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Network Pharmacology and Pathway Enrichment Analysis of *Murraya koenigii* Phytochemicals Reveals Multitarget Potential Against Breast Cancer

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ABSTRACT

Murraya koenigii (curry leaf) is a medicinal plant traditionally used in South Asian medicine and known to contain bioactive carbazole alkaloids with reported anticancer potential. This study aimed to evaluate the molecular mechanisms and multi-target potential of five key phytochemicals from *M. koenigii* such as: Koenimbine, Koenidine, Mahanimbine, Murrayazoline, and O-methylmurrayamine A, in the context of breast cancer, using a network pharmacology and pathway enrichment approach. Target prediction was performed using SwissTargetPrediction, and breast cancer–associated genes were retrieved from the GeneCards database. Intersection analysis revealed 21 overlapping genes, including major oncogenic drivers such as EGFR, ERBB2, PIK3CA, AKT1, CDK2, and AR. These targets are known to play central roles in tumor cell proliferation, hormone receptor signaling, survival, and therapeutic resistance. Functional pathway enrichment using ShinyGO demonstrated that the overlapping genes were significantly enriched in key breast cancer–related pathways, including PI3K-Akt signaling, MAPK signaling, endocrine resistance, ErbB signaling, and cell cycle control. Network visualization in Cytoscape further revealed that the phytochemicals engage interconnected targets, supporting the hypothesis of a polypharmacological mechanism. Taken together, these findings suggest that *M. koenigii* phytochemicals possess the potential to modulate multiple hallmarks of breast cancer through simultaneous interaction with diverse but functionally convergent targets. The study provides a systems-level framework for understanding the therapeutic promise of *M. koenigii* and establishes a strong foundation for further experimental validation in breast cancer models, particularly those resistant to conventional monotherapies.

Keywords: breast cancer, *Murraya koenigii*, network pharmacology, phytochemicals, target prediction.

INTRODUCTION

Breast cancer remains the most commonly diagnosed cancer and a leading cause of cancer-related death among women worldwide. Recent global data indicates that despite advancements in screening and therapy, disparities persist in early detection and treatment accessibility, especially in low-resource settings [1]. The heterogeneity of breast cancer—including its subtypes like HER2-positive, triple-negative, and luminal types—further complicates treatment strategies [2], necessitating innovative

therapeutic strategies that can target multiple pathways simultaneously.

The exploration of bioactive compounds from medicinal plants presents a promising avenue for developing multitarget anticancer agents with fewer side effects [3]. These compounds, which include flavonoids, alkaloids, terpenoids, and phenolic compounds, have demonstrated significant pharmacological activities against various diseases, including cancer [4]. *Murraya koenigii*, commonly known as curry leaf, is a medicinal plant extensively used in traditional medicine across South and Southeast Asia. This plant is rich in diverse classes of phytochemicals, including alkaloids [5], flavonoids [6], terpenoids [6], and carbazoles [7], which contribute to its wide range of pharmacological activities. Several phytochemical investigations have revealed that *M. koenigii* is a rich source of carbazole alkaloids, such as murrayazoline, O-methylmurrayamine A, mahanimbine, koenidine, and koenimbine [8]. *M. koenigii* has demonstrated various pharmacological activities such as anti-inflammatory [9], antioxidant [6], and anticancer properties [10]. However, the molecular mechanisms underlying its anticancer activity, particularly against breast cancer, remain incompletely understood.

The pharmacological effects of these phytochemicals are believed to be mediated through multi-target mechanisms, which makes them ideal candidates for network pharmacology investigations [11]. Network pharmacology is an emerging field that leverages the complexity of biological networks to understand drug actions and interactions at multiple levels [12]. This approach is particularly useful for studying phytochemicals, which often exhibit multi-target mechanisms, making them ideal candidates for network pharmacology investigations [11]. Network pharmacology integrates pharmacological data with systems biology to elucidate the complex interactions between drugs, targets, and disease-associated genes [13]. This approach enables the identification of hub targets, functional modules, and pathway-level insights that are often missed in single-target drug discovery [14]. Complementing network pharmacology, pathway enrichment analysis is crucial for identifying functional groups of genes or proteins and understanding their roles in biological processes and disease mechanisms [15]. Through the integration of compound–target prediction, protein–protein interaction networks, and KEGG or GO-based pathway analysis, researchers can uncover key molecular mechanisms and predict the synergistic or pleiotropic effects of multi-component herbal therapeutics [16].

