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RESEARCH ARTICLE

Unveiling The Therapeutic Potential of *Nyctanthes arbor-tristis* Linn. In Breast Cancer: A Network Pharmacology and Molecular Docking Approach

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Unveiling The Therapeutic Potential of Nyctanthes arbor-tristis Linn. In Breast Cancer: A Network Pharmacology and Molecular Docking Approach

SUMMARY

Nyctanthes arbor-tristis Linn., a traditionally valued medicinal plant, exhibits anticancer activity, yet its mechanisms against breast cancer remain inadequately defined. This study explored its bioactive compounds, molecular targets, and pathways using network pharmacology, molecular docking, Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) profiling. Fifty-seven bioactive compounds were identified from databases, with potential targets predicted through SwissTargetPrediction and breast cancer proteins retrieved from the Therapeutic Target Database. Protein–protein interaction and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses revealed the PI3K–Akt signalling pathway as a key mechanism. Molecular docking highlighted caffeic acid with strong binding affinity to the Epidermal Growth Factor Receptor (EGFR) kinase receptor (-27.44 kcal/mol), while lupeol was predicted to regulate the maximum number of targets. ADMET and drug-likeness profiling further identified nicotiflorin the highest drug-likeness score. Overall, lupeol and caffeic acid emerged as major active constituents, modulating breast cancer via phosphatidylinositol 3-kinase- Protein Kinase B (PI3K-Akt) and EGFR pathways. These findings provide mechanistic insights supporting the therapeutic potential of Nyctanthes arbor-tristis in breast cancer management.

Keywords: *Nyctanthes arbortristis Linn., breast cancer, network pharmacology, molecular docking, targets.*

Nyctanthes arbor-tristis Linn.'in Meme Kanserindeki Terapötik Potansiyelinin Ortaya Çıkarılması: Bir Ağ Farmakolojisi ve Moleküler Yerleştirme Yaklaşımı

ÖZ

Geleneksel olarak değerli bir tıbbi bitki olan Nyctanthes arbor-tristis Linn., antikanser aktivite göstermesine rağmen, meme kanserine karşı etkime mekanizmaları yeterince tanımlanmamıştır. Bu çalışmada, bitkinin biyoaktif bileşikleri, moleküler hedefleri ve etki yolları ağ farmakolojisi, moleküler yerleştirme (docking) ve Emilim, Dağılım, Metabolizma, Atılım ve Toksisite (ADMET) analizleri kullanılarak araştırılmıştır. Veritabanlarından elli yedi biyoaktif bileşik tanımlanmış; potansiyel hedefler SwissTargetPrediction aracılığıyla, meme kanseri ile ilişkili proteinler ise Therapeutic Target Database'den elde edilmiştir. Protein–protein etkileşimleri ve Kyoto Genleri ve Genomları Ansiklopedisi (KEGG) yolak analizleri, PI3K–Akt sinyal yolunun temel mekanizma olduğunu ortaya koymuştur. Moleküler yerleştirme analizleri, kafeik asidin Epidermal Büyüme Faktörü Reseptörü (EGFR) kinaz reseptörüne yüksek bağlanma afinitesi (-27,44 kcal/mol) gösterdiğini, lupeolün ise en fazla hedefi düzenlediğini ortaya koymuştur. ADMET ve ilaç-benzerliği analizleri sonucunda, en yüksek ilaç-benzerliği skoruna sahip bileşik olarak nikotiflorin belirlenmiştir. Genel olarak, lupeol ve kafeik asit, fosfatidilinozitol 3-kinaz–Protein Kinaz B (PI3K–Akt) ve EGFR yolakları aracılığıyla meme kanserini modüle eden başlıca aktif bileşenler olarak öne çıkmaktadır. Bu bulgular, Nyctanthes arbor-tristis'in meme kanseri tedavisinde terapötik potansiyelini destekleyen mekanistik kanıtlar sunmaktadır.

