

A Holistic *In-Silico* Characterization of Mortalin (*HSPA9*) Inhibitors from *Murraya koenigii* and *Pogostemon cablin* for *TP53* Reactivation in Breast Cancer

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Abstract

Breast cancer, a leading cause of cancer-related deaths, is characterized by therapeutic challenges, including side effects and drug resistance. The interaction between mortalin (HSPA9) and p53, a tumor suppressor, is known to play a crucial role in cancer progression, as mortalin sequesters p53 and inhibits its tumor-suppressive functions. This study evaluated the potential of bioactive compounds from *Murraya koenigii* and *Pogostemon cablin* as novel therapeutic agents for breast cancer through targeting the mortalin-p53 interaction. An *in silico* approach combining network pharmacology, molecular docking, and molecular dynamics was employed to identify potential inhibitors of this interaction. The study identified two promising candidates: 8,8''-biskoenigine (CID: 12967046) from *M. koenigii* and apigenin 7-(6''-p-coumarylglucoside) (CID: 44257826) from *P. cablin*. These compounds were screened for drug-likeness, pharmacokinetics, and binding affinity to mortalin. Molecular docking and dynamics simulations demonstrated stable binding interactions with mortalin, particularly with 8,8''-biskoenigine (−7,9 kcal/mol), which showed the strongest binding affinity and stability among the candidates. These findings highlight the potential of phytochemicals from *M. koenigii* and *P. cablin* as targeted breast cancer treatments by modulating the mortalin-p53 signaling axis. Further experimental validation is needed to confirm their efficacy *in vitro* and *in vivo*. This research offers a promising direction for the development of plant-based compounds as effective cancer therapeutics.

Keywords: Breast cancer, Mortalin, TP53, *Murraya koenigii*, *Pogostemon cablin*, 8,8''-Biskoenigine, Apigenin derivative

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Introduction

Breast cancer is the most frequently diagnosed cancer in women worldwide, with an estimated 2.3 million new cases in 2020 and around 685,000 deaths that year [1]. Despite advances in surgery, chemotherapy, and targeted therapies, current breast cancer treatments face significant limitations. Many patients experience toxic side effects, and tumors often develop therapeutic resistance - for example, long-term chemotherapy can lead to drug-resistant cancer stem cells that drive relapse [2]. These challenges underscore the urgent need for safer, more effective treatment strategies against breast cancer.

One promising avenue is the exploration of herbal medicine for anticancer drug discovery. Natural products derived from medicinal plants tend to act on multiple molecular targets and pathways, providing a holistic therapeutic effect with lower systemic toxicity [3,4]. Such multi-targeted mechanisms can reduce side effects and circumvent resistance compared to single-agent chemotherapy [5]. Indeed, many traditional remedies contain diverse bioactive compounds that act synergistically to inhibit cancer cell growth and induce apoptosis, while being well tolerated due to their long history of human use [6]. Harnessing these phytochemicals offers a valuable strategy to identify novel anticancer agents.

In this context, two Indonesian medicinal plants, curry leaf (*M. koenigii*) and patchouli (*P. cablin*), have garnered attention for their therapeutic potential. *M. koenigii* is an herb long used in Asian traditional medicine - it has been traditionally used for treating cancers and inflammation [7]. Its leaves are rich in carbazole alkaloids such as mahanimbine and girinimbine [8], which have shown noteworthy anticancer effects. For instance, mahanimbine isolated from *M. koenigii* can induce mitochondrial apoptosis and inhibit invasiveness in breast cancer cells, highlighting it as a potential therapeutic compound [9]. Meanwhile, *P. cablin*, known for its aromatic essential oil, is also widely utilized in traditional herbal therapies in Indonesia [10]. Patchouli's major sesquiterpene, patchouli alcohol, and related phytochemicals exhibit a spectrum of pharmacological activities, including anti-proliferative and pro-apoptotic effects on cancer cells

[11]. Studies have identified patchouli compounds with cytotoxic activity across various cancers (oral, lung,

breast, etc.), supporting the idea that patchouli leaves harbor multi-faceted anticancer agents [12]. These findings position *M. koenigii* and *P. cablin* as promising sources of bioactive molecules for cancer treatment.

Another critical aspect of improving breast cancer therapy is targeting key molecular pathways driving tumor progression. One such target is the mortalin-p53 interaction. Mortalin (Grp75/HSPA9) is a heat-shock family chaperone that is often overexpressed in tumor cells and plays a pivotal role in disabling the tumor suppressor p53 [13]. In normal function, p53 (encoded by *TP53*) safeguards against cancer by inducing cell-cycle arrest and apoptosis in response to stress. Mortalin binds to p53 in cancer cells, sequestering p53 in the cytoplasm and preventing its nuclear translocation and transcriptional activity [13]. This inactivation of p53 allows cancer cells to escape growth control. Notably, the mortalin-p53 protein-protein interaction is largely cancer-specific, making this pathway an appealing therapeutic target to reactivate p53 in tumors [14]. Disrupting the mortalin-p53 axis could release p53 to resume its tumor suppressor functions, triggering apoptosis in cancer cells. Therefore, small molecules capable of interfering with the mortalin-p53 interaction are highly sought after in anticancer research.

Given the multi-targeted bioactivity of *M. koenigii* and *P. cablin* phytochemicals and the importance of the mortalin-p53 interaction in breast cancer, we propose a computational investigation into the therapeutic potential of these plants. In this study, we employ an in-silico approach integrating network pharmacology, molecular docking, and molecular dynamics simulations to evaluate whether bioactive compounds from *M. koenigii* and *P. cablin* can effectively target the mortalin-p53 pathway. Computational methods enable rapid screening and mechanistic insights - for example, virtual docking can predict compound binding to mortalin, and molecular dynamics can assess the stability of the compound-protein complex [15]. This approach accelerates the discovery of promising natural compounds by identifying those with favorable interactions and pharmacological profiles in-silico.

The objective of our research is to systematically explore and identify lead compounds from curry leaves and patchouli that might disrupt the mortalin-p53 interaction and thereby restore p53 activity in breast cancer cells. By combining network pharmacology modeling with molecular docking and simulation, we aim to illuminate the mechanisms by which these herbal compounds could exert anti-breast cancer effects, providing a rationale for further experimental validation and development of novel plant-derived therapeutics targeting the mortalin-p53 axis. Although several small molecules have been proposed as mortalin-p53 disruptors, including embelin and mortaparib plus, most studies have focused on limited chemical libraries and have rarely integrated network pharmacology, multi-database enrichment, TCGA-based validation, docking grid refinement, and extended-timescale molecular dynamics in a single pipeline. To our knowledge, no previous work has comprehensively evaluated phytochemicals from *M. koenigii* and *P. cablin* against mortalin using such a holistic in-silico framework, nor directly compared their performance to experimentally validated mortalin inhibitors. This multifaceted approach enhances target prioritization, mitigates false positives in docking predictions, and yields more reliable assessments of binding affinity, conformational stability, and drug-likeness.

Materials and methods

Identification and screening of bioactive compounds

Compounds present in *P. cablin* and *M. koenigii* were selected based on previous reports by Ginting *et al.* [16] and Husna *et al.* [17], who employed GC-MS (gas chromatography-mass spectrometry) and LC-MS (liquid chromatography-mass spectrometry) techniques to profile the metabolites. The corresponding three-dimensional (3D) chemical structures were downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) as referenced by Kim *et al.* [18]. Only compounds consistently reported across at least two independent phytochemical studies or with a relative abundance above a predefined threshold were retained, in order to prioritize metabolites that are biologically relevant in traditional preparations. Before docking, all candidates were screened for oral drug-likeness using Lipinski criteria as well as in-silico

ADMET profiling (absorption, permeability, CYP450 interaction, and hepatotoxicity risk), and only compounds fulfilling basic developability criteria were advanced to structure-based analyses.

Identification of target genes

The selection of optimal breast cancer targets was conducted using data mining techniques across four databases: GeneCards (<https://www.genecards.org/>) [19], OMIM (<https://www.omim.org/>) [20], PharmGKB (<https://www.pharmgkb.org/>) [21], and The Human Protein Atlas (THPA) (<https://www.proteinatlas.org/>) [22]. Specific target genes were identified using the input query “breast cancer” and the target organism “Homo sapiens.” Some databases included initial selection parameters to ensure accurate identification of target genes.

In the GeneCards database, three main parameters were applied: The target must be a protein-coding gene, contain information related to genetic testing, and be supported by publication evidence (PMIDs). In PharmGKB, the gene pool compilation was based on clinical and variant annotations. The parameters used included the specific phenotype information “breast neoplasm” and confidence levels 3 to 4, indicating high confidence in the gene’s association with breast cancer. In the final database, THPA, the parameters included the availability of protein-level evidence and gene function annotation specific to breast cancer. The OMIM database did not apply any filtering criteria; all genes associated with breast cancer were included for further analysis.

Network pharmacology

The identification of key targets in breast cancer was performed using a combination of the STRING database (<https://string-db.org/>) and Cytoscape [22,23]. A gene pool compiled from breast cancer-related genes was used as input for the STRING database, which was utilized to construct protein-protein interaction (PPI) networks saved in TSV format [24]. Cytoscape was then used to visualize and filter the most relevant breast cancer-related genes through clustering and centrality analysis.

Clustering was conducted using the ClusterOne algorithm, while filtering was based on the highest values of degree, closeness centrality, and betweenness

centrality [25]. For cross-validation, an additional PPI network analysis was performed using the CytoHubba plugin, focusing on six key centrality metrics: degree centrality (DC), betweenness centrality (BC), closeness centrality (CC), edge percolated component (EPC), maximal clique centrality (MCC), and maximum neighborhood component (MNC) [26].