In this study, we employed a network pharmacology approach combined with pathway enrichment analysis to systematically investigate the anti-breast cancer potential of selected phytochemicals from *M. koenigii*. Our goal is to identify the molecular targets and signaling pathways modulated by these compounds, offering a mechanistic basis for their multitargeted therapeutic effects. The results provide novel insights into the systems-level action of *M. koenigii* constituents and support their development as promising candidates for integrative breast cancer therapy.

MATERIALS & METHODS

1. Phytochemical Selection

Five bioactive carbazole alkaloids derived from *M. koenigii*—namely Koenimbine, Mahanimbine, Koenidine, Murrayazoline, and O-methylmurrayamine A—were selected based on their previously reported anticancer and cytotoxic potential. These compounds were identified through literature mining of peer-reviewed articles and confirmed via compound entries in PubChem, ensuring structural availability and unique compound identifiers (CIDs). The SMILES (Simplified Molecular Input Line Entry System) notation for each compound was retrieved from PubChem to serve as the input format for target prediction tools.

2. Target Prediction

The SwissTargetPrediction platform (http://www.swisstargetprediction.ch/) was utilized to predict potential human protein targets for each compound based on their SMILES representation. The tool leverages a combination of 2D and 3D molecular similarity to known ligands with experimentally validated targets. Predictions were restricted to the Homo sapiens species. Target lists for each compound were downloaded in tabular format and filtered to include only those targets with a probability score ≥ 0.1 to reduce noise and ensure biological relevance. The target lists were then consolidated across all five compounds to generate a unified candidate target pool for downstream analysis.

3. Retrieval of Breast Cancer-Associated Genes

To identify therapeutic relevance in the context of breast cancer, disease-associated genes were collected from publicly accessible databases GeneCards (https://www.genecards.org/) Using the search term "breast cancer", gene lists were downloaded and scored based on relevance. For GeneCards, genes with a relevance score > 10 were retained. Redundant entries were removed, and the resulting gene list was curated to ensure high-confidence cancer relevance.

4. Overlapping Target Identification and PPI Network Construction

To identify key targets of *M. koenigii* phytochemicals that are also associated with breast cancer, an intersection analysis was performed between the predicted compound targets and the curated breast cancer gene list. This was done using Venny comparison tools (https://bioinfogp.cnb.csic.es/tools/venny/) and later validated manually. The resulting overlapping genes were submitted to the STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins) (https://string-db.org/) to construct a protein–protein interaction (PPI) network. The interaction network was built using a minimum interaction score of 0.7 (high confidence) and limited to human proteins.

5. Pathway Enrichment Analysis

To investigate the biological relevance of the identified gene targets, pathway enrichment analysis was performed using ShinyGO v0.76 (http://bioinformatics.sdstate.edu/go/), a web-based tool that integrates KEGG and Reactome pathway databases. ShinyGO employs a hypergeometric test to assess pathway overrepresentation, and p-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) method. A q-value (adjusted p-value) threshold of < 0.05 was applied to identify statistically significant pathways. The output included ranked KEGG pathways based on fold enrichment and FDR values, as well as pathway diagrams with mapped genes and dot plots summarizing the most enriched pathways. This analysis enabled the identification of core cancer-related signaling routes such as PI3K-Akt, MAPK signaling, endocrine resistance, and cell cycle regulation, all of which are relevant to breast cancer progression and treatment resistance. These enriched pathways were used to interpret the network pharmacology results and to explore how *M. koenigii* compounds may exert multi-target effects through coordinated modulation of breast cancer–associated molecular pathways.

6. Network Visualization

The integrated compound–target relationships were visualized using Cytoscape v3.9.1, an open-source software platform for visualizing molecular interaction networks. The visualization was constructed as a bipartite network, where phytochemical compounds were represented as hexagonal nodes and predicted breast cancer–related target genes as elliptical nodes. Edge connections between compounds and genes were based on target prediction results from SwissTargetPrediction, filtered by overlapping relevance to breast cancer as identified through GeneCards. The network

layout was manually arranged to enhance readability and exported as a high-resolution image for inclusion in the final report. Node attributes such as shape and color were assigned to distinguish compound types and target classes, enabling visual differentiation between compound–gene interactions.

