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In Silico Study of Bioactive Compounds from *Syzygium malaccense* Targeting HER2 and Progesterone Receptors in Breast Cancer

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Abstract

Breast cancer remains one of the most common causes of cancer-related deaths among women, with progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) being key molecular targets in its progression. This study aimed to explore the potential of bioactive compounds from *Syzygium malaccense* that have exhibited anticancer activities, as targeted inhibitors for PR and HER2, using computational approaches. A total of 155 compounds were initially screened for anticancer potential using the Prediction of Activity Spectra for Substances (PASS), identifying 80 compounds for further analysis. Drug-likeness and pharmacokinetic predictions indicated that several compounds complied with the Rule of Five (RO5) and had favorable absorption and distribution profiles, suggesting their suitability as oral drug candidates. Molecular docking revealed that quercetin exhibited favorable interactions with PR, particularly involving the Arg 766 residue, while myricetin demonstrated strong binding affinity to HER2, surpassing trastuzumab, and interacting with key residues Asp 863, Lys 753, Ala 751, and Leu 796. Molecular dynamics simulations confirmed the stability of the Myricetin-HER2 complex under physiological conditions over 15 ns, supporting its potential as a HER2 inhibitor. These findings highlight myricetin and quercetin as promising natural compounds for breast cancer therapy targeting HER2 and PR, respectively. However, further experimental validation, including *in vitro* and *in vivo* studies, is necessary to confirm their therapeutic efficacy and safety. Overall, this study supports *Syzygium malaccense* as a valuable source of natural bioactive compounds for breast cancer drug discovery.

Keywords: *in silico screening,* Syzygium malaccense*, breast cancer, progesterone receptor, HER2*.

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INTRODUCTION

Breast cancer remains one of the leading causes of cancer-related deaths among women worldwide (Bray, *et al*., 2024). Among its molecular subtypes, other than the well-studied estrogen receptor (ER) as biomarker for this cancer type, progesterone receptor-positive (PR+) and human epidermal growth factor receptor 2-positive (HER2+) breast cancers stand out due to their clinical behavior and therapeutic implications (Yersal & Barutca, 2014). The PR receptor supports tumor growth by mediating hormone-dependent cell proliferation, while HER2, a member of the ErbB receptor family, is frequently amplified in aggressive tumors, driving proliferation through downstream signaling cascades (Hsu & Hung, 2016). Overexpression of HER2 occurs in approximately 15%-20% of breast cancer cases and is associated with poor clinical outcomes and higher recurrence rates (Zhao, *et al*., 2025). Meanwhile, although PR+ tumors are often hormone-responsive, resistance to endocrine therapy remains a persistent clinical challenge (Lu, *et al*., 2025).

Current treatment strategies for these subtypes, including anti-HER2 monoclonal antibodies, tyrosine kinase inhibitors (TKIs), and selective hormone modulators, have significantly improved patient survival (Swain, *et al*., 2023). However, treatment failure due to drug resistance and systemic toxicity continues to highlight the need for safer and more effective therapeutic alternatives (Amalia, *et al*., 2019). Natural products, especially those derived from medicinal plants, offer a promising reservoir of bioactive compounds with potential anticancer properties.

Syzygium malaccense, commonly known as Malay apple, is a tropical plant traditionally consumed as fruit and utilized in folk medicine. Recent studies have shown that its fruit extract possesses antioxidant and cytotoxic activities against various cancer cell lines (Vadu, *et al*., 2023), effects largely attributed to its rich content of flavonoids, anthocyanins, and phenolic acids. Despite these promising findings, the molecular mechanisms underlying the interactions of its bioactive compounds with breast cancer-related targets remain insufficiently understood. In particular, the role of HER2 and PR, both of which play central roles in the pathogenesis and progression of breast cancer, has not been extensively explored in the context of *S. malaccense* bioactive compounds. Therefore, this study aims to evaluate the potential interactions of *S. malaccense* compounds with HER2 and PR, providing an initial assessment of their relevance as breast cancer therapeutic candidates. Nonetheless, it remains possible that these findings may also uncover meaningful polypharmacological potential in future investigations.

In silico approaches such as molecular docking and molecular dynamics simulations provide valuable tools to predict and analyze ligand-protein interactions prior to experimental validation. These methods help accelerate drug discovery by identifying promising candidates based on their binding affinity and stability with molecular (Prasetiawati, *et al*., 2023). Hence, this study aimed to investigate the therapeutic efficacy of bioactive compounds extracted from *Syzygium malaccense* against PR and HER2 breast cancer targets employing an *insilico* screening methodology.