GO and KEGG enrichment analysis

The breast cancer-related gene pool was further validated using GO and KEGG enrichment analysis through SRplot [26,27]. This method was employed to examine the functional annotations of the breast cancer gene pool. The GO enrichment analysis included three categories: Biological process (BP), molecular function (MF), and cellular component (CC). Meanwhile, the KEGG enrichment analysis identified specific biological pathways associated with breast cancer [28]. GO and KEGG enrichment significance were evaluated using enrichment *p*-values, *q*-values, and false discovery rate (FDR)-adjusted *q*-values generated by SRplot.

The cancer genome atlas

The Cancer Genome Atlas (TCGA) analysis was conducted using the UALCAN database (<https://ualcan.path.uab.edu/>) to obtain the mRNA expression levels of *TP53* and *HSPA9*, along with their promoter methylation status [29]. The TCGA dataset used was for invasive breast carcinoma, with *TP53* and *HSPA9* as the input target genes. This analysis enabled a comparison between the expression levels of *TP53* and *HSPA9* in normal versus tumour tissues. Additional mutation analysis was conducted using cBioPortal (<https://www.cbioportal.org/>) by querying the target gene *TP53* [30]. Data were obtained from The Cancer Genome Atlas (TCGA) cohort, focusing on mutation frequency, classification, and distribution, with particular attention to breast cancer samples.

Molecular docking

Docking simulations were performed using AutoDock Vina v1.1.2, implemented via Perl script version 5 (<https://www.perl.org/>) [31]. Several parameters were applied, including an exhaustiveness value of 16, several modes set to 10, and an energy range of 4. The target protein structure was retrieved from the RCSB database with PDB ID: 4KBO [32]. Protein

screening was conducted based on network pharmacology results, a resolution of less than 3 Å, and X-ray diffraction crystallography.

Protein preparation was carried out using AutoDock Tools version 1.5.7 [31]. The preparation steps included removing non-essential ions, including sodium ion (Na⁺, NA501), as well as water molecules, adding hydrogen atoms (polar only), and assigning Gasteiger charges. The protein model was also refined and optimized by filling in missing residues. Protein preparation was performed using chain A as the main chain for molecular docking. Grid box mapping and construction focused on the 260 - 288 amino acid region, the known binding site of mortalin with p53. The grid box was divided into 10 segments to evaluate binding affinity and Root Mean Square Deviation (RMSD) values.

Bioactive compounds from *M. koenigii* (38 active compounds) and *P. cablin* (24 active compounds) were sourced from the KNApSACk database (<https://www.knapsackfamily.com/>) by selecting the organism of interest and retrieving compound names along with their SMILES representations [33]. The SMILES strings were input into the PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) database to download 3D structures in SDF format. Compound identity was verified by matching the retrieved structures with the corresponding names and SMILES under the “Names and Identifiers” section in PubChem. Compound models for docking were prepared using Open Babel and RDKit. In Open Babel, energy minimization was performed using the MMFF94 force field with default parameters, followed by the addition of hydrogen atoms (all-atom explicit) and Gasteiger charges. The optimized ligands were then converted to PDBQT format for docking analysis. Open Babel was also used to convert ligand files from SDF to PDBQT format.

After preparing both the protein and ligands, docking simulations were executed. Redocking was not performed in this study, as the target protein mortalin (PDB ID: 4KBO) does not possess a native ligand. Instead of native ligand coordinates, literature-based experimental data were used to determine the specific amino acid binding sites between mortalin and p53 [34]. Mortaparib plus and embelin were used as positive controls both are known novel inhibitors with well-

characterized experimental profiles. To enhance reproducibility, all docking runs were performed in triplicate with identical grid parameters and different random seeds, and the best-scoring pose was required to be consistently recovered across runs. The resulting protein-ligand complexes were visualized using PyMOL (3D) and BIOVIA Discovery Studio (2D) for interaction profiling. Docking results were evaluated based on binding affinity values, Root Mean Square Deviation (RMSD), and protein-ligand interaction profiles, which were further analyzed through molecular visualization.

Molecular dynamics

Molecular dynamics simulations were conducted for 100 nanoseconds under identical AMBER14 force-field settings, with the following parameters: pH 7.4, 0.9% NaCl concentration, water density of 0.997 g/cm³ in a cubic simulation cell, NVT ensemble, and a

temperature of 310 K. Trajectories sampled every 250 ps to allow downstream comparative analyses (RMSD, SASA, RG, RMSF, and number of H-bonds) All simulations were carried out using YASARA Dynamic v.21.8.27. Binding energy calculations were performed using the Poisson-Boltzmann (PBS) method with YASARA's built-in binding energy macros [35].

Principal Component Analysis (PCA) and Dynamic Cross-Correlation Matrix (DCCM)

PCA and DCCM analyses were performed using the Bio3D package based on (10.1186/s12859-014-0399-6) and (10.29090/psa.2025.01.24.1659) [36]. The GROMACS trajectory output (xtc) was converted to CHARMM or NAMD format (dcd) using VMD, and the resulting files were analyzed using the Bio3D package in RStudio. The bioinformatics screening workflow and computational methods for identifying potential breast cancer compounds are shown in **Figure 1**.

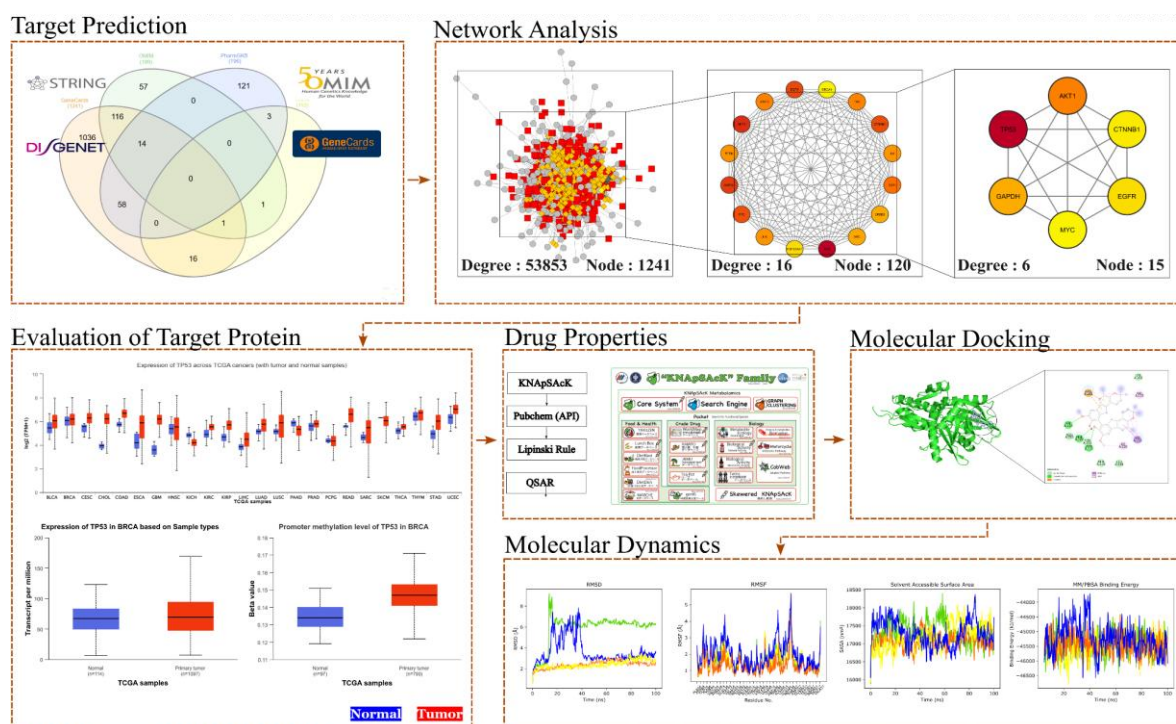


Figure 1 Workflow of bioinformatics screening and computational methods for identifying potential anti-breast cancer compounds.

Results and discussion

The development of cancer around the world has become a special concern, including breast cancer. Side effects and limitations in the development of cancer drugs are serious challenges for the future [37]. The

indication of drug resistance in some treatment methods also makes cancer more complicated to handle [38]. This shows the importance of finding more effective and efficient treatment approaches. One early step to address

this problem is to search for new drugs that target breast cancer specifically.

Various pathways can be used in breast cancer therapy. One potential target mechanism inhibits the interaction between p53 and mortalin. This is supported by validating potential targets based on network pharmacology and enrichment analysis. The results of the six databases used for cross-validation show that p53

is an essential target in breast cancer (**Figures 2(B)** and **2(C)**). The analysis was also carried out by looking at the protein-protein interaction (PPI) results using Cytoscape and the CytoHubba plugin (**Figure 3**). Based on these results, it can be concluded that p53 is indeed the best target, given its highest and most dominant centrality value.