Anahtar Kelimeler: *Nyctanthes arbor-tristis Linn., meme kanseri, ağ farmakolojisi, moleküler yerleştirme, hedefler.*

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INTRODUCTION

Cancer is a major global public health concern, with an estimated 2,001,140 new cases and 611,720 related deaths projected in the United States in 2024 (Siegel, Giaquinto, & Jemal, 2024) and approximately 4.8 million new cases and 3.2 million cancer-related deaths reported in China (Xia et al., 2022). One of the most common malignancies in the world is still breast cancer. Despite extensive research on its prevention and tumorigenesis over the past decades (Britt, Cuzick, & Phillips, 2020), its incidence continued to rise by 0.5% annually from 2014 to 2018 (Siegel, Miller, Fuchs, & Jemal, 2022). Breast cancer is an incurable metastatic disease that can spread to the brain, liver, lungs, and bones. However, early detection significantly improves prognosis and increases the chances of survival (DeSantis et al., 2016). According to the World Health Organization (WHO), early detection is the cornerstone of breast cancer management, as it significantly improves outcomes and survival rates. Various modern medicines are prescribed for breast cancer treatment, including antiestrogens like raloxifene and tamoxifen, which may help prevent the disease in individuals at high risk. In some cases, preventive mastectomy is considered for those with a significantly increased likelihood of developing breast cancer. Treatment options for people with breast tumours include radiation therapy, surgery, chemotherapy, hormone therapy, and targeted therapy. The main goals of management in situations of distant metastases are to increase survival and improve quality of life (Akram, Iqbal, Daniyal, & Khan, 2017). The adverse effects of conventional breast cancer treatments have driven interest in alternative therapies. Herbal medicine is regarded as a natural option, as certain plants may possess bioactive compounds with potential anticancer properties (Abdull Razis & Noor, 2013).

The quantity of bioactive chemicals in plants has led to extensive research into herbal medicine as a possible source of drugs against cancer (Dehelean et al., 2021). Through a variety of mechanisms, such as causing cancer cells to undergo apoptosis (programmed cell death), preventing angiogenesis (the growth of new blood vessels supplying tumours), and altering important signalling pathways involved in cancer progression and metastasis, some compounds derived from plants have shown promising anticancer properties (Abdulridha et al., 2020). Herbal medicine has been used for centuries to treat a variety of illnesses, including cancer. Herbal treatments have long been utilised to treat cancer in traditional medical systems like Ayurveda and Traditional Chinese Medicine (TCM), which laid the groundwork for current studies into their potential as therapeutics. However, with the rise of industrial medicine, the use of herbal treatments declined for some time. Recent advancements in technology have helped overcome challenges associated with natural compounds, reigniting interest in their integration into pharmaceutical development. Traditional treatments continue to be important, since the World Health Organisation estimates that 80% of the world's population uses them (Jenča et al., 2024). Advancements in biomolecular science have enhanced our understanding of the pharmacological effects of herbal compounds, including their anticancer, anti-inflammatory, and antiviral properties (Jenča et al., 2024). Research on the anticancer effects of herbal remedies is still ongoing. To get around issues including poor solubility, low bioavailability, and non-specific distribution, sophisticated drug delivery systems such as nanoparticles have been created (Coy-Barrera, Ogungbe, & Schmidt, 2023; Gaikwad et al., 2023; Garcia-Oliveira et al., 2021; Singh, Singh, Kanwar, & Chauan, 2020). The therapeutic efficacy, targeted delivery, and bioavailability of herbal anticancer medicines are all enhanced by these methods. However, issues including the requirement for standardisation, possible drug interactions, and variations in the quality of bioactive compounds must be addressed. Furthermore, many herbal substances' exact processes are still unknown, necessitating more investigation to clarify their biological pathways and interactions (Atanasov et al., 2021; Mali, 2023).