RESULT AND DISCUSSION

Phytochemical Selection and In Silico Target Profiling of *Murraya koenigii* Constituents in Breast Cancer

Table 1 shows five major carbazole alkaloids were selected from *M. koenigii* leaves: Koenimbine, Mahanimbine, Koenidine, Murrayazoline, and O-methylmurrayamine A. These compounds were previously isolated and structurally characterized via spectroscopic methods (1D and 2D NMR) as reported by Arun et al. [8]. In that study, the compounds demonstrated significant in vitro anticancer activity, particularly murrayazoline and O-methylmurrayamine A, which were shown to induce apoptosis in DLD-1 colon cancer cells via mitochondrial membrane depolarization, reactive oxygen species (ROS) regulation, and downregulation of the Akt/mTOR signaling pathway.

Although the original study emphasized colon cancer models, the shared molecular targets and signaling pathways such as PI3K-Akt, mTOR, and Bcl-2 family proteins are also well-established in breast cancer progression [17,18]. Therefore, these compounds were selected for further target prediction and network pharmacology analysis in the context of breast cancer, using bioinformatics tools. Canonical SMILES strings and PubChem CIDs were retrieved for each compound to support downstream in silico analyses.

Table 1. Canonical SMILES and PubChem CIDs of selected *M. koenigii* phytochemicals based on their previously reported anticancer activity [8]

Bioactive Compounds	CID	Canonical SMILES
Koenimbine	97487	CC1=CC2=C(C3=C1OC(C=C3) (C)C) NC4=C2C=C(C=C4) OC
Murrayazoline	375143	CC1=CC2=C3C4=C1OC5(CCC(C4C5) C(N3C6=CC=CC=C62) (C)C) C
O-methylmurrayamine A	14892681	CC1=CC2=C(C3=C1OC(C=C3) (C)C) NC4=C2C=CC(=C4) OC
Koenidine	278055	CC1=CC2=C(C3=C1OC(C=C3) (C)C) NC4=CC(=C(C=C42) OC) OC
Mahanimbine	167963	CC1=CC2=C(C3=C1OC(C=C3) (C)CCC=C(C)C) NC4=CC=CC=C42

The five selected phytochemicals from Murraya koenigii—Koenimbine, Koenidine, Mahanimbine, Murrayazoline, and O-methylmurrayamine A demonstrated diverse target profiles with relevance to breast cancer pathways, as predicted using SwissTargetPrediction (probability ≥ 0.1). These results reveal potential multitarget mechanisms that could underlie the reported anticancer effects of these compounds.

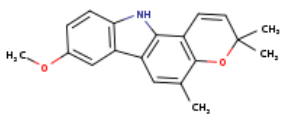
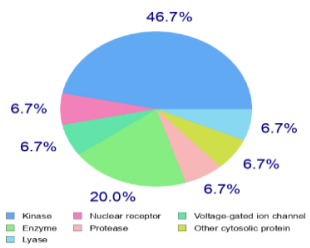
1. Koenimbine was predicted to interact with several critical regulators of breast cancer, including EGFR, AKT1, CDK4, and ERBB2 (HER2). These targets are well-established in breast tumorigenesis, especially in hormone receptor-positive and HER2-positive subtypes [19]. EGFR and AKT1 are central nodes in the PI3K-Akt and MAPK pathways, mediating cell proliferation, migration, and survival [20]. CDK4, a key regulator of G1 phase progression, is also a validated drug target in breast cancer [21]. The broad interaction profile of Koenimbine suggests strong potential for multitarget modulation across growth and survival signaling axes.
2. Koenidine, on the other hand, showed a distinct target spectrum involving Platelet-Derived Growth Factor Receptor Alpha (PDGFRA), 6-Phosphofructo-2-Kinase/Fructose-2,6-Bisphosphatase 3 (PFKFB3), CLK1, and DYRK2. These proteins are associated with angiogenesis, glycolytic