MATERIALS AND METHODS

Screening of Bioactive Compounds

The selection of bioactive compounds from *Syzygium malaccense* was based on an extensive literature review using the keyword "bioactive compound of *Syzygium malaccense*" (Batista, *et al*., 2017; Feltrin, *et al*., 2020; Ismail, *et al*., 2010; Karioti, *et al*., 2007; Nunes, *et al*., 2016; Pino, *et al.*, 2004; Rajan & Bhat, 2019; Reis, *et al*., 2021; Savi, *et al*., 2020; Tukiran & Putri, 2021). Reported compounds from various scientific articles were then compiled, curated, and verified for their relevance before being incorporated into this study as the compound dataset.

Prediction of Anticancer Activity Using PASS Online

An initial prediction of anti-breast cancer activity of compounds derived from *Syzygium malaccense* was conducted using the Prediction of Activity Spectra for Substances (PASS Online) web server. Compounds with a probability of activity value greater than 0.5 were considered potentially active and selected for further analysis (Filimonov, *et al.*, 2014).

Molecular Geometry Optimization of Bioactive Compounds

The three-dimensional molecular structures of bioactive compounds derived from *Syzygium malaccense* were subjected to geometry optimization using MarvinSketch software. The optimization process was performed using the Merck Molecular Force Field (MMFF94) method to obtain the most stable conformations for further analysis.

Drug-Likeness Evaluation Based on Lipinski's Rule of Five

The secondary metabolites of *Syzygium malaccense* used in this study were retrieved from the PubChem database. Ligand structures were prepared using MarvinSketch, where energy minimization was performed with the MMFF94 force field and the resulting structures were saved in MOL2 format. These MOL2 files were then reopened in MarvinSketch, protonated at physiological pH (7.4), and saved in PDB format. Following structural preparation, the physicochemical properties of each compound were evaluated using the Lipinski's Rule of Five via the online tool provided by SCFBio (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) to assess their oral drug-likeness potential (Jayaram, *et al*., 2012).

Pharmacokinetic and Toxicity Prediction via PreADMET Server

The pharmacokinetic and toxicity profiles of selected *Syzygium malaccense* compounds were predicted using the PreADMET web server (http://preadmet.bmdrc.kr). The analysis focused on key pharmacokinetic parameters, including human intestinal absorption (HIA), Caco-2 cell permeability, and plasma protein binding (PPB), as well as toxicity-related properties such as mutagenicity and carcinogenicity. Chemical structures of the compounds were uploaded through the platform's interface, and the resulting data were analyzed to evaluate the drug-likeness and safety potential of each compound (Dulsat, *et al*., 2023).

Target Protein Selection and Preparation

The three-dimensional crystal structures of the target proteins were obtained from the Protein Data Bank (https://www.rcsb.org/) with PDB ID 1SQN (Mokashi & Bhatia, 2024) for the progesterone receptor and PDB ID 3PP0 (Mutiah, *et al*., 2021) for the HER2. Protein-ligand complexes were separated using Discovery Studio Visualizer®. Both protein and ligand structures were prepared using AutoDock Tools®, including the removal of water molecules, addition of polar hydrogen atoms, and charge assignment. The prepared structures were saved in PDBQT format. Structural validation and binding affinity calculations were performed using AutoDock Vina®.

Molecular Docking

To ensure the accuracy of the docking protocol, validation was first carried out by re-docking the native ligands into the active sites of the PR and HER2. Once validated, the main docking simulations were performed using AutoDock Vina® with the same parameters. The docking results were then visualized and analyzed using Discovery Studio Visualizer®. The analysis focused on the binding affinity and key amino acid residues that interacted directly with the ligands at the active sites of the target proteins.

Molecular Dynamics

Molecular dynamics simulations were performed using the OpenMM toolkit (Eastman, *et al*., 2017) to evaluate the stability of the ligand-protein complex formed between the HER2

receptor and the ligand with the lowest binding energy obtained from molecular docking results. The simulation began with the generation of topologies for both the protein and the ligand. The ff19SB force field was used to construct the receptor topology, while the ligand was parameterized using the GAFF2 force field. The system was solvated using the TIP3P water model. Following system preparation, equilibration and production phases were carried out for a total simulation time of 15 nanoseconds. Subsequent analyses included the evaluation of Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and Radius of Gyration (Rg) to assess the structural stability and flexibility of the complex throughout the simulation.

RESULTS

Screening of Anti-Breast Cancer Activity

A total of 155 bioactive compounds from *Syzygium malaccense* were identified and selected for this study based on literature screening from previously published scientific journals. The complete list of these compounds is provided in Supplementary Table S1. The bioactive compounds of Malay apple were evaluated for their potential anti-breast cancer activity using the PASS Online prediction tool. Out of 155 compounds, 80 were predicted to possess anti-breast cancer activity based on their probability scores. This screening aimed to provide an early estimation of each compound's pharmacological potential. The PASS Online output includes values for probability of activity (Pa) and probability of inactivity (Pi), which represent the likelihood that a given compound exhibits the predicted biological activity. Compounds with Pa>0.5 were considered to have significant potential and were selected for further analysis. The results of the screening are presented in Supplementary Table S2.