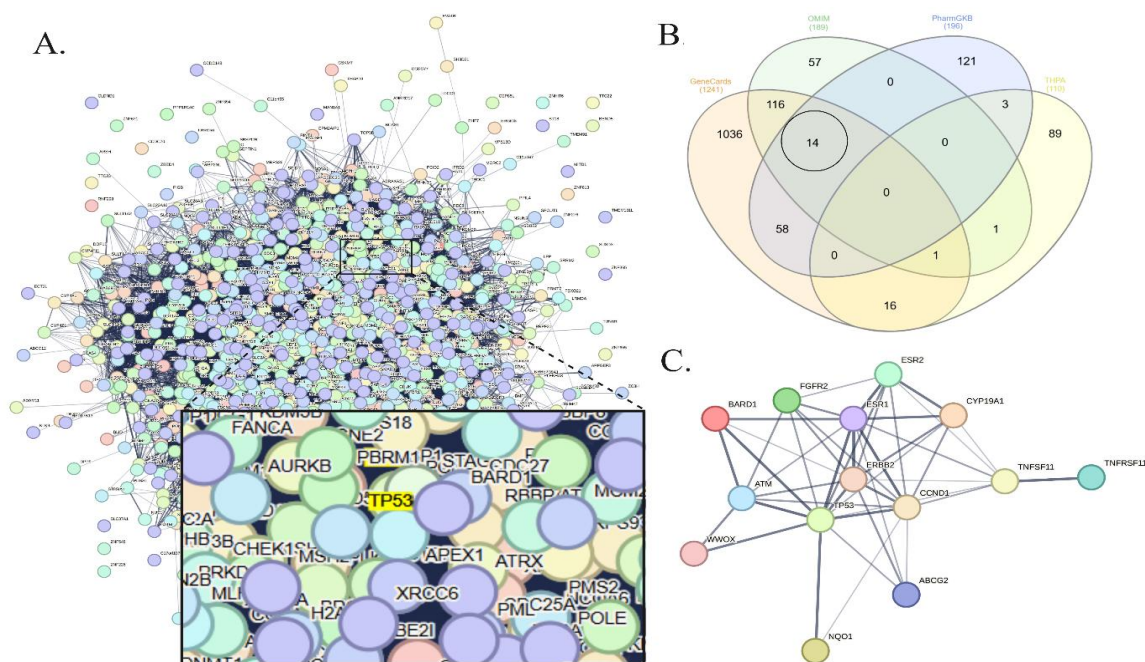


Figure 2 Venn diagram and protein-protein interaction (PPI) network of breast cancer-related genes. (A) STRING protein-protein interaction. (B) Venn diagram from four databases. (C) Protein-protein interaction (PPI) network of 14 commonly identified breast cancer-related genes from GeneCards, OMIM, PharmGKB, and The Human Protein Atlas.

Data mining

The identification of potential targets was analyzed based on four main databases, namely GeneCards, OMIM, PharmGKB, and The Human Protein Atlas. A total of 1736 targets (genes/proteins) were obtained from the data mining results of the four main databases. **Figure 2(A)** shows the visualization result of the protein-protein interaction (PPI) constructed using the STRING database. It can be seen that in one of the nodes (proteins), there is *TP53* as a potential target. The four main databases were also compared, and based on the overlap results, 14 main target genes/proteins related to breast cancer were identified (**Figures 2(B)** and **2(C)**). **Figures 2(A) - 2(C)** are provided in the supplementary Materials.

Network pharmacology

Network pharmacology was applied as an advanced method for determining potential protein targets in breast cancer. This gene set is a combination of four pure databases with no overlap. The protein-protein interaction (PPI) network, as shown in **Figure 3(A)**, was obtained from the STRING database, with a total of 1,241 nodes (proteins) and 53,853 edges. This PPI network was clustered using the ClusterONE algorithm, and a cluster with a *p*-value of 0.424 was selected, with a total of 188 nodes. A further screening step was performed to eliminate most proteins by applying the following criteria: degree > 120, betweenness centrality > 0.01, and closeness centrality > 0.5. From this filter, 16 nodes with a degree of 120 were identified. A second round of filtering, with the

criteria: degree > 154, betweenness > 0.02, and closeness centrality > 0.6, identified the main potential target, *TP53*, followed by *AKT1*, *GAPDH*, *EGFR*, *CTNBB1*, and *MYC*.

As cross-validation, **Figure 3(B)** shows the results of network pharmacology based on the CytoHubba algorithm using six types of centrality: Degree centrality (DC), closeness centrality (CC), betweenness centrality (BC), maximum neighborhood component (MNC), maximum clique centrality (MCC), and edge percolated component (EPC). Using this algorithm, the top 10 genes were identified for each parameter. The results show that *TP53* is also the best target across 5 of the 6 parameters used. **Figures 3(A)** and **3(B)** are provided in the supplementary Materials.

Notably, while multiple canonical oncogenic drivers (e.g. HER2, PIK3CA, AKT1, and BRCA1/2) emerged as central nodes, the mortalin-p53 axis provided a unique combination of high network

centrality and cancer-specific protein-protein interaction, which is less likely to perturb normal tissue homeostasis compared to upstream kinases. This observation justified the focus on mortalin as a druggable node to indirectly restore p53 function in breast cancer. The process of selecting potential protein targets is crucial for breast cancer treatment. Therefore, a screening of 1241 nodes (potential protein targets) was conducted to identify the leading candidate, p53. Centrality was used to measure the direct pharmacological connections of each node, determining its influence within the network [39,40]. Several parameters, such as betweenness and closeness centrality, are used as considerations for analyzing the shortest paths and how close a node is to others [40]. In the context of breast cancer, the *TP53* gene often appears as a target with a high centrality value in various network pharmacology parameters. This shows its crucial role in regulating the cell cycle and apoptosis.

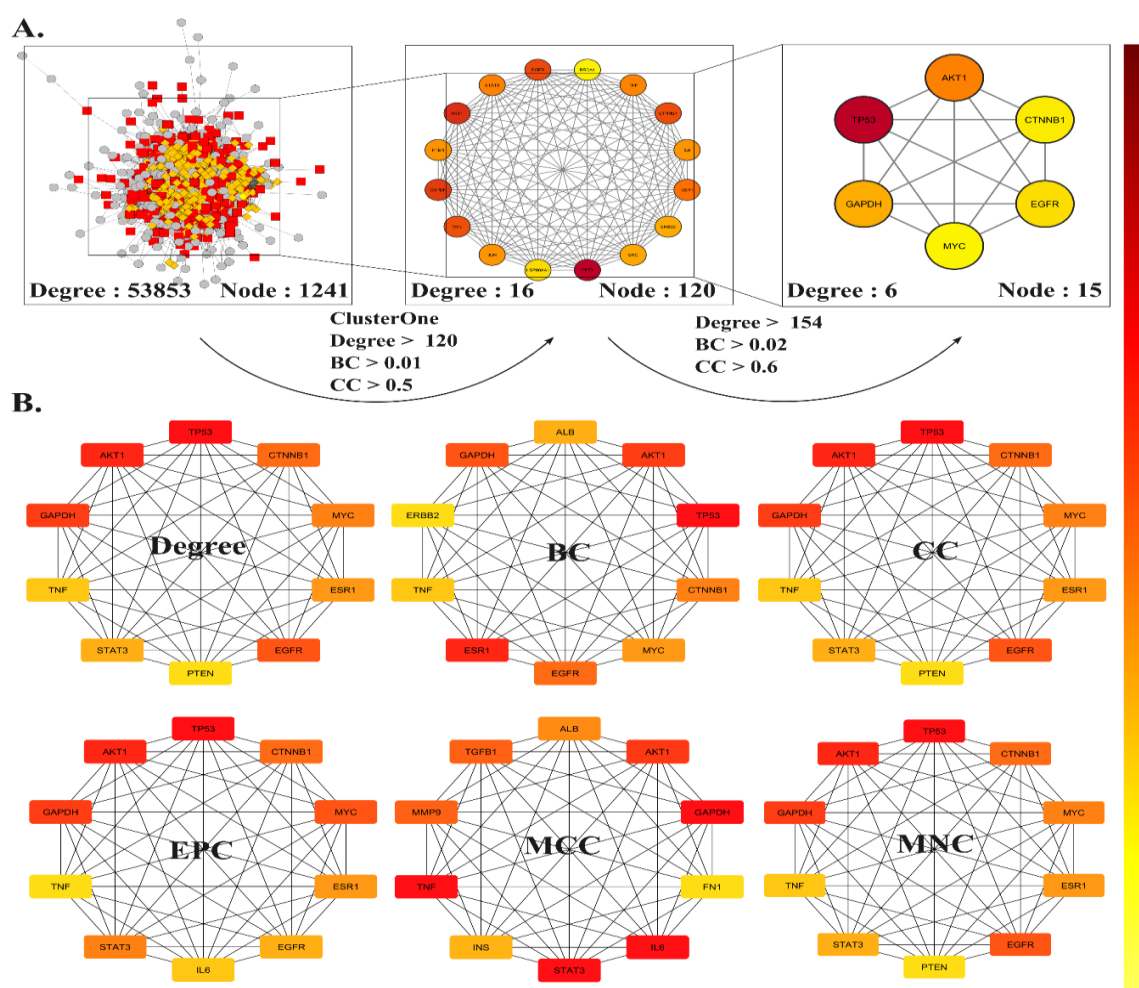


Figure 3 Network pharmacology approach reveals central genes from PPI network analysis. (A) Identification of hub genes using cluster one. (B) CytoHubba analysis using six topological centralities.

As a further analysis, the gene pool was re-analysed with gene ontology (GO) and KEGG enrichment and further analysis using TCGA. In line with TCGA datasets, the overexpression of HSPA9 (mortalin) in breast tumors relative to normal tissues underscores its function as an oncogenic chaperone that impairs p53 activity through cytoplasmic sequestration. The phytochemical candidates identified herein, by selectively inhibiting mortalin, are anticipated to reinstate TP53-mediated transcriptional programs without perturbing upstream signaling pathways critical for normal cellular homeostasis.

GO and KEGG enrichment analysis

Enrichment analysis was performed using SRplot to examine gene ontology (GO) and KEGG enrichment analysis (Figures 4 and 5). This classification was done to look deeper into the functions of the genes identified in breast cancer, through molecular function (MF), biological process (BP), and cellular component (CC), as shown in Figure 4. The results (Figure 4) indicate that these genes are involved in epithelial cell proliferation and migration, cellular signaling activities through kinases and receptors, and gene expression regulation. The gene function annotations show their relevance to breast cancer. Figure 4 is provided in the supplementary Materials.