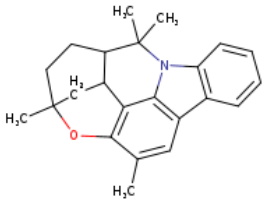
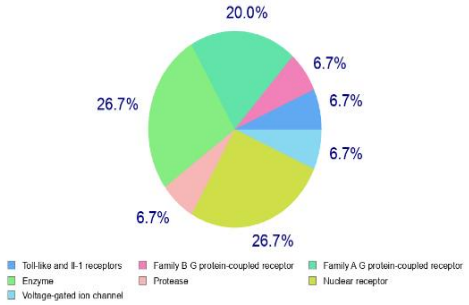
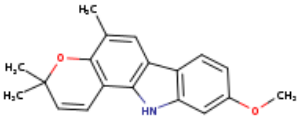
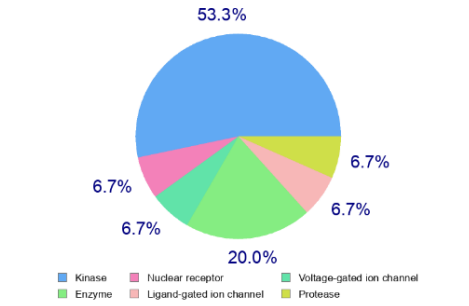
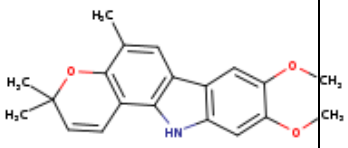
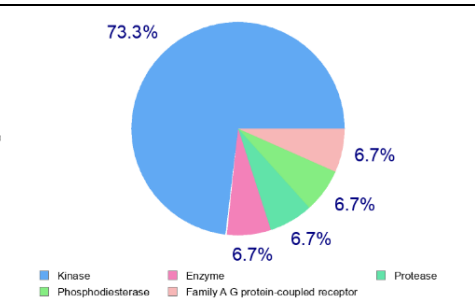
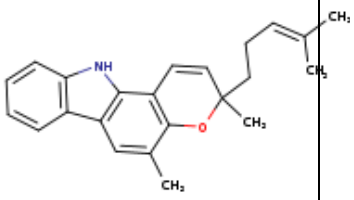
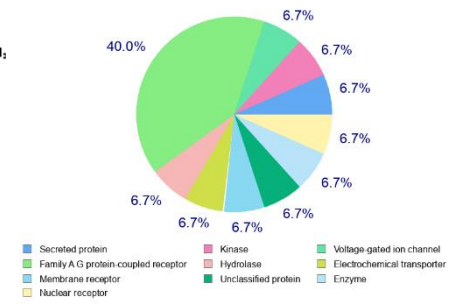
metabolism, and RNA splicing, respectively [22–25]. Although not traditionally associated with frontline breast cancer pathways, many of these targets are increasingly recognized in cancer biology for their roles in tumor microenvironment modulation [26], energy metabolism [27], and cell cycle checkpoint control [28]. This implies that Koenidine may exert indirect or supportive anticancer effects by disrupting tumor metabolism and cellular homeostasis.

3. Mahanimbine was predicted to engage with ERBB2, CDK2, MET, and ALK, a set of targets enriched in HER2-driven and invasive breast cancer phenotypes [29]. The co-targeting of CDK2 and HER2 suggests potential synergy in inhibiting both cell cycle progression and receptor-mediated proliferation [30]. Furthermore, the presence of MET and ALK, which are associated with drug resistance and epithelial-to-mesenchymal transition, indicates that Mahanimbine may also impact aggressive breast cancer subtypes and recurrence.
4. Murrayazoline showed a smaller but focused set of predicted targets, including RET, CDK4, and ALK [31]. RET and ALK fusions have been detected in subsets of triple-negative breast cancer (TNBC) and HER2-low cases [32], making Murrayazoline a potentially useful compound for precision medicine approaches in these hard-to-treat populations [8]. Its predicted targeting of CDK4 also aligns with cell cycle control, reinforcing its potential role in antiproliferative strategies [33].
5. O-methylmurrayamine A exhibited strong multitarget potential, with predicted interactions involving EGFR, ERBB2, BRAF, SRC, STAT3, and BCL2. This set of targets spans key oncogenic [34], signaling [35], and apoptotic pathways [36]. Particularly, the interaction with BCL2, a key anti-apoptotic protein often overexpressed in breast cancer, suggests this compound may directly modulate cell survival mechanisms [8]. Its shared targets with Koenimbine further suggest that these compounds may have overlapping yet complementary pharmacological profiles.

Collectively, these findings highlight the pharmacological diversity of *M. koenigii* alkaloids and their potential to modulate multiple hallmarks of breast cancer. While compounds such as Koenimbine and O-methylmurrayamine A appear to target classical oncogenic drivers, others like Koenidine and Murrayazoline may offer less conventional but biologically relevant activities. This supports the hypothesis that the therapeutic efficacy of *M. koenigii* may arise from polypharmacology, where multiple compounds act simultaneously on different targets and pathways to achieve synergistic anticancer effects. These predictions provide a strong rationale for further in vitro and in vivo validation, particularly in HER2-positive and triple-negative breast cancer models.