Lipinski's Rule of Five Analysis, Pharmacokinetics and Toxicity

The online portal (http://scfbio-iitd.res.in) was used to predict the potential of the tested compounds as oral drugs based on Lipinski's Rule of Five, which assesses drug-likeness. According to this rule, optimal oral drugs should have a molecular weight <500 Da, Log *P*<5, hydrogen bond donors <5, and hydrogen bond acceptors <10 to ensure good absorption and membrane permeability (Az-Zahra, *et al*., 2022). Table 1 presents a summary of the pharmacokinetic and toxicity profiles of 80 tested compounds. A total of 64 compounds complied with Lipinski's Rule of Five (RO5), while 16 did not.

Pharmacokinetic absorption was further predicted using HIA and Caco2 permeability, while distribution was estimated by Plasma Protein Binding (PPB). HIA predicts intestinal absorption, with values >70% indicating good absorption (Sagitasa, *et al*., 2021). Caco2 assays estimate membrane permeability, with >70 nm/sec reflecting lipophilic properties (Suherman, *et al*., 2020). PPB assesses how strongly a compound binds to plasma proteins, with >90% indicating strong binding (Nusantoro & Fadlan, 2020).

Toxicity was evaluated using Ames and carcinogenicity predictions. A positive Ames result suggests potential mutagenicity, while carcinogenicity tests confirm cancer risk (Hartanti, *et al*., 2022). Based on pharmacokinetic parameters, 57 compounds were predicted to have good absorption, 16 with moderate absorption, and 7 with poor absorption. In terms of distribution, 74 compounds exhibited moderate membrane permeability and 6 compounds showed low permeability. Furthermore, 38 compounds were predicted to have strong plasma protein binding (PPB), while 42 exhibited weak binding. Regarding toxicity profiles, 26 compounds were predicted to be non-mutagenic, 54 mutagenic, 22 carcinogenic, and 58 non-carcinogenic.

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Table 1. Results of lipinski's rule of five analysis, pharmacokinetics, and toxicity analysis.

No	Compound	Lipinski's Rule of Five Analysis					Pharmacokinetics and Toxicity Analysis				
		MW (< 500 Da)	Hydrogen Bonds		Log P (<5)	Result	Absorption	Distribution		Toxicity	
			Donor (<5)	Acceptor (<10)			HIA (%)	CaCO-2 Cell (nm sec)	Plasma Protein Binding (%)	Ames Test	Carcinogenity
1	*Quercitrin*	428	0	11	0	Not Met	24.94**	7.37**	64.95**	Non-mutagen	Negative
2	*Procyanidin B2*	552	0	12	-0.71	Not Met	19.51***	13.67**	100*	Non-mutagen	Negative
3	*Procyanidin B1*	552	0	12	0	Not Met	19.51***	13.67**	100*	Non-mutagen	Negative
4	*Procyanidin A2*	552	0	12	-2.83	Not Met	35.29**	9.23**	100*	Non-mutagen	Negative
5	*2',4'-Dihydroxy-6'-Methoxy-3-Methyldihidrochalcone*	268	0	4	0.57	Met	92.76*	18.49**	96.08*	Mutagen	Negative
6	*Stercurensin*	268	0	4	0.57	Met	93.03*	18.43**	92.06*	Mutagen	Negative
7	*Kaempferol-3-glucoside*	428	0	11	0	Not Met	25.17**	11.14**	57.57**	Non-mutagen	Negative
8	*Isorhamnetin-3-glucoside*	456	0	12	0	Not Met	21.6**	9.93**	47.83**	Non-mutagen	Negative
9	*Isoquercitrin*	444	0	12	0	Not Met	11.77***	9.43**	59.15**	Non-mutagen	Negative
10	*(-)-Epicatechin*	276	0	6	-1.56	Met	66.7**	0.65***	100*	Mutagen	Negative
11	*Quercetin*	292	0	7	0	Met	63.48**	3.41***	93.23*	Mutagen	Negative
12	*(+)-Catechin*	276	0	6	-1.56	Met	66.7**	0.65***	100*	Mutagen	Negative
13	*Morin*	292	0	7	0	Met	63.49**	17.1**	91.62*	Mutagen	Negative
14	*Myricitrin*	444	0	12	0	Not Met	11.64***	6.14**	65.37**	Non-mutagen	Negative
15	*Chlorogenic Acid*	336	0	9	0	Met	20.42**	18.71**	41.96**	Mutagen	Positive
16	*Myricetin-3-(3"galloylrhamnoside)*	592	0	16	0	Not Met	4.07***	7.47**	100*	Non-mutagen	Positive
17	*Mearnsitrin*	456	0	12	0	Not Met	21.45**	6.21**	55.6**	Non-mutagen	Negative
18	*(-)-Epicatechin gallate*	424	0	10	-2.88	Not Met	40.58**	13.21**	100*	Non-mutagen	Negative
19	*Desmanthin I*	592	0	16	0	Not Met	4.07***	13.67**	100*	Non-mutagen	Positive
20	*Rutin*	580	0	16	0	Not Met	2.86***	7.91**	43.89**	Non-mutagen	Negative
21	*Ursolic Acid*	408	0	3	0.42	Met	95.99*	21.86**	100*	Non-mutagen	Positive
22	*(-)-Beta Caryophyllene*	180	0	0	1.56	Met	100*	23.63**	100*	Mutagen	Negative
23	*(-)-B-pinene*	120	0	0	0.84	Met	100*	23.49**	100*	Mutagen	Negative