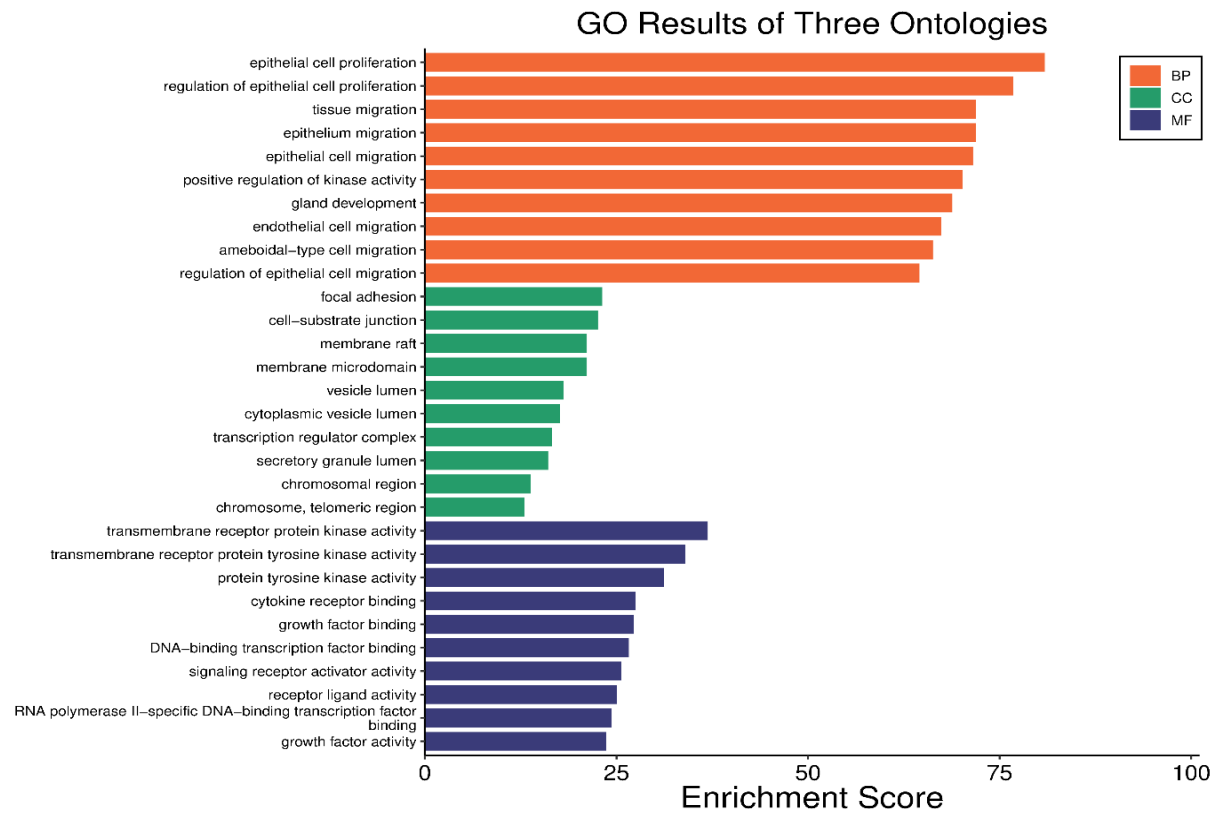


Figure 4 Gene ontology (GO) enrichment analysis of the breast cancer-related gene pool based on biological process (BP), molecular function (MF), and cellular component (CC).

Figures 4 and 5 show indications that the obtained gene pool is closely related to breast cancer, in terms of epithelial cell proliferation, tissue migration, epithelial cell migration, and kinase activity in cancer. The pathway shown in Figure 5 highlights several genes, including TP53, as a potential target, followed by other genes such as BRCA1, BRCA2, ESRI, PIK3CA, AKT1,

and others. In the literature, these candidate genes are highly relevant to breast cancer. The BRCA gene is a tumor suppressor gene that is associated with gene mutations and increased cancer risk [41]. In addition, genes such as EGFR, PIK3CA, and AKT1 are highly relevant to proliferation, anti-apoptosis, and cell regulation [42,43].

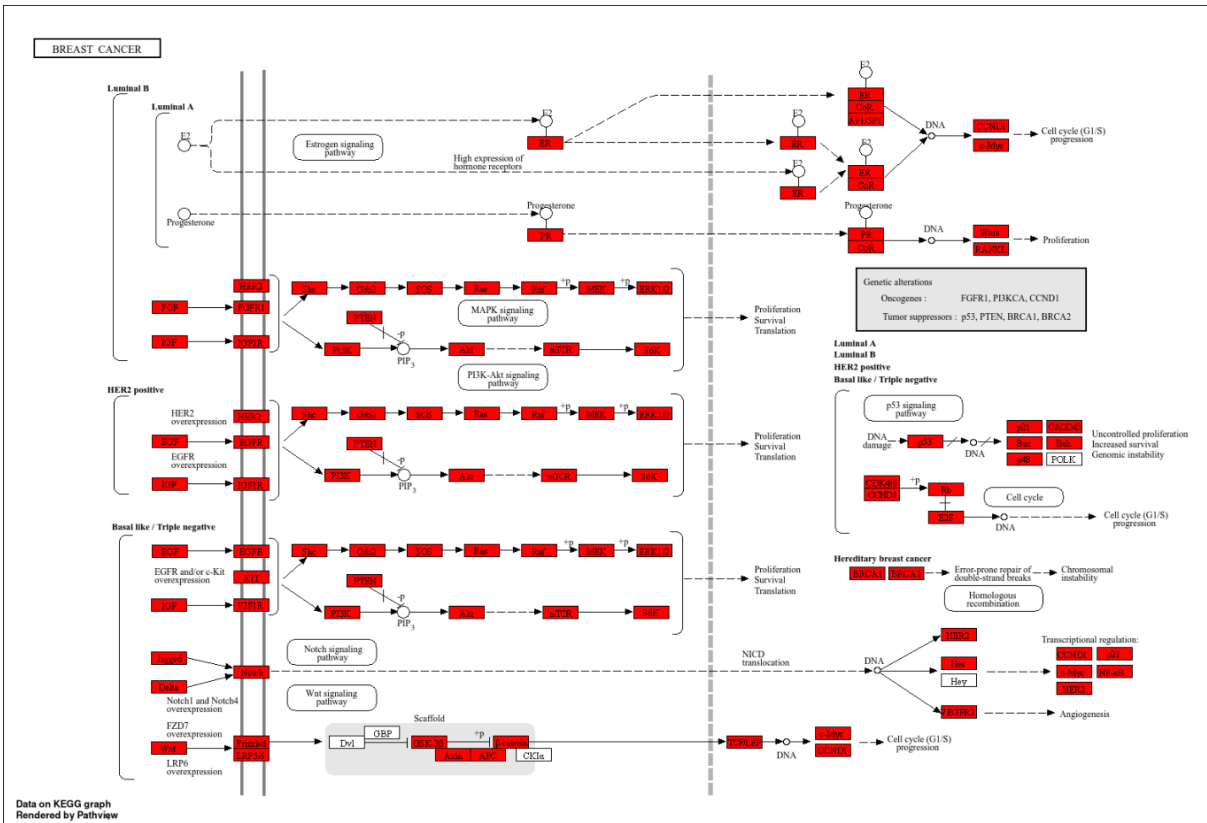


Figure 5 KEGG pathway enrichment analysis highlighting significant breast cancer-related pathways.

Validation of protein targets using KEGG pathway enrichment analysis (**Figure 5**) shows several potential metabolic and disease pathways based on the obtained gene set. One of the best targets is the breast cancer pathway (KEGG ID: hs05224), which is highly significant based on a p -value of 5.8×10^{-45} and a q -value of 2.2×10^{-45} (**Table 1**). The pathway information

obtained also includes several key genes important in the breast cancer signaling system, such as *TP53*, *PIK3CA*, *HER2*, *BRCA1/2*, and *CCND1*. The gene pool obtained can also be identified as playing an important role in the pathways of several other cancers, such as MicroRNAs in cancer, colorectal cancer, and prostate cancer.

Table 1 The best KEGG pathway enrichment analysis of selected breast cancer-related genes.

	ID	Description	p -value	q -value
KEGG	hsa05206	MicroRNAs in cancer	1.83792E-87	2.7859E-85
KEGG	hsa04151	PI3K-Akt signaling pathway	2.55756E-53	1.93836E-51
KEGG	hsa05210	Colorectal cancer	2.02658E-51	1.02395E-49
KEGG	hsa05224	Breast cancer	5.88666E-47	2.23073E-45
KEGG	hsa05215	Prostate cancer	2.27802E-46	6.906E-45

Network pharmacology through enrichment analysis was used to support the holistic evaluation of *M. koenigii* and *P. cablin* [44]. Although *TP53* was determined as a potential target gene, it can also be seen that *M. koenigii* and *P. cablin* have biological functions against other targets. A series of analyses were used to

define the overall function of plant compounds against multiple targets in overcoming cancer [23,39]. By using this method, it will not only focus on one potential target but also expand the holistic analysis in the context of molecular target-based drug development [45].

