratinib exhibited a predicted binding energy of −7.1 kcal/mol. These results indicate that α-humulene, caryophyllene oxide, and β-caryophyllene demonstrated relatively favorable predicted interactions with ERα, although their docking scores remained less favorable than that of tamoxifen.

The favorable interaction of α-humulene with ERα may be associated with its lipophilic and nonpolar structure, which facilitates hydrophobic contacts within the predominantly hydrophobic ligand-binding pocket of the receptor. β-Caryophyllene showed a similar pattern, with its extensive hydrocarbon framework providing a structural basis for hydrophobic interactions and van der Waals contacts. Caryophyllene oxide also showed a favorable docking score, potentially because the epoxide group provides an additional polar feature that may contribute to ligand orientation and interaction within the binding site. These findings are consistent with previous reports describing the biological activities of sesquit-

erpenoids and support the possibility that these compounds may contribute to the pharmacological potential of clove leaf waste (Fidyt, *et al*., 2016; Saini, *et al*., 2024).

In contrast, eugenol exhibited a relatively less favorable docking score against ERα. This result may be related to its smaller molecular structure and greater polarity compared with the hydrophobic sesquiterpenoids. However, the relatively weaker predicted binding affinity does not exclude eugenol from contributing to the biological activity of clove leaf waste. Its anticancer effects have been associated with several mechanisms beyond direct receptor binding, including induction of apoptosis, modulation of oxidative stress, and inhibition of cancer cell proliferation (Begum, *et al*., 2022). Consequently, molecular docking results should be interpreted as one component of the overall assessment of biological potential rather than as a definitive measure of pharmacological efficacy.

Interestingly, neratinib, which is primarily recognized as a HER2-targeted therapeutic agent, also demonstrated a predicted interaction with ERα in the present docking simulation, with a binding energy of −7.1 kcal/mol. This observation emphasizes that docking simulations can identify potential interactions between compounds and molecular targets outside their primary pharmacological targets. However, the predicted interaction should not be interpreted as evidence that neratinib has established therapeutic activity against ERα. Instead, the result reflects the ability of the molecule to occupy the computationally defined binding site under the conditions applied in this study.

Binding-Site and Interaction Analysis

Visualization of the docking poses showed that the clove leaf waste-derived compounds and reference ligands were positioned within the ERα binding pocket, indicating that the docking protocol identified a biologically relevant region of the receptor. The location of the ligands within the binding site provides additional structural information to complement the calculated docking scores (Friesner, *et al*., 2026). The ligand–receptor interactions involved several types of non-covalent forces, including hydrophobic interactions, hydrogen bonding, van der Waals forces, and, where structurally applicable, π-related interactions.

The interaction patterns varied according to the chemical characteristics of each ligand. The nonpolar sesquiterpenoids, particularly α-humulene and β-caryophyllene, predominantly relied on hydrophobic contacts and van der Waals interactions (Banwo, *et al*., 2026). These interactions are consistent with the largely hydrophobic character of the ERα ligand-binding pocket and may facilitate favorable ligand accommodation. Caryophyllene oxide, owing to the presence of its epoxide group, has the potential to establish additional polar interactions, whereas eugenol contains hydroxyl and methoxy groups that can participate in hydrogen bonding and other polar interactions. Such differences in interaction profiles may contribute to variations in the predicted docking energies among the tested compounds (Wahman, *et al*., 2025).

The reference compounds also displayed interactions within the target binding sites, providing a useful basis for comparative interpretation. Tamoxifen showed a particularly favorable interaction with ERα, consistent with its established role as an ER-targeting therapeutic agent. The similarity in the general binding location of several natural compounds and reference ligands suggests that the identified compounds may interact with functionally relevant regions of the receptor. Nevertheless, similarity in docking pose or binding location does not necessarily imply an identical mechanism of action. Experimental studies are required to determine whether these interactions translate into receptor antagonism, agonism, modulation, or no meaningful biological effect.