Table 1. Results of lipinski's rule of five analysis, pharmacokinetics, and toxicity analysis continuous.

No	Compound	Lipinski's Rule of Five Analysis					Pharmacokinetics and Toxicity Analysis				
		MW (< 500 Da)	Hydrogen Bonds		Log *P* (<5)	Result	Absorption	Distribution		Toxicity	
			Donor (<5)	Acceptor (<10)			HIA (%)	CaCO-2 Cell (nm sec)	Plasma Protein Binding (%)	Ames Test	Carcinogenicity
24	*(-)-caryophyllene oxide*	196	0	1	1.12	Met	100*	56.34**	90.84*	Mutagen	Positive
25	*(-)-Epigallocatechin gallate*	440	0	11	-3.4	Not Met	20.71**	12.04**	100*	Non-mutagen	Negative
26	*(2E,6E)-Farnesyl acetate*	236	0	2	0	Met	100*	55.05**	100*	Non-mutagen	Positive
27	*(E)-3,7,11- trimethyl-1,6,10-dodecatrien-3-ol*	196	0	1	0	Met	100*	26.61**	100*	Non-mutagen	Negative
28	*2',4'-Dihydroxy-6'-methoxy-3',5'-Dimethylchalcone*	280	0	4	0.18	Met	93.23*	20.13**	91.52*	Non-mutagen	Negative
29	*2-ethylhexyl p-methoxycinnamate*	264	0	3	0.79	Met	98.76*	55.98**	94.07*	Non-mutagen	Positive
30	*3-buten-2-one*	64	0	1	-0.15	Met	100*	30.44**	62.78**	Mutagen	Negative
31	*3-hexen-1-ol*	88	0	1	0.23	Met	100*	25.12**	87.64**	Mutagen	Negative
32	*3-Methyl-2-buten-1-ol*	76	0	1	-0.07	Met	100*	21.72**	81.16**	Mutagen	Negative
33	*8-Methylpinocembrin*	256	0	4	-0.31	Met	95.57*	5.06**	97.89*	Mutagen	Negative
34	*allo-aromadendrene*	180	0	0	1.64	Met	100*	23.49**	100*	Mutagen	Negative
35	*alpha-copaene*	180	0	0	1.09	Met	100*	23.63**	100*	Non-mutagen	Negative
36	*alpha-fenchol*	136	0	1	0.06	Met	100*	24.23**	100*	Mutagen	Negative
37	*alpha-humulene*	180	0	0	0	Met	100*	23.63**	100*	Non-mutagen	Positive
38	*alpha seline*	180	0	0	0	Met	100*	23.63**	100*	Mutagen	Negative
39	*alpha-ylangene*	180	0	0	1.09	Met	100*	23.63**	100*	Non-mutagen	Negative
40	*aromadendrene*	180	0	0	1.64	Met	100*	23.49**	100*	Mutagen	Negative
41	*Aurentiacin*	280	0	4	0.73	Met	95.62*	36.48**	90.50*	Mutagen	Negative
42	*Benzaldehyde*	100	0	1	0.16	Met	100*	21.87**	4.50**	Mutagen	Negative
43	*Beta Pinene*	120	0	0	0.84	Met	100*	23.49**	100*	Mutagen	Negative
44	*Beta Seline*	180	0	0	0.82	Met	100*	23.49**	100*	Mutagen	Negative
45	*Beta Sitosterol*	364	0	1	1.77	Met	100*	52.37**	100*	Non-mutagen	Positive
46	*Beta Elemene*	180	0	0	1.06	Met	100*	23.49**	100*	Mutagen	Negative
47	*borneol*	136	0	1	0.06	Met	100*	24.23**	100*	Mutagen	Negative
48	*Caffeic acid*	172	0	4	-2.52	Met	82.30*	21.10**	40.29**	Mutagen	Negative

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Table 1. Results of lipinski's rule of five analysis, pharmacokinetics, and toxicity analysis continuous.