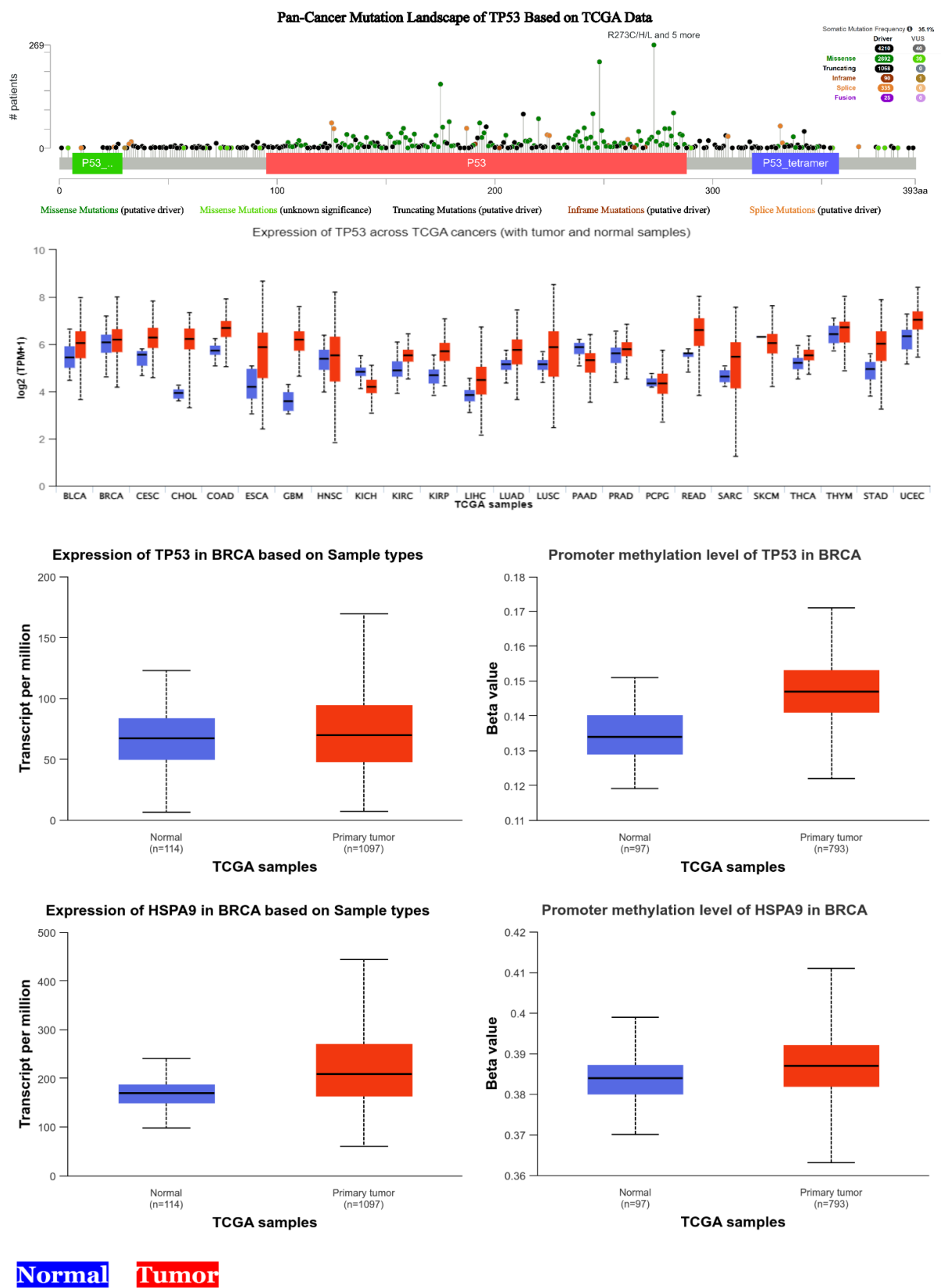


Figure 6 mRNA expression, methylation, mutation analysis of *TP53* and *HSPA9* from the cancer genome atlas (TCGA) analysis.

TCGA

After *TP53* was identified as a potential target, an analysis was conducted to see the differential mRNA expression using The Cancer Genome Atlas (TCGA) analysis. The results were obtained from TCGA samples in the UALCAN database. **Figure 6(A)** shows the expression of *TP53* across TCGA cancers, including tumour and normal cell expression data. The expression of *TP53* in the TCGA results is nearly equivalent to that in normal cells, with a statistical significance (*p*-value) of 6.1×10^{-4} . However, for mortalin, the significance in breast cancer is quite considerable, with a *p*-value of 10^{-12} . This makes mortalin a potential target for further analysis in breast cancer.

The TCGA results (**Figure 6**) show that the expression of p53 in breast cancer is nearly the same as in normal patients. In contrast, the expression of heat shock protein 9 (mortalin) in tumor-bearing patients is higher compared to normal patients. This can be used as a key parameter for the use of *HSPA9*/mortalin in cancer treatment. Overexpression of *HSPA9* is strongly associated with the development of breast cancer [46]. The interaction between p53 and mortalin causes p53 to become inactive [13]. Mortalin has a function similar to murine double minute 2 (MDM2), as a gene that regulates p53 activity [47]. MDM2 functions as the main regulator of p53 by preventing DNA binding and facilitating the degradation of p53 through the ubiquitin-proteasome pathway [48,49]. Meanwhile, mortalin works by binding to p53 and blocking the translocation of p53 into the cell nucleus [50]. This prevents p53, which should drive the cell cycle into the G0 phase, from doing so, due to its interaction with mortalin. Therefore,

one potential strategy for breast cancer treatment is to inhibit mortalin, allowing p53 to translocate into the nucleus [13].

As supplementary data to the expression and methylation analyses, mutation profiling was performed to validate the results of the TCGA analysis via cBioPortal [30]. The results showed that TP53 mutations account for 35.1% of cancer cases, predominantly consisting of missense mutations with non-random distributions and hotspot regions such as R237. Variants such as Y220C were observed to contribute to the majority of cancers, including breast cancer. Mutations in TP53 cause loss of function; therefore, TP53 reactivation is a promising therapeutic strategy to restore its function as a tumor suppressor gene.

Optimization of grid box

In the molecular docking method, all compounds were attached to the segmented protein coordinates. In an effort to create a very small grid box, the grid box coordinates were divided into ten, as shown in **Figure 7**. The results were divided into 2 based on the type of plant: **Figure 7(A)** shows the distribution of RMSD values for *M. koenigii*, while **Figure 7(B)** shows the RMSD distribution for *P. cablin*. The amino acid segments 260 - 262, 266 - 268, and 284 - 286 are potential coordinate points. The RMSD values from docking for all compounds, as shown in the graph, have the smallest values. On average, the three potential segments have RMSD values below 5 Å. However, among the three candidates, the amino acid segment 260 - 262 has the smallest average RMSD distribution value.

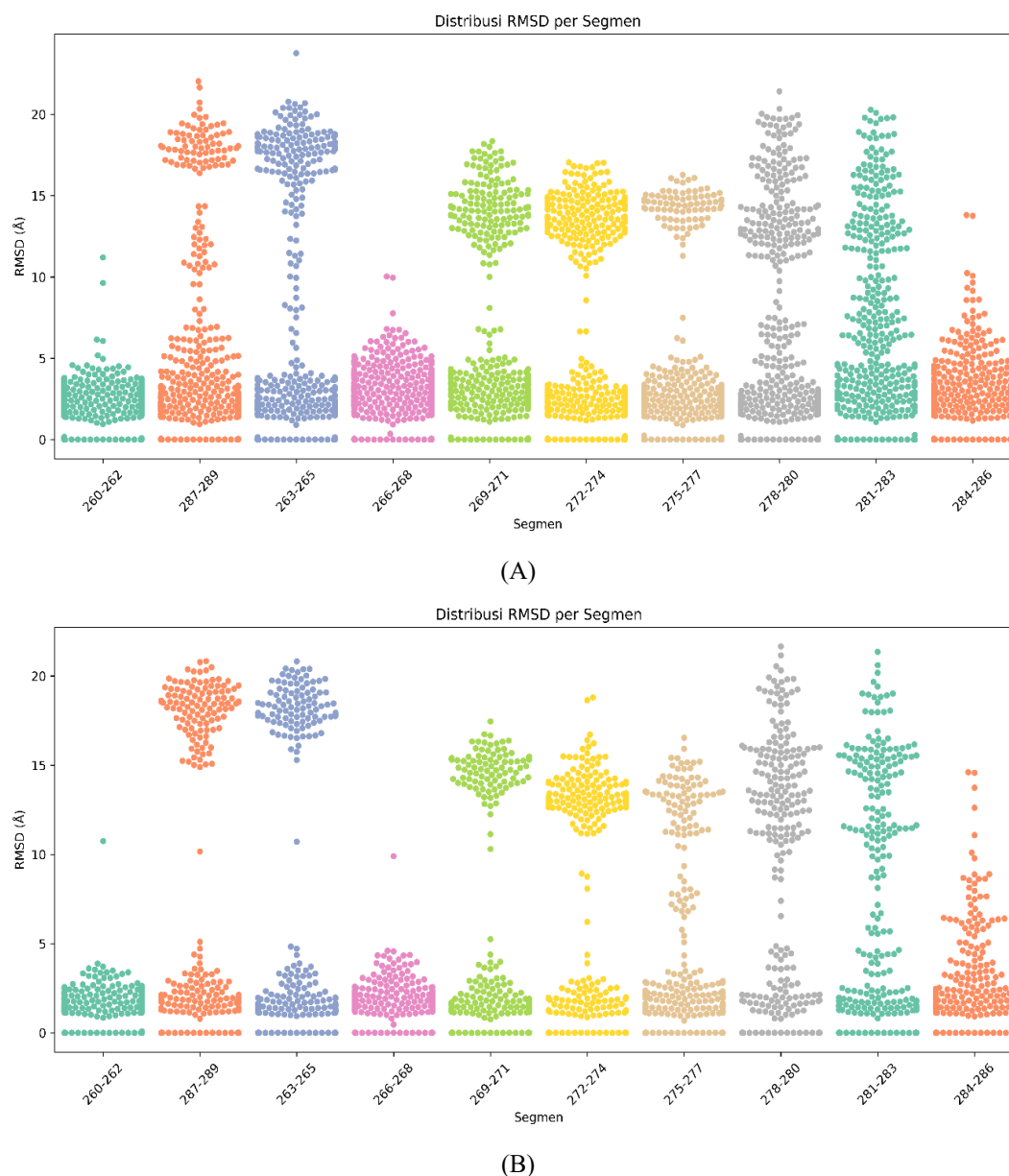


Figure 7 Grid box segmentation and RMSD distribution for docking on mortalin's 260 - 288 residue region. (A) The segmentation docking results of *M. koenigii* and (B) *P. cablin*.

After the potential target for breast cancer treatment was identified using network pharmacology and TCGA analysis, docking tests were conducted to see the binding affinity of the test plant compounds (*M. koenigii* and *P. cablin*) against mortalin. This analysis was done to find the best drug candidate with an effective mechanism to inhibit the active site of mortalin with p53. Based on the UniProt and RCSB databases,

there was no availability of 3D structures with a native natural ligand [32]. Therefore, the grid box determination was not done through a re-docking process but through literature studies on the amino acid attachment points of p53 on mortalin [34]. Two controls, mortaparib plus and embelin, were also used as standard parameters in the docking study.