Table 2. Swiss Target Prediction of *M. koenigii* Bioactive Compound

Bioactive Compounds	Structure	Target Prediction
Koenimbine		

Murrayazoline		
O-methylmurrayamine A		
Koenidine		
Mahanimbine		

Intersection of Predicted Phytochemical Targets with Breast Cancer-Associated Genes

To evaluate the potential relevance of *M. koenigii* phytochemicals in breast cancer therapy, we performed an intersection analysis between the predicted target genes of five selected compounds and genes associated with breast cancer obtained from the GeneCards database. As shown in Figure 1, a total of 21 genes were shared between the predicted targets and breast cancer-related genes.

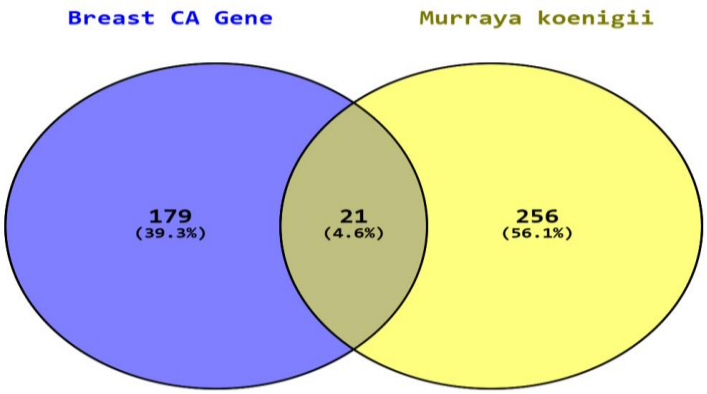


Figure 1. Venn diagram showing the overlap between predicted target genes of *M. koenigii* phytochemicals and breast cancer–associated genes obtained from GeneCards

This overlap represents approximately 4.6% of the total union set, indicating that while *M. koenigii* compounds act on a broad range of targets, a subset directly intersects with key oncogenic drivers of breast cancer. The overlapping genes include well-established cancer-associated targets such as EGFR, ERBB2, PIK3CA, AKT1, CDK4, and BCL2, many of which play crucial roles in breast tumor proliferation, survival, and drug resistance [37]. Specifically, EGFR was predicted as a target of four compounds: Koenidine, Koenimbine, Mahanimbine, and O-methylmurrayamine A, highlighting its prominence as a multitarget node. Likewise, ERBB2 (HER2), frequently overexpressed in HER2-positive breast cancer [38], was targeted by Koenimbine, Mahanimbine, and O-methylmurrayamine A. Other genes of interest include:

- AKT1, RET, MET, and BRAF: central to PI3K-Akt and MAPK signaling [39].
- CDK2/CDK4, AR, and PGR: involved in cell cycle and hormone receptor pathways [40].
- BCL2 and STAT3: mediators of apoptosis and inflammatory signaling [36].

Notably, Koenidine emerged as the compound with the most diverse overlap, targeting multiple core regulators including CHEK2, PIK3CA, CDK2, AR, SRC, and CYP family members. Meanwhile, O-methylmurrayamine A displayed the broadest multitarget profile, sharing targets with all major signaling categories relevant to breast cancer.

These findings support the hypothesis that *M. koenigii* phytochemicals exert anticancer effects not by targeting a single protein, but through multitarget engagement of key oncogenic pathways. The 21 shared genes are prime candidates for further validation as they bridge predicted compound activity and breast cancer molecular etiology.

Table 2. Bioactive Compounds from *M. koenigii* and Targeted Breast Cancer Genes

Bioactive Compound	Targeted Breast Cancer Genes
Koenidine	CHEK2, EGFR, PIK3CA, AKT1, CDK4, AR, SRC, CYP17A1, CYP19A1, CDK2, PGR
Koenimbine	EGFR, ERBB2, AKT1, AR, FGFR1, STAT3, CYP17A1, NTRK1, CYP19A1, CDK2, PGR
Mahanimbine	EGFR, ERBB2, MET, ALK, AR, MDM2, CDK2, PGR
Murrayazoline	RET, ALK, CDK4, AR, MDM2
O-methylmurrayamine A	EGFR, ERBB2, BRAF, AR, SRC, CYP17A1, NTRK1, CDK2, BCL2, PGR

Integrative Network-Based Analysis of *Murraya koenigii* Targets in Breast Cancer

To better understand the functional context of the 21-breast cancer–associated genes targeted by *M. koenigii* phytochemicals, we constructed a protein–protein interaction (PPI) network using the STRING database. The resulting network revealed a highly interconnected cluster of genes, suggesting their involvement in a shared set of biological pathways central to breast cancer progression. Prominent among these were AKT1, EGFR, PIK3CA, and ERBB2 (HER2) genes widely recognized as key oncogenic drivers [41]. These proteins displayed extensive connectivity, serving as central hubs in the network. Notably, interactions such as AKT1–MDM2 and AKT1–PIK3CA yielded high combined confidence scores of 0.999 and 0.998, respectively, supported by experimental, co-expression, and curated database evidence. These genes are crucial to core cancer pathways, including PI3K-Akt signaling, cell cycle regulation, and apoptosis inhibition, which are commonly dysregulated in aggressive and treatment-resistant forms of breast cancer [42,43].