No	Compound	Lipinski's Rule of Five Analysis					Pharmacokinetics and Toxicity Analysis				
		MW (< 500 Da)	Hydrogen Bonds		Log P (<5)	Result	Absorption	Distribution		Toxicity	
			Donor (<5)	Acceptor (<10)			HIA (%)	CaCO-2 Cell (nm sec)	Plasma Protein Binding (%)	Ames Test	Carcinogenicity
49	*citronellyl acetate*	176	0	2	0.21	Met	100*	44.81**	100*	Non-mutagen	Positive
50	*E-Beta-Ocimene*	120	0	0	0	Met	100*	23.63**	100*	Mutagen	Positive
51	*Ellagic acid*	296	0	8	-1.93	Met	61.39**	20.48**	88.40**	Mutagen	Negative
52	*Epigalocatechin Gallate*	440	0	11	-3.4	Not Met	20.71**	12.04**	100*	Non-mutagen	Negative
53	*Ethyl (E)-2-butenoate*	104	0	2	-0.25	Met	100*	50.49**	96.52*	Mutagen	Positive
54	*Ethyl acetate*	80	0	2	-0.27	Met	99.78*	37.20**	79.98**	Mutagen	Positive
55	*Ferulic acid*	184	0	4	-1.47	Met	90.60*	21.11**	50.41**	Mutagen	Negative
56	*Gallic acid*	164	0	5	-2.96	Met	53.69**	13.84**	65.38**	Mutagen	Negative
57	*gamma – Elemene*	180	0	0	0	Met	100*	23.49**	100*	Mutagen	Negative
58	*Gamma-Butyrolactone*	80	0	2	-0.41	Met	99.78*	20.78**	78.40**	Mutagen	Negative
59	*Gamma-Muurolene*	180	0	0	1.64	Met	100*	23.63**	100*	Mutagen	Negative
60	*isopulegol*	136	0	1	9.47	Not Met	100*	38.71**	100*	Mutagen	Negative
61	*isopulegyl acetate*	176	0	2	0.58	Met	100*	53.74**	99.14*	Mutagen	Positive
62	*limonene*	120	0	0	1.02	Met	100*	23.63**	100*	Mutagen	Negative
63	*linalool*	136	0	1	0.09	Met	100*	29.35**	100*	Mutagen	Negative
64	*methyl salicylate*	144	0	3	-0.23	Met	93.05*	20.78**	44.29**	Mutagen	Negative
65	*Mustakone*	196	0	1	0	Met	100*	40.02**	100*	Mutagen	Positive
66	*Myltayl-4(12)-ene*	180	0	0	0.58	Met	100*	23.49**	81.32**	Mutagen	Negative
67	*myrcene*	120	0	0	0	Met	100*	23.63**	100*	Mutagen	Negative
68	*Myricetin*	308	0	8	-2.29	Met	40.96**	0.99***	96.78*	Mutagen	Negative
69	*p-Coumaric acid*	156	0	3	-2.09	Met	92.09*	21.10**	63.05**	Mutagen	Positive
70	*Pentyl acetate*	116	0	2	-0.34	Met	100*	52.87**	95.17*	Mutagen	Positive
71	*phytol*	256	0	1	0	Met	100*	37.62**	100*	Non-mutagen	Positive
72	*Pinocembrin*	244	0	4	-0.36	Met	92.35*	2.47***	98.45*	Mutagen	Negative
73	*Sibirene*	180	0	0	0.74	Met	100*	23.64**	100*	Mutagen	Negative
74	*Spathulenol*	196	0	1	0.84	Met	100*	54.42**	82.88**	Mutagen	Positive
75	*t-Cinnamic acid*	140	0	2	-1.26	Met	97.84*	21.03**	60.85**	Mutagen	Negative

Table 1. Results of lipinski's rule of five analysis, pharmacokinetics, and toxicity analysis (continuous).

No	Compound	Lipinski's Rule of Five Analysis					Pharmacokinetics and Toxicity Analysis				
		MW (< 500 Da)	Hydrogen Bonds		Log *P* (<5)	Result	Absorption	Distribution		Toxicity	
			Donor (<5)	Acceptor (<10)			HIA (%)	CaCO-2 Cell (nm sec)	Plasma Protein Binding (%)	Ames Test	Carcinogenicity
76	*terpinolene*	120	0	0	1.02	Met	100*	23.63**	93.16*	Mutagen	Positive
77	*Trans-sesquisabinene hydrate*	196	0	1	0	Met	100*	21.21**	100*	Mutagen	Positive
78	*Uvangoletin*	256	0	4	0	Met	92.55*	16.01**	96.38*	Mutagen	Negative
79	*Vanillic acid*	160	0	4	-1.48	Met	85.36*	19.93***	52.10**	Mutagen	Negative
80	*Z-Beta-Ocimene*	120	0	0	0	Met	100*	23.63**	100*	Mutagen	Positive

Description:

HIA(%):
70-100 is well absorbed (*)
20-70 absorbed enough (**)
<20 poorly adsorbed (***)