Overall, the molecular docking results indicate that several compounds present in clove leaf waste, particularly α-humulene, β-caryophyllene, and caryophyllene oxide, possess favorable predicted interactions with both HER2 and ERα. The results are particularly noteworthy because these two proteins represent important molecular targets in distinct but interconnected breast cancer signaling pathways. The ability of individual compounds to interact with both targets raises the possibility of multitarget activity; however, this interpretation remains hypothetical and requires experimental confirmation.

Biological Implications and Study Limitations

Taken together, the GC–MS and molecular docking findings provide complementary evidence for the potential value of clove leaf waste as a source of bioactive compounds. The GC–MS analysis demonstrated the presence of several compounds with previously reported biological activities, while the docking analysis provided preliminary structural evidence of their potential interactions with HER2 and ERα. Among the tested compounds, α-humulene and β-caryophyllene showed particularly favorable predicted interactions with HER2, whereas α-humulene, caryophyllene oxide, and β-caryophyllene exhibited favorable interactions with ERα. These findings suggest that sesquiterpenoid constituents may make an important contribution to the potential anti-breast cancer activity of clove leaf waste.

Nevertheless, the docking results should be interpreted cautiously. A more negative docking energy indicates a more favorable predicted ligand–protein interaction within the computational model, but it does not directly demonstrate higher biological potency, receptor inhibition, pharmacological efficacy, or therapeutic superiority over approved drugs. Molecular docking also does not fully capture protein flexibility, solvent effects, entropic contributions, ligand dynamics, or the complex biochemical environment of living cells. Therefore, the present findings should be considered preliminary computational evidence rather than confirmation of anti-breast cancer activity.

Further studies are required to validate these predictions through molecular dynamics simulations, binding free-energy calculations such as MM-PBSA or MM-GBSA, and experimental assays. *In vitro* studies using appropriate breast cancer cell lines are necessary to determine cytotoxicity, antiproliferative activity, receptor modulation, and selectivity, whereas *in vivo* studies are needed to evaluate efficacy, toxicity, pharmacokinetic characteristics, and systemic responses. Such validation is particularly important because pharmacological activity depends not only on ligand–target binding but also on absorption, distribution, metabolism, excretion, bioavailability, and toxicity (Kavarthapu, *et al*., 202; Waks, *et al*., 2019).

Despite these limitations, the integration of GC–MS profiling and molecular docking provides a rational preliminary framework for identifying bioactive compounds from clove leaf waste. The findings highlight that an agricultural by-product containing eugenol and several biologically active sesquiterpenoids may represent a valuable source for further anti-breast cancer research. More importantly, the present results provide a scientific basis for advancing the investigation from computational screening toward *in vitro* and *in vivo* validation, while supporting the broader potential of clove leaf waste as a sustainable source of natural bioactive compounds.

CONCLUSION

This study successfully applied a molecular docking approach to screen potential anti-breast cancer compounds derived from clove (*Syzigium aromaticum*) leaf waste by targeting two key molecular drivers of breast cancer, namely HER2 and ERα. The docking simulations demonstrated that all tested bioactive compounds

eugenol, α-humulene, β-caryophyllene, and caryophyllene oxide were able to recognize and bind within the active sites of both target proteins, indicating specific and biologically relevant interactions rather than nonspecific surface binding.

The results revealed that several terpenoid compounds derived from clove leaf waste exhibited binding affinities comparable to or more favorable than the reference drugs, Tamoxifen and Neratinib, under identical docking conditions. Among these, α-humulene consistently demonstrated the most favorable binding profile across both HER2 and ERα targets, indicating its potential as the most promising lead compound for further development.

In the HER2 system, α-humulene demonstrated lower binding energy compared to the other tested compounds, including neratinib, and was positioned within the active site, indicating its potential to interact with key residues involved in receptor activity. Similarly, in the ERα system, α-humulene, along with β-caryophyllene and caryophyllene oxide, occupied the ligand-binding pocket and showed favorable interaction profiles, although tamoxifen exhibited stronger binding affinity, consistent with its established clinical role. Overall, these findings suggest that α-humulene may represent a promising compound based on its consistent interaction profile across multiple targets. However, it should be noted that these results are limited to computational predictions of binding affinity and interaction patterns. Further validation using dynamic simulations and experimental approaches is required to assess binding stability and biological activity.