In addition to these canonical pathways, the network also encompassed regulators of hormone response (AR, PGR)[44], apoptotic suppression (BCL2, STAT3) [45,46], and cell cycle control (CDK2, CDK4, CHEK2)[47], indicating the compounds' ability to modulate multiple hallmarks of cancer. The tight integration of these targets within a single molecular network implies that *M. koenigii* constituents act not through isolated pathways but by influencing a cohesive and functionally interdependent oncogenic module. Such polypharmacological behavior offers a potential advantage over single-target agents, as it may reduce compensatory pathway activation and therapeutic resistance. Taken together, these findings highlight the multitargeted therapeutic promise of *M. koenigii* phytochemicals and provide strong justification for further experimental validation through in vitro and in vivo assays to assess their efficacy, selectivity, and synergistic potential in breast cancer treatment.

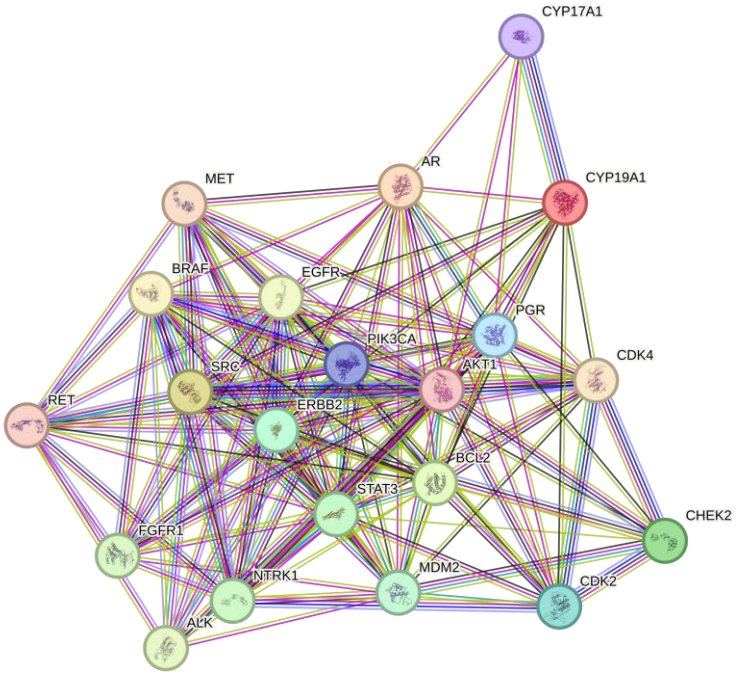


Figure 2. Protein–protein interaction (PPI) network of 21 overlapping genes between *M. koenigii* phytochemical targets and breast cancer–associated genes, constructed using the STRING database

Enrichment of Breast Cancer–Relevant Pathways by *Murraya koenigii* Target Genes

Pathway enrichment analysis using ShinyGO revealed that the 21 overlapping genes between *M. koenigii* target predictions and breast cancer–associated genes are significantly enriched in canonical pathways involved in breast cancer biology. These include the PI3K-Akt signaling, MAPK cascade, estrogen and progesterone receptor signaling [48], and cell cycle control pathways [40], as visualized in the KEGG pathway map (Figure 3). The highlighted targets (e.g., EGFR, HER2, PIK3CA, AKT1, CDK4, PGR) represent key regulatory nodes frequently altered in various breast cancer subtypes, supporting the relevance of the predicted targets. This integrative result reinforces the hypothesis that *M. koenigii* phytochemicals can simultaneously modulate multiple molecular pathways essential for breast tumor progression and survival.