CaCo-2(nm) sec):
>70 high permeability (*)
4-70 medium permeability (**)
<4 low permeability (***)

PPB (%):
>90 tightly bound (*)
<90 weakly bound (**)

Docking Validation

Docking parameter validation occurs prior to the docking process for the test ligands. The docking parameter is considered valid if it can re-docking the native ligand that has been withdrawn from the native ligand or the ligand complex to its original position with an RMSD value less than 2 Å (Astuty & Komari, 2022). The RMSD values obtained from re-docking of native ligands are summarized in Table 2. The redocking results showed that the progesterone receptor (PDB ID: 1SQN) exhibited an RMSD value of 0.3846 Å, while the HER2 receptor (PDB ID: 3PP0) demonstrated an RMSD value of 0.6678 Å. The RMSD values were declared valid and ready for use in molecular docking simulations of the test compounds.

Table 2. Docking validation results.

PDB ID	Grid Center			Grid Box			RMSD (Å)
	X	Y	Z	X	Y	Z	
1SQN	-2.357	1.546	25.869	40	40	40	0.3846
3PP0	16.387	17.394	26.218	40	40	40	0.6678

Molecular Docking Simulation

Docking validation was performed by redocking the native ligand into the binding site of the receptor. The redocking results showed an RMSD value of <2 Å (Astuty & Komari, 2022), which is in accordance with the standard criteria for docking validation and indicates that the applied protocol can reliably reproduce the ligand binding pose. Therefore, the docking parameters used in this study are considered valid, and the predicted ligand-receptor interactions can be regarded as reliable. The complete list of these compounds is provided in Supplementary Table S3 and Supplementary Table S4. The docking of Malay apple compounds on progesterone receptor and HER2, quercetin-PR, and myricetin-HER2 complex was predicted to have the highest binding activity based on the similarity of hydrogen bonds and lower bond free energy values. Quercetin is bound to essential amino acids of PR in Arg 766, and myricetin is bound to HER2 amino acid residues in Asp 863, Lys 753, Ala 751, and Leu 796 (Figure 1). The similarity of amino acid residues bound by the test compounds, the native ligand, and the

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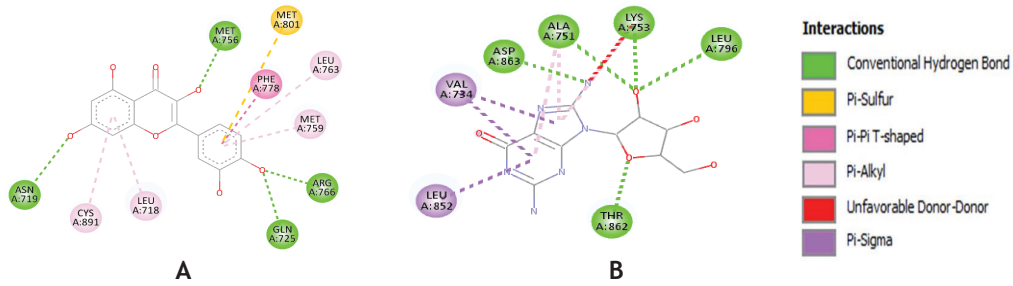

Figure 1. The 2-dimentional docking visualization from molecular docking between bioactive compounds in *Syzygium malaccense* (A) : complex PR-quercetin, (B) : complex HER2- myricetin).

reference compounds indicates that the test compounds are predicted to exhibit comparable pharmacological activity, as these residues constitute an essential part of the receptor's active site (Mutiah, *et al*., 2021). Molecular docking simulation results can be seen in Table 3.

Molecular Dynamic Simulation

Molecular Dynamics (MD) simulations were conducted to assess the stability of ligand–receptor interactions under physiological conditions (Chairunisa, *et al*., 2023). Since the docking results show stronger binding between myricetin-HER2 compared to the reference drug (trastuzumab), we then focus on evaluating the binding dynamics between these complexes. The simulations, performed for 15 ns using OpenMM on Google Colab connected to Google Drive, evaluated the HER2-ligand complexes. The RMSD analysis showed that myricetin had an average fluctuation of 2.14 Å (maximum 3.04 Å), higher than the natural ligand O3Q (1.62 Å) and the reference ligand Trastuzumab (1.92 Å). All ligands were considered stable with RMSD values below 5 Å (Chairunisa, *et al*., 2023). RMSF analysis indicated that myricetin caused higher fluctuations in several residues (Ala 706 to Asp 993), while stable residues included Val 905, Trp 913, and Asp 863. Both O3Q and Trastuzumab showed similar fluctuation patterns, but trastuzumab had

Table 3. Results of molecular docking simulation and analysis of linked amino acid residues.