Nyctanthes arbor-tristis Linn., a member of the Oleaceae family, is commonly known in India as harsingar or night-flowering jasmine. It has long been used in Ayurvedic, Siddha, and Unani medicine and remains integral to traditional healing practices among indigenous tribal communities (Chabattula et al., 2024). Native to South and Southeast Asia, particularly India, Nepal, Pakistan, and Thailand, *Nyctanthes arbor-tristis* Linn. thrives in dry deciduous forests and rocky soils up to 1500 m altitude. The plant typically flowers between July and October and is now widely cultivated across Asia, Africa, and other tropical regions for its medicinal and ornamental value**.** Ethnomedicinally, its leaves act as laxative, diuretic, and diaphoretic agents; flowers are employed to treat skin and eye disorders; the bark is used against malaria and bronchitis; and the seeds serve as anthelmintic and are applied in alopecia. Tribal communities in India traditionally consume leaf extracts mixed with honey to manage malaria, fever, and blood disorders. Beyond its therapeutic applications, *Nyctanthes arbor-tristis* Linn. holds deep spiritual and cultural significance, being planted in temple gardens for its fragrant blossoms and symbolic purity (Tipugade, Sawale, & Jadhav, 2025a). Phytochemically, the plant is rich in benzoic acid, carotene, oleanolic acid, friedelin, essential oils, tannic acid, and lupeol, among other compounds. These bioactive constituents confer a broad spectrum of pharmacological properties, including antioxidant (Gahtori et al., 2024), anti-inflammatory (Sana et al., 2024), anti-cancer (Karan, Maity, Pal, Singha, & Jana, 2019), immunomodulatory (Bharshiv, Garg, & Bhatia, 2016), anti-leishmania, anti-allergic, and antimicrobial properties (ALNadhari, Alsakkaf, & Albarakat, 2024). Using network pharmacology and molecular docking, the current study explores the multiple bio-actives–many targets interaction paradigm in an effort to clarify the likely mode of action of *Nyctanthes arbor-tristis* Linn. By establishing connections between compounds, genes, and diseases, the network pharmacology technique sheds light on the target proteins' regulatory mechanisms (Sekaran et al., 2024). By examining the compounds' binding affinities to druggable receptor proteins, molecular docking was used to evaluate the compounds' therapeutic potential (Nxumalo et al., 2024).

It was hypothesized that the bioactive constituents of *Nyctanthes arbor-tristis* Linn. exert anticancer effects against breast cancer through multi-target and multi-pathway modulation, primarily involving key signaling cascades such as the PI3K-Akt and EGFR pathways. By integrating network pharmacology and molecular docking, the study aimed to delineate the compound–target–pathway interactions underlying the plant's therapeutic efficacy. This approach was expected to provide mechanistic insights into how phytochemicals from *Nyctanthes arbor-tristis* synergistically regulate oncogenic proteins, thereby offering a scientific rationale for its traditional use and identifying potential lead compounds for future drug development.

MATERIALS AND METHODS

Mining of phytoconstituents and targets

A list of reported phytoconstituents was obtained from Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/) and the IMPPAT: Indian Medicinal Plants, Phytochemistry, and Therapeutics Database (https://cb.imsc.res.in/imppat/#browse). The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) provided the molecular formula, molecular weight, PubChem CID, InChI key, and canonical SMILES. Targets associated with breast cancer were retrieved from the Therapeutic Target Database (http://db.idrblab.net/ttd/) and GeneCards: The Human Gene Database (https://www.genecards.org/) using the keyword "Breast cancer" and cross-referenced with proteins regulated by the bio-actives of *Nyctanthes arbortristis* Linn., as identified from bio-actives-regulated proteins in SwissTargetPrediction (http://www.swisstargetprediction.ch/) (Daina, Michielin, & Zoete, 2019).

Drug-likeness and ADMET profile of bio-actives

By entering their SMILES, each bioactive compound's drug-likeness score was acquired from MolSoft (https://molsoft.com/mprop/) (Gad et al., 2020). Additionally, the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile of each bioactive was predicted utilizing SwissADME (http://www.swissadme.ch/index.php#) (Daina, Michielin, & Zoete, 2017) and PreADMET (https://preadmet.webservice.bmdrc.org/toxicity/) (Maliehe, Tsilo, & Shandu, 2020). P-glycoprotein (P-gp) inhibition, P-gp substrate potential, Colon Adenocarcinoma-2 (Caco-2) permeability, blood–brain barrier permeability, human intestinal absorption, and CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 enzyme inhibition were all evaluated utilizing these tools (Gandla et al., 2023).

Gene expression and enrichment analysis

The gene codes of the regulated proteins were found using the UniProt database (https://www.uniprot.org/), and they were subsequently queried in STRING (https://string-db.org/) for Gene Ontology (GO) analysis (Szklarczyk et al., 2017). Similarly, the pathways associated with breast cancer were identified using the KEGG database (https://www.genome.jp/kegg/). Additionally, Cytoscape version 3.7.2 (https://cytoscape.org/) was utilised to build a network of bioactive compounds, proteins, and pathways (Shannon et al., 2003), excluding any duplication.