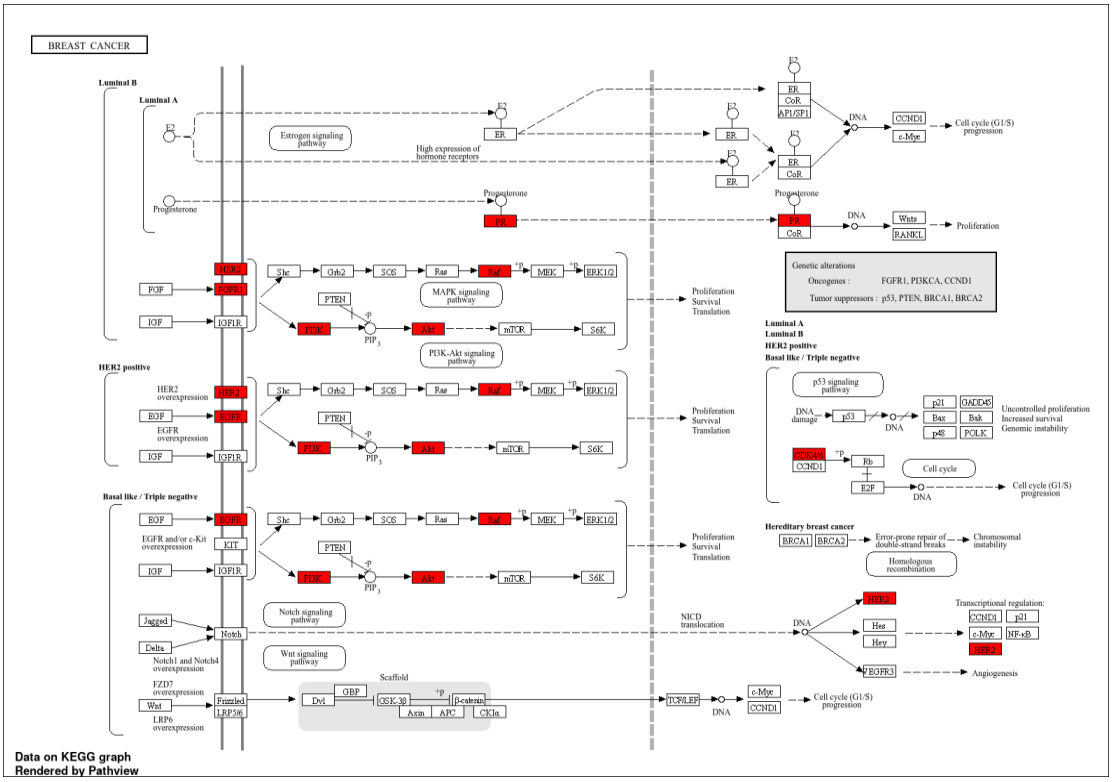


Figure 3. KEGG pathway map of breast cancer highlighting the genes targeted by *M. koenigii* phytochemicals

The KEGG pathway enrichment analysis (Figure 3) revealed that the 21 overlapping gene targets of *M. koenigii* phytochemicals which also associated with breast cancer are significantly involved in a wide array of cancer-related pathways. Notably, Pathways in cancer, PI3K-Akt signaling, and ErbB signaling pathway showed the highest fold enrichment and strongest statistical significance, as indicated by large dot sizes and red to pink coloration (low FDR) (Figure 4). These pathways encompass key regulators such as EGFR, ERBB2, PIK3CA, AKT1, and CDK4, all of which play central roles in tumor proliferation, cell survival, and therapeutic resistance in various breast cancer subtypes [49]. The presence of the Breast cancer–specific KEGG pathway among the top-ranked enrichments further validates the functional relevance of these genes to breast tumorigenesis. These findings suggest that *M. koenigii* phytochemicals act on clinically actionable targets, many of which are used for diagnostic classification and treatment

selection in HER2-positive, luminal A/B, and triple-negative breast cancers [50,51].