Table 2 Top five compounds with the strongest binding affinity from *M. koenigii* and *P. cablin*.

	Compound	Pubchem Compound Identifier (CID)	Binding affinity (kcal/mol)	Hydrogen bond	RMSD (Å)
Control	Mortaparib plus	-	-5.9	GLN424	2.064
	Embelin	3218	-5.6	ARG202, ARG218, ASN221	0.528
<i>Murraya koenigii</i>	Bismurrayaquinone A	10364815	-8.1	PHE262	0.088
	8,8"-Biskoenigine	12967046	-7.9	VAL264, PHE262, ASN221	0.163
	21,23:24,25- Diepoxytirucalla-1,7- diene-3,21-diol	163184181	-7.2	ASP233, ARG202	2.771
	1-(2-hydroxy-3-methyl- 9H-carbazol-1-yl)-3- methyl-9H-carbazol-2-ol	10408200	-7.1	ARG218	0.089
	Bicyclomahanimbicine	77994099	-7.1	-	1.681
<i>Pogostemon cablin</i>	Apigenin 7-(6"-p- coumarylglucoside)	44257826	-6.9	THR224, ASN221, ARG202, LYS206, PHE262	2.465
	Apigenin	5280443	-6.4	THR224, ASN 221, VAL219, GLY260	2.070
	Rhamnetin	5281691	-6.3	ASP233, GLN424, VAL219	1.881
	Ombuin	5320287	-6.2	THR224, ASP233	2.203
	Pachypodol	5281677	-6.1	ASP233, PHE262, ASN221	2.108

Molecular docking

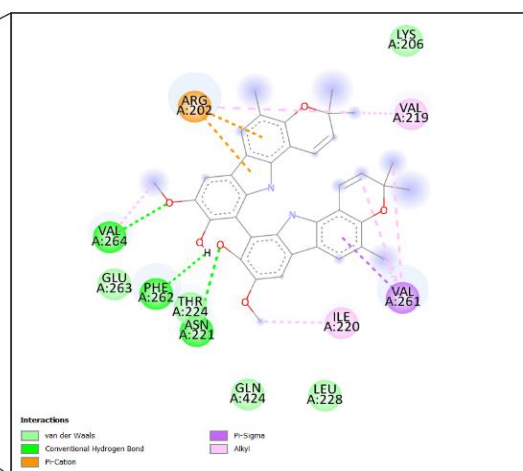
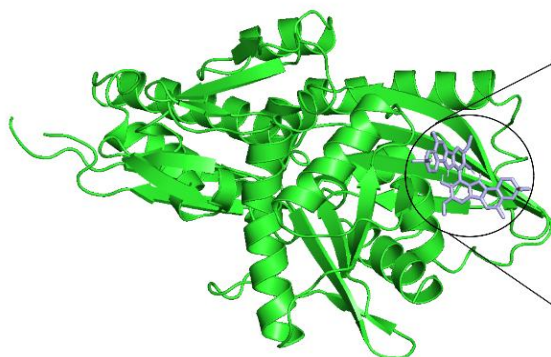
Molecular docking was performed on the mortalin complex (PDB ID: 4KBO) with compounds from *nilam* (patchouli) and *temurui* (curry leaf) plants. The results of virtual screening from molecular docking can be seen in the table. **Table 2** shows the results for binding affinity, root mean square deviation (RMSD), and protein-ligand interactions for the best compounds. The RMSD value represents the average deviation of each atom's position relative to the reference structure. In

general, RMSD values below 2 Å are often associated with good structural stability, whereas values up to 3 Å are typically considered acceptable [51]. This parameter is used to evaluate the ligand's binding position in relation to the desired coordinate points. Meanwhile, the parameters of intermolecular interactions are evaluated based on the number of bonds present in the protein-ligand system. Hydrogen bonds are one of the most important types of bonds in the protein-ligand system [52]. The number of hydrogen bonds reflects the

strength of the bond, which is further reinforced by other types of bonds. **Figure 8** shows the best candidate

compounds from each plant, which are apigenin 7-(6"-p-coumarylglucoside) and 8,8"-biskoenigine.

A.



B.

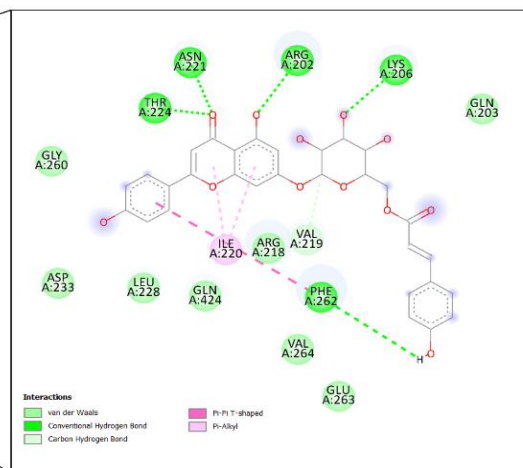


Figure 8 Three-dimensional (3D) and two-dimensional (2D) visualisation of ligand-mortalin interaction using BIOVIA Discovery Studio. (A) The visualization of 8,8"-biskoenigine and (B) apigenin 7-(6"-p-coumarylglucoside).

The two controls were selected based on validated literature sources *in vitro* and *in vivo*. Embelin is a phenolic compound found in *Embelia ribes* Burm. f. (Primulaceae) [53]. In-silico, *in vitro*, and *in vivo* tests found that embelin can inhibit mortalin and restore the function of p53 [53,54]. Embelin also has the potential to suppress various signaling pathways in cancer, such as STAT3, TNF- α , and metastatic pathways [54,55]. Another control is mortaparib plus, which also functions as an inhibitor of mortalin and other targets such as PARP1 [56]. Both controls are indicated to have anti-cancer functions in several potential pathways and reactivation of p53 function [50]. Therefore, both compounds were used as controls for analysis in molecular docking and molecular dynamics.

The best grid box was shown in the 260 - 262 amino acid segment, which was obtained from the graph results (**Figure 7**). This was important to do because if a grid box was made for all amino acids, which is 260 - 288 AA, it would result in a grid box area that is very large. The huge grid box makes the docking strategy similar to blind docking. Blind docking gives less accurate results and is often misused in molecular biology analysis [57]. To avoid blind docking, a grid box division for smaller amino acids was performed. Therefore, the grid box was divided into several segments, and the best point was determined. By using a grid box in the 260 - 262 AA segment, the docking results of the candidate compounds showed relatively small RMSD values.

The best compounds from the docking results were 8,8''-biskoenigine and apigenin 7-(6''-p-coumarylglucoside). As seen in **Table 2**, the binding affinity values of the test compounds were more negative compared to the control, mortaparib plus. Based on the Autodock Vina algorithm, the smaller the binding affinity value, the stronger the bond in a protein-ligand complex system [58]. However, it should be understood that the docking method needs to be supported with further analysis, such as molecular dynamics. This is done to understand the dynamics of each molecule, even down to the atomic level, and its significance in the stability of the protein-ligand interaction [36].

The docking results showed that the compound 8,8''-biskoenigine had a binding affinity value of -7.9 kcal/mol and an RMSD of 0.163 Å. Meanwhile, the docking result for the compound apigenin 7-(6''-p-coumarylglucoside) had a binding affinity value of -6.9 kcal/mol with an RMSD of 2.465 Å. Two-dimensional visualizations can also be seen in **Figure 8**, which show several hydrogen bonds and other types of interactions. The docking results show interactions such as van der Waals, hydrogen, pi-cation, pi-sigma, and alkyl bonds. There are several hydrogen bonds at residues Val A:264, Phe A:262, and Asn A:221. Based on data analysis, some residues often interact with all candidate compounds. Residues Ile 220, Phe 262, Thr 224, Asn 221, Val 261, and Leu 228 were observed multiple times forming bonds with compounds from *M. koenigii* and *P. cablin* plants. This shows that these residues have potential functional roles in the binding site. Hydrogen bonds do play a key role in determining a strong interaction, but several other bonds also contribute to the creation of a protein-ligand complex [59]. The next results from molecular dynamics show further analysis to observe the dynamics of the protein-ligand complex.

Pharmacokinetic and toxicity (ADMET) analysis

Based on the results of the docking screening, several selected compounds exhibited binding affinities comparable to or lower than those of the reference

inhibitor. For further evaluation to assess drug feasibility, ADMET analysis was conducted to examine the bioavailability and safety of the candidate compounds [60]. Regarding absorption, several points are worth noting for the drug candidate compounds. Compound 8,8''-biskoenigine exhibited extremely high intestinal absorption of 100%. However, for the compound apigenin 7-(6''-p-coumarylglucoside), absorption (61.71%) and permeability were low. This may be attributed to the relatively large molecular size exceeding 500 Da, which can limit the passive diffusion of the compound across the intestinal membrane [61].

In other parameters, the distribution indicates that the compound has limited permeability across the blood-brain barrier and into the central nervous system. This is supported by the CNS permeability values (log PS), where apigenin 7-(6''-p-coumarylglucoside) showed the lowest value (-3.974), followed by 8,8''-biskoenigine (-2.584). This indicates a low tendency for distribution into the central nervous system. Meanwhile, regarding metabolism, the candidate compounds do not inhibit CYP1A2 but interact with CYP3A4, indicating the involvement of these enzymes in the metabolic processes.

Drug screening is primarily focused on oral safety, so toxicity analysis is required to assess drug suitability. Several parameters are used, namely the AMES test, hERG I, skin sensitization, and hepatotoxicity. Both drug candidate compounds showed no potential for toxicity in these four parameters. The AMES test was conducted to assess mutagenic potential at the genetic level to prevent genetic diseases [62]. Meanwhile, skin sensitization and hepatotoxicity tests were used to assess potential for allergic reactions and liver damage.