Receptor	Compound	ΔG (kcal/mol)	Conventional Hydrogen Interaction
Progesteron	Native ligand (Norethindrone)	-12.0	Arg 766
	Quercetin	-9.9	Arg 766, Gln 725, Asn 719, Met 756
HER2	Native ligand (O3Q)	-11.4	Asp 863, Met 801
	Trastuzumab (Comparative drug)	-7.8	Asp 863, Ala 751, Lys 753, Leu 796, Thr 862
	Myricetin	-10.3	Asp 863, Lys 753, Met 801, Ala 751, Leu 796

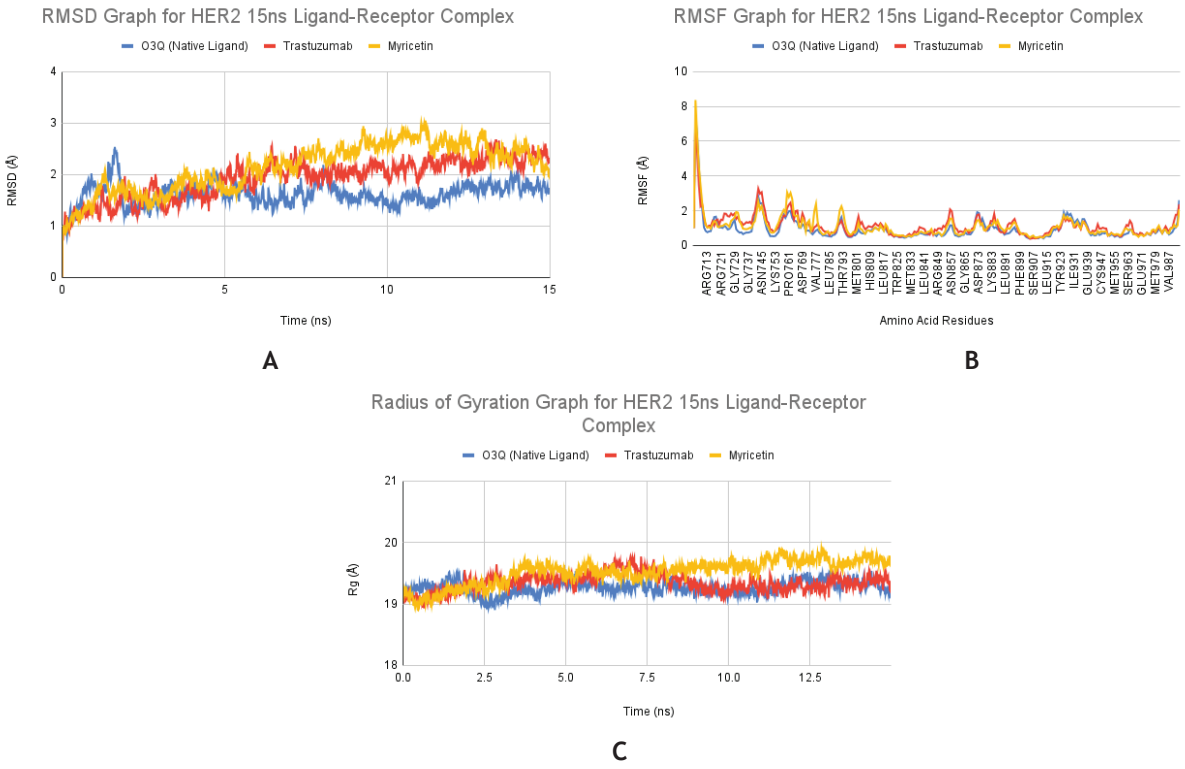

Figure 2. Results of molecular dynamics simulation for myricetin and HER2 complex for 15 ns showed (A) RMSD, (B) RMSF, and (C) radius of gyration graphs.

more stable residues, suggesting better overall stability. Radius of Gyration (Rg) results demonstrated that all ligands maintained structural stability throughout the simulation, although transient fluctuations were observed for myricetin at 1156 ps, O3Q at 1313 ps, and trastuzumab at 731 ps, indicating brief conformational changes. Overall, trastuzumab exhibited greater stability compared to myricetin (Figure 2).

DISCUSSION

This study provides new insights into the potential of bioactive compounds from *Syzygium malaccense* as therapeutic agents for breast cancer by targeting progesterone receptor (PR) and HER2, two key molecular targets involved in breast cancer progression. Initial screening using PASS analysis revealed that 80 out of 155 compounds showed potential anticancer activity, indicating that *S. malaccense* could be a valuable source of secondary metabolites. These findings align with previous studies highlighting the therapeutic potential of tropical medicinal plants in cancer drug discovery.

Drug-likeness and pharmacokinetic analyses revealed that several compounds fulfilled the Rule of Five (RO5) criteria and exhibited favorable absorption and distribution profiles, supporting their potential as oral drug candidates. However, these predictions did not cover toxicity, metabolism, or long-term safety aspects, underscoring the need for further validation through *in vivo* studies. Consistent with previous reports, natural products often exhibit strong pharmacological activities despite not fully complying with RO5, due to their complex structures and specific interactions with biological

targets (Doak, *et al*., 2014). Therefore, structural optimization of these compounds is recommended to enhance their pharmacokinetic profiles and reduce potential toxicity.