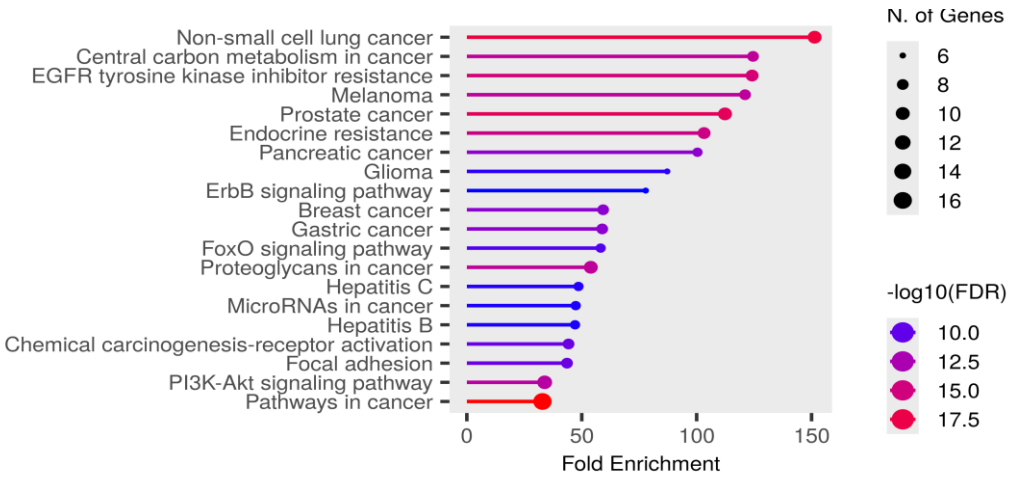


Figure 4. Dot plot of KEGG pathway enrichment results generated using ShinyGO for the 21 overlapping genes between *M. koenigii* phytochemical targets and breast cancer-associated genes.

M. koenigii Compounds Modulate Key Oncogenic Pathways

This compound–target interaction network demonstrates how phytochemicals derived from *Murraya koenigii* may modulate multiple genes implicated in breast cancer. In the network, five selected compounds: Koenimbine, Koenidine, Mahanimbine, Murrayazoline, and O-methylmurrayamine A, are shown to interact with 21 target genes that are both predicted through SwissTargetPrediction and recognized in breast cancer according to GeneCards. These targets span key oncogenic pathways, including PI3K-Akt, ERBB/HER2 signaling, hormone receptor signaling, and cell cycle regulation [47,48,52]. Notably, Koenimbine was predicted to act on critical proliferation and survival regulators [53] such as EGFR, ERBB2, AKT1, CDK2, and AR, while Koenidine interacts with targets involved in DNA repair and endocrine signaling such as CHEK2, PIK3CA, CDK4, and PGR [54]. O-methylmurrayamine A, the most broadly connected compound in the network, is associated with EGFR, ERBB2, BCL2, and STAT3, suggesting its ability to affect both growth and apoptotic pathways [8].

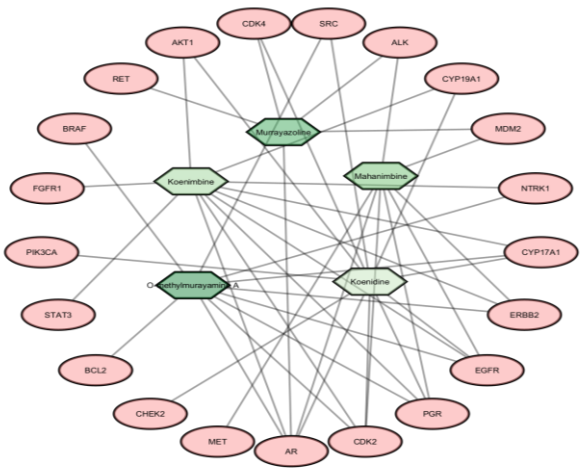


Figure 5. Compound–target interaction network showing five phytochemicals from *Murraya koenigii* (green hexagonal nodes) and their overlapping predicted gene targets associated with breast cancer (pink elliptical nodes).

The visual clustering of these targets around distinct but interlinked biological processes reinforces the concept of polypharmacological action, where multiple compounds synergistically modulate a shared signaling network [55]. Mahanimbine and Murrayazoline, for example, are linked to RET, ALK, MET, and NTRK1, kinases that are clinically relevant in triple-negative breast cancer and receptor-tyrosine kinase–driven subtypes [56–58]. The convergence of these interactions within well-characterized breast cancer pathways underscores the potential of *M. koenigii* compounds to overcome limitations of single-target therapies. By simultaneously disrupting several molecular circuits including hormone receptor activity, oncogenic kinases, and apoptosis evasion, these phytochemicals offer a compelling mechanistic rationale for further preclinical investigation in breast cancer models.

CONCLUSION

This study highlights the polypharmacological potential of *Murraya koenigii* phytochemicals against breast cancer through in silico target prediction and pathway enrichment analysis. Five selected compounds were found to collectively target 21 breast cancer–associated genes, including key regulators such as EGFR, ERBB2, AKT1, and CDK2. These targets are enriched in critical pathways like PI3K-Akt, MAPK, and hormone signaling, suggesting multi-pathway modulation. The findings support the therapeutic relevance of *M. koenigii* as a source of multitarget agents and provide a strong rationale for further experimental validation in breast cancer models.

Declaration by Authors

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Conflict of Interest: The authors declare that they have no conflict of interest.

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