Molecular docking

Ligand preparation

The ligands' 2D structures were downloaded to the workspace in .sdf format after being obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) (Setlur, Chandrashekar, Bhattacharjee, Kumar, & Niranjan, 2023; Tipugade, Sawale, & Jadhav, 2025b).

Preparation of macromolecule

The docking simulation study of bioactive compounds was conducted on EGFR kinase (PDB ID: 1XKK), obtained from the Protein Data Bank (https://www.rcsb.org). The protein structure was prepared using the Molsoft ICM Pro, which involved adding hydrogen atoms, removing water molecules, assigning partial charges, determining protonation states, and applying restraints. After the ligand was removed, the binding sites were identified, and a grid box was used to create a grid (Bello, 2018; Ongko et al., 2022).

Ligand–protein docking

Each bioactive compound was docked into the appropriate binding site inside the grid utilising Molsoft ICM Pro's Glide module. It is a rapid and efficient docking program designed for docking small molecules into receptor-binding sites. The program incorporates a scoring function that considers shape complementarity, electrostatic interactions, and van der Waals forces between the ligand and receptor. Key active site interactions and corresponding scoring functions were thoroughly evaluated (Agu et al., 2023).

RESULTS

Mining of phytoconstituents and targets involved in breast cancer

In the pursuit of identifying potential phytoconstituents and their molecular targets in breast cancer, an extensive database mining approach was employed. The Dr. Duke and IMPPAT databases revealed fifty-seven bio-actives in the plant *Nyctanthes arbor-tristis* Linn., all of which altered proteins implicated in breast cancer. According to the GeneCards: The Human Gene Database, 3,722 proteins have been linked to breast cancer; of these, 144 have been altered by the bio-actives from *Nyctanthes arbortristis* Linn. The list of bio-actives, together with their molecular formula, molecular weight, and PubChem CID, is summarised in Table S1.

Drug-likeness score and ADMET profile of bio-actives

Computational analysis was carried out to evaluate the drug-likeness, pharmacokinetic behavior, and

toxicity profiles (ADMET properties) of the identified bioactive compounds. Among them, Nicotiflorin showed the highest drug-likeness score of 0.90 out of 18 compounds with positive scores (Table S2.). The pharmacokinetic characteristics of each bioactive were visualized in a heat map, showing their water solubility, intestinal permeability (Caco-2), skin permeability, and human intestinal absorption. Additional parameters such as P-glycoprotein substrate or inhibitor activity, blood–brain barrier (BBB) permeability, and cytochrome P450 (CYP) enzyme interactions were also analyzed (Figure S1.).

Phenylacetaldehyde and 4-Methoxybenzaldehyde demonstrated the highest intestinal absorption and permeability values, while Tetratriacontane and Tritriacontane showed the greatest BBB permeability. Cassiarins A and B were predicted to have strong P-glycoprotein inhibitory activity. Several other compounds, including Melanin, Syringic acid, Naringetol, Loganin, Chlorogenic acid, Astragalin, Nicotiflorin, and Crocin, were identified as potential P-glycoprotein substrates, suggesting possible roles in drug transport and metabolism.

In terms of toxicity prediction, α-Carotene and Melanin showed potential mutagenic (AMES) and hepatotoxic effects. Likewise, compounds such as 4-hydroxy hexahydrobenzofuran-7-one, Linolenic acid, Ursolic acid, Oleanolic acid, Betulinic acid, and Lignoceric acid were predicted to be hepatotoxic. Overall, the ADMET analysis provided valuable insights into the absorption, distribution, metabolism, excretion, and toxicity properties of NAT bioactives, helping to identify those with promising pharmacokinetic potential and safety profiles.

Enrichment and network analysis

Enrichment and network analyses were performed to explore the molecular mechanisms modulated by bio-actives. Lupeol was predicted to modulate the highest number of genes (33), as shown in Table S3. Likewise, all regulated proteins' protein–protein interactions are depicted in Figure S2. NFKB1 was the main target of the greatest amount of bioactives in the network of bioactives, targets, and pathways that was created. Similarly, Table S4 shows that the PI3K-Akt pathway was primarily controlled by affecting the greatest number of genes (22).