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REFERENCES

Ariazi, E.A., Ariazi, J.L., Cordera, F., and Jordan, V.C., 2006, Estrogen receptors as therapeutic targets in breast cancer, *Current topics in medicinal chemistry*, **6**(3), 181-202.

Begum, S.N., Ray, A.S., and Rahaman, C.H., 2022, A comprehensive and systematic review on potential anticancer activities of eugenol: From pre-clinical evidence to molecular mechanisms of action, *Phytomedicine*, **107**, 154456.

Burley, S.K., Bhikadiya, C., Bi, C., Bittrich, S., Chen, L., Crichlow G.V., *et al.*, 2023, RCSB Protein Data Bank: Powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences, *Nucleic Acids Research*, 51(D1), D488–D497.

Banwo, O.M., Babarinde, C.O., Ogunlakin, A.D., Olaoluwa, O.O., Badmos, S.T., Mohammed, I., and Guido, F., 2026, Essential oils of *Ficus recurvata* and *Ficus sagittifolia* (Moraceae) as potential BCL-2 inhibitors in cancer therapy, *In Silico Pharmacology*, **14**(1), 95.

Burstein, H.J., 2020, Systemic therapy for estrogen receptor–positive, HER2-negative breast cancer, *New England Journal of Medicine*, **383**(26), 2557–2570.

Caesar, L.K., and Cech, N.B., 2019, Synergy and antagonism in natural product extracts, *Natural Product Reports*, **36**(6), 869–888.

Cheikhyoussef, A., Cheikhyoussef, N., Rahman, A., and Hussein, A.A., 2022, Clove (*Syzygium aromaticum*) phenolics: Extraction, compositions, and biological activities. In Clove (*Syzygium aromaticum*) (pp. 215-233), Academic Press.

Dallakyan, S., and Olson, A.J., 2015, Small-molecule library screening by docking with PyRx, *Methods*

in Molecular Biology, **1263**, 243–250.

Du, X., Li, Y., Xia, Y.L., Ai, S.M., Liang, J., Sang, P., *et al.*, 2016, Insights into protein–ligand interactions: mechanisms, models, and methods, *International journal of molecular sciences*, **17**(2), 144.

Elkhalifa, A.E.O., Al-Shammari, E., Kuddus, M., Adnan, M., Sachidanandan, M., Awadelkareem, A.M., and Ashraf, S.A., 2023, Structure-based multi-targeted molecular docking and dynamic simulation of soybean-derived isoflavone genistin as a potential breast cancer signaling proteins inhibitor, *Life*,**13**(8), 1739.

El-Saber Batiha, G., Alkazmi, L.M., Nadwa, E.H., Rashwan, E.K., and Batiha, G.E.S., 2020, *Syzygium aromaticum* (clove): Chemical composition, pharmacological activities, and health benefits, *Biomolecules*, **10**(2), 202.

Eghtedari, M., Jafari Porzani, S., and Nowruzi, B., 2021, Anticancer potential of natural peptides from terrestrial and marine environments: a review, *Phytochem Lett.*, **42**, 87–103.

Fidyt, K., Fiedorowicz, A., Strządała, L., and Szumny, A., 2016, β-caryophyllene and β-caryophyllene oxide—natural compounds of anticancer and analgesic properties, *Cancer medicine*, **5**(10), 3007-3017.

Fuentes, N., and Silveyra, P., 2019, Estrogen receptor signaling mechanisms, *Advances in Protein Chemistry and Structural Biology*, **116**, 135–170.

Friesner, R.A., Murphy, R.B., Repasky, M.P., Frye, L.L., Greenwood, J.R., Halgren, T.A., *et al.*, 2006, Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein–ligand complexes, *Journal of medicinal chemistry*, **49**(21), 6177-6196.

Guo, L., Shao, W., Zhou, C., Yang, H., Yang, L., Cai, Q., *et al.*, 2023, Neratinib for HER2-positive breast cancer with an overlooked option, *Molecular Medicine*, **29**(1), 134.