Molecular docking analysis showed that quercetin interacted favorably with PR, particularly at the key residue Arg 766. Although its binding energy was higher than that of the native ligand, the interaction profile suggests that quercetin may act as a modulator of PR activity. This finding is in line with previous reports demonstrating the antiproliferative effects of quercetin on hormone-responsive breast cancer cells (Altundag, *et al*., 2020). Meanwhile, myricetin exhibited a stronger binding affinity toward HER2, even surpassing the reference ligand trastuzumab. Its interaction with essential residues such as Asp 863, Lys 753, Ala 751, and Leu 796 suggests that myricetin may potentially inhibit HER2 oncogenic activity (Mutiah, *et al*., 2021).

The subsequent Molecular Dynamics (MD) simulation confirmed the stability of the myricetin-HER2 complex under physiological conditions for 15 ns, supporting the potential of myricetin as a HER2 inhibitor. Although the 15 ns simulation provided meaningful insights into the stability of the ligand-receptor complexes, we recognize that longer simulations (*e.g*., 50-100 ns) would offer stronger reliability and capture broader conformational changes. Therefore, extending the MD simulation duration will be an important direction for future studies. These results are consistent with previous studies showing that flavonoids like myricetin can modulate tyrosine kinase receptor activity. Nevertheless, the main limitation of this study lies in its reliance on computational approaches without experimental validation. Critical factors such as bioavailability, metabolic stability, and off-target effects require further investigation to confirm the therapeutic efficacy of these compounds.

In this study, trastuzumab was selected as the reference control in the molecular docking and molecular dynamics analyses, considering its established role as a standard clinical therapy for HER2-positive breast cancer. However, it should be acknowledged that Trastuzumab interacts with the extracellular domain of HER2 rather than the ATP-binding site. Therefore, although Lapatinib was not directly employed as a comparator, in this study the tested compounds were first compared with the native ligand of HER2 kinase (PDB ID: 3PP0), which is itself a tyrosine kinase inhibitor. This allows for the molecular interaction profiles of the compounds to still be evaluated in relation to a mechanistically relevant ATP-binding site inhibitor, thereby minimizing the limitation regarding the absence of a tyrosine kinase inhibitor comparator.

This study has several limitations that should be acknowledged. First, the use of trastuzumab as a comparator in molecular docking and molecular dynamics analysis is less relevant due to its different mechanism of action compared to ligands binding at the ATP-binding site. A tyrosine kinase inhibitor such as lapatinib would have been more representative; however, this simulation was not conducted in the present work, thus constituting one of the limitations of the study. Second, the investigation was restricted to *in silico* analysis and has not yet been validated through *in vitro* or *in vivo* experiments, which will be essential for confirming the findings. Nevertheless, the current funding support has enabled this study to be carried out as an initial step toward the development of potential HER2 inhibitors. These findings are expected to provide a foundation for further studies aimed at designing derivatives with enhanced activity against HER2.

Phytochemical investigations have reported that this plant contains considerable amounts of flavonoids, particularly myricetin and quercetin (Arumugam, *et al*., 2019; Oldoni, *et al*., 2025; Prasniewski, *et al*., 2021), which are suggested to contribute to its pharmacological activities. Based on these findings, future studies are recommended to conduct *in vitro* and *in vivo* experiments to

validate the anticancer activity of myricetin and quercetin, particularly in PR+ and HER2+ breast cancer models. Pharmacokinetic and toxicological studies in animal models are also needed to assess their safety profiles. Additionally, chemical structure modification, such as functional group addition or prodrug design, could be explored to enhance their pharmacological properties.

CONCLUSION

Myricetin and quercetin, two bioactive compounds derived from *Syzygium malaccense*, exhibited promising potential as targeted therapeutic agents against breast cancer. Myricetin exhibited strong binding affinity toward the HER2 receptor, interacting with key residues Asp 863, Lys 753, Ala 751, and Leu 796, and showed stable complex formation during a 15 ns molecular dynamics simulation, suggesting its potential as a HER2 inhibitor. Quercetin, on the other hand, displayed favorable interactions with the progesterone receptor (PR), particularly involving Arg 766, and achieved the best binding energy among the tested ligands, although still lower than the natural ligand. These findings suggest that myricetin and quercetin could serve as potential candidates for HER2 and PR-targeted breast cancer therapy, respectively. However, as a future direction, these in silico results should be validated through experimental studies using breast cancer cell lines such as MCF-7 for PR-positive validation and SK-BR-3 or BT-474 for HER2-positive validation, along with cell viability and apoptosis assays to confirm the predicted inhibitory potential.

SUPPLEMENTARY

Supplementary material: https://bit.ly/SupplData

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