Overall, the candidate compounds in this study are not small molecules, which have limitations in certain pharmacokinetic parameters. However, these parameters are not the sole determinants of a compound's potential as a drug. Other factors to consider include, among others, low toxicity and further evaluation of stability and drug-drug interactions [63].

Table 3 ADME and toxicity profile of selected compounds.

Category	Parameter	Unit	Pubchem Compound ID (CID)			
			Mortaparib+	Embelin	apigenin 7-(6''-p-coumarylglucoside)	8,8''-biskoenigine
Absorption	Caco2 permeability	Log Papp in 10 ⁻⁶ cm/s	1.332	0.503	0.025	-0.448
	Intestinal absorption	%	94.978	89.155	61.715	100
	Water solubility	Log mol/L	-4.276	-4.511	-3.046	-3.011
Distribution	BBB permeability	Log BB	0.364	-0.06	-1.66	-1.413
	CNS permeability	Log PS	-1.907	-2.625	-3.974	-2.584
Metabolism	CYP3A4 substrate	Yes/No	Yes	No	Yes	Yes
	CYP1A2 inhibitor	Yes/No	Yes	No	No	No
Excretion	Total clearance	Log ml/min/kg	0.442	1.518	0.051	-1.737
	Renal OCT2 substrate	Yes/No	No	No	No	No
Toxicity	AMES toxicity	Yes/No	No	No	No	No
	hERG I inhibitor	Yes/No	No	No	No	No
	Skin sensitisation	Yes/No	No	Yes	No	No
	Hepatotoxicity	Yes/No	Yes	No	No	No

Molecular dynamics

RMSD (Root Mean Square Deviation) is a score that provides information on conformational changes in a macromolecule after the interaction process with a particular ligand. RMSD can also be used to evaluate the standard deviation of conformational changes and sample stability under simulation conditions. The standard RMSD value for a protein receptor (RMSD Bb) is ≥ 3 Å [64], as a sign that the protein has undergone conformational changes that differ from its native condition. The standard value < 2 Å is generally used for ligand configuration RMSD [55].

The RMSD values of the complexes (**Figure 9(A)**) from the four samples generally have a similar fluctuation pattern to the RMSD of ligand movement (RMSD LigMov), where a significant increase in values above 5 Å occurs at 10 - 40 ns of simulations of apigenin 7-(6''-p-coumarylglucoside) and mortaparib plus control samples (**Figure 9(D)**). Meanwhile, the backbond RMSD (Bb) values show that both complexes exhibit the highest values between 3 - 4 Å (**Figure 9(B)**). This trend indicates that the instability of the complex does not occur solely due to the ligand's inhibitory effect, but also reflects the instability of the binding of both ligands

to mortalin. In addition, only the apigenin 7-(6''-p-coumarylglucoside) and embelin ligand configuration RMSD values exceed 2 Å (**Figure 9(C)**). Thus, it can be predicted that, although the mortaparib plus control releases its binding to the mortalin binding pocket, its conformation remains unchanged, unlike apigenin 7-(6''-p-coumarylglucoside). Meanwhile, the 8,8''-biskoenigine compound is predicted to have conformational stability and bind to mortalin.

Furthermore, based on the number of hydrogen bonds in the complex (solute HBond), the mortalin-apigenin 7-(6''-p-coumarylglucoside) and mortalin-8,8''-biskoenigine complexes have higher average and maximum HBond values compared to the mortalin complex with both drug controls (**Figure 9(E)**). The same pattern is also seen in the number of HBonds formed between the ligand and the protein (**Figure 9(F)**). Although the number of HBonds of the ligand and the average HBond of the biskoenigin complex are slightly lower compared to apigenin 7-(6''-p-coumarylglucoside), the maximum value of the number of HBonds formed in the mortalin-8,8''-biskoenigin complex is higher. Based on the MMPBSA value, which describes the binding energy of the ligand, it can also be

seen that the binding affinity of the biskoeningine compound is better than apigenin 7-(6"-p-coumarylglucoside) and embelin (**Figure 9(I)**). This tendency is predicted to help stabilize the movement of the 8,8"-biskoeningine ligand and the mortalin-8,8"-biskoeningine complex.

The inhibitory effect of each ligand can be indirectly observed based on solvent-accessible surface area (SASA) and radius of gyration (RG). SASA (Solvent Accessible Surface Area) is described as the surface area of a protein that is sufficiently exposed or accessible to interact with surrounding solvent molecules Miller *et al.* 1987. This can provide insights into conformational changes, protein-ligand interactions, and protein-protein interactions. SASA is considered a determining factor in protein stability and folding/unfolding studies and is characterized by a hypothetical solvent sphere centered on the molecular surface, with van der Waals contacts to the molecular surface [65]. Higher SASA values indicate an expansion of the protein structure. In the analysis of potential inhibitor compounds, large fluctuations in SASA values are expected during simulation, indicating decreased complex stability and conformational changes, such as unfolding [66]. The SASA patterns of the four compounds showed fluctuations with a continuous increasing trend. Furthermore, the radius of gyration (RG) is a parameter that describes the equilibrium conformation of the entire simulation system. The RG value can also be interpreted as the radius of gyration of the dynamic motion of a complex, either protein-protein or protein-compound, with respect to the solvent, and thus represents a way to predict simulation-based estimates of sample solubility in aqueous solutions or solvents [67]. The lowest value indicates the folded state

of the protein, while the highest value indicates its conformational state when unfolded [68]. The highest fluctuations occurred in the complex of mortalin with mortaparib plus and apigenin 7-(6"-p-coumarylglucoside) due to significant ligand release and movement (**Figure 9(G)**). Meanwhile, a more stable but increasing fluctuation pattern occurred in the complex of mortalin with embelin and 8,8"-biskoeningine. This trend suggests that the inhibitory effect of apigenin 7-(6"-p-coumarylglucoside) resembles mortaparib plus, while 8,8"-biskoeningine shows inhibitory activity similar to embelin, which, although it takes longer, is much more stable. This hypothesis is also supported by the similarity of the RG patterns of each logan pair (**Figure 9(H)**).

RMSF (Root Mean Square Fluctuation) is a score that provides more detailed information about conformational changes because it is linked to fluctuations that occur at the amino acid residue level of the target protein. The results show that the amino acids at the mortalin inhibition site, namely Phe262, Val264, and Asn221, are maintained stable in all complexes. The inhibitory effect, as evidenced by an increase in the RMSF value, is observed at nos. 260 - 350. Residues Glu313, Lys316, Ser320, Gly388, and Arg391 are known to be binding sites for adenosine diphosphate (ADP), which plays a role in the catalytic activity of mortalin [69]. The increase in RMSF values in this key amino acid region indicates the inhibition mechanism of the four ligands. Mortalin-mortaparib plus has the highest average RMSF value (**Figure 9(J)**). Meanwhile, the mortalin-8,8"-biskoeningine complex shows the second-highest maximum RMSF value after the mortaparib control.

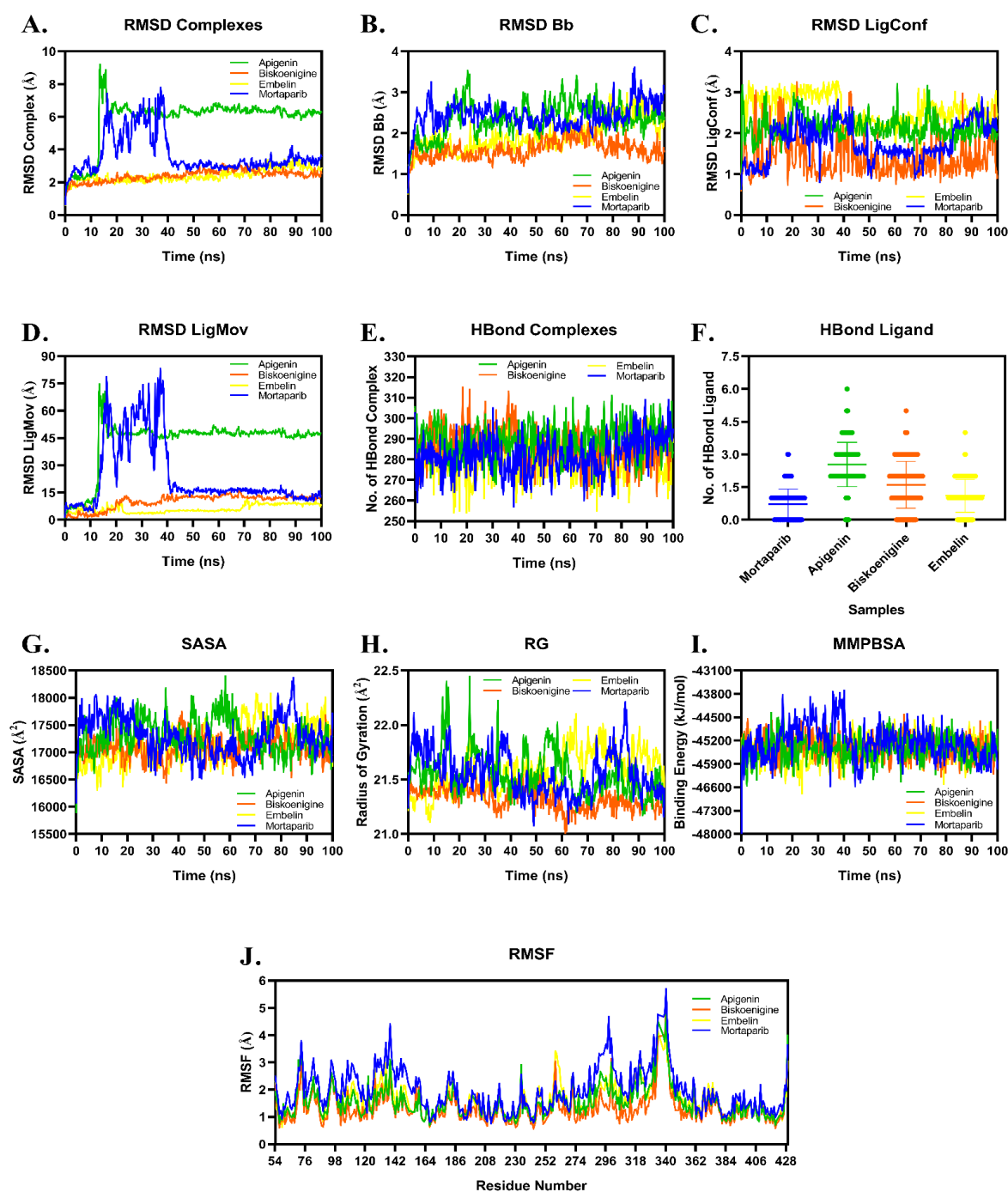


Figure 9 Molecular dynamics simulation analysis. (A) root mean square deviation of complex (RMSD Complexes), (B) root mean square deviation of backbone (RMSD Bb), (C) root mean square deviation of ligand configuration (RMSD LigConf), (D) root mean square deviation of ligand movement (RMSD LigMov), (E) hydrogen bond count complex (HBond Complexes), (F) hydrogen bond count ligand (HBond Ligand), (G) solvent-accessible surface area (SASA), (H) Radius of Gyration (RG), (I) binding energy (MMPBSA), and (J) root mean square fluctuation (RMSF).