Hemaiswarya, S. and Doble, M., 2013, Combination of phenylpropanoids with 5-fluorouracil as anti-cancer agents against human cervical cancer (HeLa) cell line, *Phytomedicine*, **20**(2), 151-158.

Hanker, A.B., Sudhan, D.R., and Arteaga, C.L., 2020, Overcoming endocrine resistance in breast cancer, *Cancer Cell*, **37**(4), 496–513.

Harbeck, N., Penault-Llorca, F., Cortes, J., Gnant, M., Houssami, N., Poortmans, P., *et al.*, 2019, Breast cancer, *Nature Reviews Disease Primers*, **5**, 66.

Jordan, V.C., 2021, The new biology of estrogen-induced apoptosis applied to treat and prevent breast cancer, *Endocrine-Related Cancer*, **28**(3), T1–T12.

Jiang, H., Zhong, S., Schwarz, P., Chen, B., and Rao, J., 2022, Chemical composition of essential oils from leaf and bud of clove and their impact on the antifungal and mycotoxin inhibitory activities of clove oil-in-water nanoemulsions, *Industrial Crops and Products*, **187**, 115479.

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., *et al.*, 2023, PubChem 2023 update, *Nucleic Acids Research*, **51**(D1), D1373–D1380.

Kitchen, D.B., Decornez, H., Furr, J.R., and Bajorath, J., 2023, Docking and scoring in virtual screening for drug discovery: Methods and applications, *Nature Reviews Drug Discovery*, **22**(9), 709–726.

Li, D., Jiang, K., Teng, D., Wu, Z., Li, W., Tang, Y., *et al.*, 2022, Discovery of new estrogen-related receptor α agonists via a combination strategy based on shape screening and ensemble docking, *Journal of Chemical Information and Modeling*, **62**(3), 486-497.

Letlora, M.E., Baharuddin, A.A., Wael, S., Jaber, Z.M., and Rehena, J.F., 2024, Evaluation of tea herbal based ingredients clove leaves (*Syzygium aromaticum*) of Maluku Indonesia, *Rumphius Pattimura Biological Journal*, **6**(2), 078-083.

Loibl, S., Poortmans, P., Morrow, M., Denkert, C., and Curigliano, G., 2021, Breast cancer, *The Lancet*, **397**(10286), 1750–1769.

Meng, X.Y., Zhang, H.X., Mezei, M., and Cui, M.,

2011, Molecular docking: A powerful approach for structure-based drug discovery, *Current Computer-Aided Drug Design*, **7**(2), 146–157.

Moasser, M.M., 2023, The oncogene HER2: Its signaling and targeting in cancer, *Oncogene*, **42**, 1581–1594.

Modi, S., Saura, C., Yamashita, T., Park, Y.H., Kim, S.B., Tamura, K., and Verma, S., 2021, Trastuzumab deruxtecan in previously treated HER2-positive breast cancer, *New England Journal of Medicine*, **384**(12), 1143–1154.

Nahar, L., Al-Groshi, A., Kumar, A., and Sarker, S.D., 2022, Arbutin: occurrence in plants, and its potential as an anticancer agent, *Molecules*, **27**(24), 8786.

Nilsson, S. and Gustafsson, J.Å., 2011, Estrogen receptors: Therapies targeted to receptor subtypes, *Clinical Pharmacology & Therapeutics*, **89**(1), 44–55.

Nusaly, W.N., Watuguly, T.W., Pattiasina, E.B., and Wael, S., 2023, Identification of medicinal plants and benefits in the village of negeri lima, maluku district, *Biopendix: Jurnal Biologi, Pendidikan dan Terapan*, **10**(1), 156-170.

O'Boyle, N.M., Banck, M., James, C.A., Morley, C., Vandermeersch, T., and Hutchison, G.R., 2011, Open Babel: An open chemical toolbox, *Journal of Cheminformatics*, **3**, 33.

Patil, R., Das, S., Stanley, A., Yadav, L., Sudhakar, A., and Varma, A.K., 2010, Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of drug-designing, *PloS one*, **5**(8), e12029.