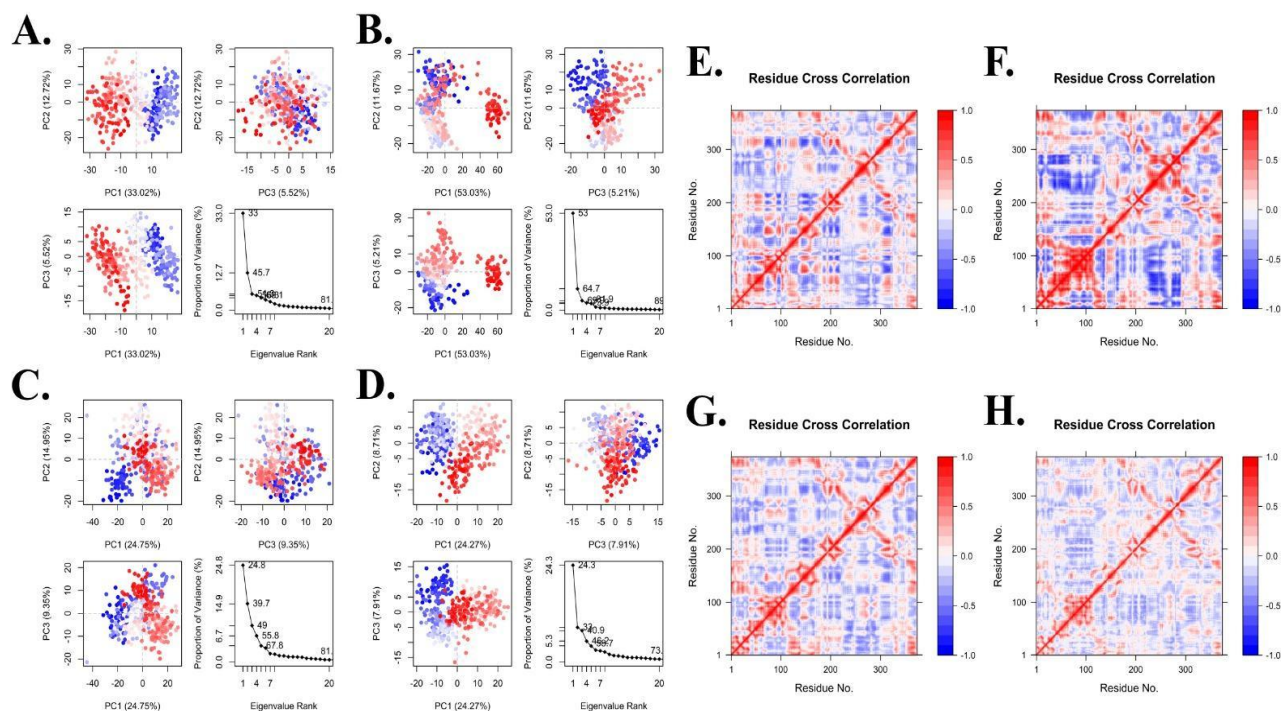


Figure 10 Principal component analysis (PCA) (A - D) and dynamics cross-correlation matrix (DCCM) (E - H) plots of protein-ligand complexes. (A) Embelin, (B) Mortaparib plus, (C) Apigenin, (D) Biskoenigine, (E) Embelin, (F) Mortaparib plus, (G) Apigenin, (H) Biskoenigine.

In aggregate, the docking analysis and 100-ns molecular dynamics simulations converge on 8,8"-biskoenigine as the most promising mortalin inhibitor, showing stronger binding affinity than the positive controls, favorable interactions with critical functional residues (Phe262, Val264, Asn221), and lower conformational fluctuation combined with more favorable binding energy profiles. Apigenin 7-(6"-p-coumarylglucoside) consistently ranked as a second-line candidate with slightly weaker yet pharmacologically attractive stability, suggesting that both scaffolds as prime targets for experimental validation.

PCA and DCCM

In line with other trajectory analyses such as RMSDBb, RMSDLigMov, RMSDLig, and H-Bond, the results of the analysis of conformational fluctuation variation of the mortalin-8,8"-biskoenigine complex showed an increase in protein conformational stability characterized by the low value of the cumulative variation of the first two PCs, PC1 (24.27%) and PC2 (8.71%) of 32.98%. This result is much lower than the mortalin complex against the two control ligands, mortaparib plus and embelin, with a cumulative value of

the first two PCs of 64.7% and 45.74%, respectively (**Figure 10**). In addition, the test ligand Apigenin 7-(6"-p-coumarylglucoside) had a cumulative value of the first two PCs of 39.7%, slightly higher than the first two PCs of 8,8"-biskoenigine. Also, the DCCM showed a positive movement towards increased stability in 8,8"-biskoenigine characterised by increased positive correlation (red) in residues close to the diagonal and decreased anticorrelation plot (blue) when compared to the mortaparib complex plot with high anticorrelation plot (blue). In addition, epigenein and embelin tend to have similar correlation distributions.

PCA and DCCM successfully validated the results of other MD trajectories which showed an increase in the stability of the mortalin and 8,8"-biskoenigine complexes, through a decrease in the variation of conformational fluctuations during simulations as well as an increase in the correlation of intermolecular interactions in the protein structure during simulations [36]. Combined trajectory analysis of molecular dynamics through four main parameters: RMSD, RMSF, RG, HBond and elaboration with PCA and DCCM provide a perspective for protein-ligand binding stability [21]. These results strengthen the analysis that

the compound 8,8"-biskoenigine is the best candidate. However, apigenin 7-(6"-p-coumarylglucoside) is also a compound that deserves further testing, to ensure its accurate function in molecular biology functions.

Thus, 8,8"-biskoenigine and apigenin 7-(6"-p-coumarylglucoside) from *M. koenigii* and *P. cablin* show potential as candidate inhibitors of mortalin in breast cancer therapy. The best drug candidates are based on stability and the potential for strong binding in the protein-ligand complex system. The following series of bioinformatics analyses need to be further validated through experimental tests to achieve higher accuracy.

This study has several strengths, including the integration of multi-database network pharmacology, stringent KEGG/GO enrichment, TCGA-based expression and methylation analyses, optimized grid definition to avoid blind docking artefacts, and orthogonal validation of docking results using long-timescale molecular dynamics, PCA, and DCCM. Nonetheless, the work remains purely computational and is constrained by the quality and completeness of public databases, the absence of an experimentally co-crystallized mortalin-ligand structure, and the well-known limitations of current scoring functions and force fields in fully capturing entropic and solvent effects. Consequently, the predicted affinity and stability of 8,8"-biskoenigine and apigenin 7-(6"-p-coumarylglucoside) should be interpreted as hypothesis-generating rather than definitive proof of efficacy.

Based on a translational perspective, the identification of mortalin-targeting phytochemicals from widely used medicinal plants is attractive, as these compounds could be developed either as lead structures for semi-synthetic optimization or as standardized phytopharmaceutical formulations. Future studies should therefore prioritize experimental validation of mortalin inhibition and p53 reactivation in relevant breast cancer cell lines, dose-response characterization, and selectivity profiling against non-tumorigenic mammary epithelial cells. In parallel, structure-activity relationship (SAR) studies and medicinal chemistry optimization of the 8,8"-biskoenigine and apigenin glycoside scaffolds may enhance potency, improve ADMET properties, and ultimately support preclinical in vivo evaluation.

Conclusions

In conclusion, this study provides computational evidence to support future experimental investigations of bioactive compounds from *Muraya koenigii* and *Pogostemon cablin* in the context of breast cancer using an integrated *in silico* approach. Network pharmacology analysis identified the TP53 target as a key factor in breast cancer, suggesting a therapeutic strategy to disrupt the mortalin-p53 interaction to facilitate p53 translocation into the nucleus. Through a comprehensive workflow combining network pharmacology, molecular docking, molecular dynamics, and ADMET, 8,8'-biskoenigine (CID: 12967046) and apigenin 7-(6"-p-coumarylglucoside) (CID: 44257826) were identified as promising compounds. The candidate compounds exhibited lower binding affinities compared to the control, indicating promising binding strength. Molecular dynamics simulation analysis also demonstrated stable interaction profiles, with patterns comparable to those observed for Mortaparib Plus and Embelin as reference compounds. These findings support the reliability of the computational approach while highlighting its application in the prioritization of potential lead compounds during the early stages of drug discovery. However, further validation through in vitro and in vivo studies is required to confirm their biological activity and clinical relevance.

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Declaration of Generative AI in Scientific Writing

No use of generative AI in scientific writing

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Supplementary Materials

The supplementary file contains the research data to ensure transparency and reproducibility. All data can be accessed via the following link: <https://doi.org/10.5281/zenodo.20032280>