Peluso, P., and Chankvetadze, B., 2022, Recognition in the domain of molecular chirality: from noncovalent interactions to separation of enantiomers, *Chem. Rev.*, **122**(16), 13235-13400.

Pinzi, L. and Rastelli, G., 2019, Molecular docking: Shifting paradigms in drug discovery, *International Journal of Molecular Sciences*, **20**(18), 4331.

Prashar, A., Locke, I.C., and Evans, C.S., 2006, Cytotoxicity of clove (*Syzygium aromaticum*) oil and its major components to human skin cells, *Cell Proliferation*, **39**(4), 241-248.

Pagadala, N.S., Syed, K., and Tuszynski, J., 2017, Software for molecular docking: A review, B*iophysical Reviews*, **9**(2), 91–102.

Kavarthapu, R., Anbazhagan, R., and Dufau, M.L., 2021, Crosstalk between PRLR and EGFR/HER2 signaling pathways in breast cancer, *Cancers*, **13**(18), 4685.

Kiralan, M. and Ketenoglu, O., 2022, Food applications of clove (*Syzygium aromaticum*) extracts, *In Clove (Syzygium aromaticum)* (pp. 607-617). Academic Press.

Shi, X., Zhang, W., Bao, X., Liu, X., Yang, M., and Yin, C., 2023, Eugenol modulates the NOD1-NF-κB signaling pathway via targeting NF-κB protein in triple-negative breast cancer cells, *Frontiers in Endocrinology*, **14**, 1136067.

Saini, H., Gupta, P.K., Mahapatra, A.K., Rajagopala, S., Tripathi, R., and Nesari, T., 2024, Deciphering the multi-scale mechanism of herbal phytoconstituents in targeting breast cancer: a computational pharmacological perspective, *Scientific reports*, **14**(1), 23795.

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F., 2021, Global cancer statistics 2020, *CA: A Cancer Journal for Clinicians*, **71**(3), 209–249.

Thakur, N., Chitme, H.R., Tripathi, Y.C., and Varshney, V.K., 2025, Multitarget *in Vitro* and *in Silico* Evaluation of Neolitsea Pallens Leaf Essential Oil: GC–MS Profiling, Molecular Docking, and ADMET Analysis, *ChemistrySelect*, **10**(44), e02787.

Trott, O. and Olson, A.J., 2010, AutoDock Vina: Improving the speed and accuracy of docking, *Journal of Computational Chemistry*, **31**(2), 455–461.

Waks, A.G. and Winer, E.P., 2019, Breast cancer treatment: A review, *JAMA*, **321**(3), 288–300.

World Health Organization, 2023, Breast cancer. WHO.

Wael, S., Nuringtyas, T.R., Wijayanti N Astuti P., 2018, Secondary metabolites production in clove (*Syzygium aromaticum*): chemical compounds, *Journal of biological science*. **18**(7), 399-406.

Wahman, R., Mohamed, S., Bayoumi, S., Morsy, R., Shafie, S., Abdelraheem, N., *et al*., 2025, Investigating the potential scabicidal activities of three essential oils: gaining mechanistic insights through in vitro contact bioassay and molecular docking, *BMC Complementary Medicine and Therapies*, **25**(1), 185.

Watuguly, T.W., Leiwakabessy, F., Rehena, J.F., Mose, W., and Wael, S., 2022, Prevention of breast cancer with the breast self-examination method (BSE) in the Negeri Lima village, Leihitu District, Maluku Province, *Bioedupat: Pattimura Journal of Biology and Learning*, **2**(2), 32-36.

Wang, H., 2025, The Role of HER2 in Cancer: Molecular Mechanisms, Clinical Impact, and Advances in Targeted Therapies, Open access peer-reviewed chapter, *Role of HER2 - From Molecular Mechanisms to Targeted Therapies*.

Zhang, Z., Yang, L., Hou, J., Tian, S., and Liu, Y., 2021, Molecular mechanisms underlying the anticancer activities of licorice flavonoids, *Journal of Ethnopharmacology*, **267**, 113635.