Similarly, the gene ontology analysis of protein interactions (Figure S3.) identified 917 biological processes, with response to oxygen-containing compounds (GO:1901700) exhibiting the lowest false discovery rate. This process was modulated through 74 genes *CFTR, MAPK1, APEX1, SERPINE1, NFKB1, MAPK14, SIRT2, CHRM3, PDGFRA, CDK4, PDGFRB, PIK3CA, STAT3, CDK2, CYSLTR2, HPGD, NOS3, MC4R, MMP3, ACHE, CHRM1, P2RY12, PTPN2, HSD11B2, NOS2, PRKCD, HSP90AA1, ESR2, C5AR1, BLM, RET, MAP3K5, PDE3A, DNMT1, CES1, NTRK3, STAT1, EGLN1, PTGS2, CNR1, CHUK, CACNA1B, GRIN1, HSP90AB1, PTPN1, TLR4, CNR2, RXRB, AR, F7, RORB, RPS6KA3, AKR1C3, CDK1, NFE2L2, PTK2B, GSTP1, ABCC1, KDM1A, OPRM1, CHRM2, ESR1, CHRM4, GLRA1, SLC2A1, RXRA, CDK5, PIK3R1, HDAC2, IDO1, HIF1A, NR4A1*, *FPR2* and *ACACA* against a background of 1,547 genes, with a strength of 0.82. Similarly, 134 molecular functions were identified in which Catalytic activity (GO:0003824) scored the lowest false discovery rate via modulation of 94 genes (*CFTR, HSD17B10, MIF, MAPK1, APEX1, DHODH, DUSP3, MAPK14, CTSD, FLT3, PIN1, SIRT2, ALOX12, CHRM3, PDGFRA, CDK4, ADAM10, PDGFRB, MAP2K2, BRD4, PIK3CA, HDAC4, F13A1, CDK2, GGPS1, HPGD, NOS3, MMP3, ANPEP, GUSB, ACHE, CHRM1, F2, PTPN7, TERT, PTPN2, AURKB, HSD11B2, METAP2, NOS2, PRKCD, HSP90AA1, TDP1, TRIM24, MAOA, CTSB, BLM, RET, MAP3K5, PDE3A, DNMT1, CES1, NTRK3, TOP1, PTGS1, EGLN1, PTGS2, CHUK, HSP90AB1, PTPN1, NOX1, HDAC8, TLR4, F7, CSNK2B, PIK3CD, XDH, RPS6KA3, NQO2, AKR1C3, DUT, GRK5, PRCP, CDK1, PTK2B, GSTP1, ABCC1, FKBP1A, KDM1A, CHEK1, ITK, PRKCA, TOP2A, CDK5, HDAC2, IDO1, CAPN1, RPS6KA1, ADK, PAK4, ACACA, PTPN11, CYP3A4, PIK3CB*)

against 5522 background genes at a strength of 0.37. Likewise, 84 cellular components were identified in which intrinsic plasma membrane (GO:0005886) scored the minimum false discovery rate by modulation of 84 genes (*CFTR, HSD17B10, MIF, MAPK1, SERPINE1, DUSP3, TFPI, FLT3, SIRT2, ALOX12, CHRM3, PDGFRA, ADAM10, PDGFRB, KCNH2, MAP2K2, PIK3CA, STAT3, NPC1, CYSLTR2, HPGD, NOS3, MC4R, ANPEP, ACHE, ADORA2B, CHRM1, P2RY12, F2, PTPN7, TERT, PTPN2, TLR8, METAP2, NOS2, PRKCD, HSP90AA1, TDP1, CTSB, C5AR1, RET, MAP3K5, NTRK3, ADORA1, PTGS2, ADRB1, CNR1, CHUK, CACNA1B, GRIN1, HSP90AB1, NOX1, TACR2, TLR4, CNR2, AR, F7, S1PR3, PIK-3CD, GRK5, PRCP, NFE2L2, PTK2B, ABCC1, FKBP1A, OPRM1, SCN4A, ITK, CHRM2, ESR1, PRKCA, CHRM4, FCGRT, GLRA1, SLC2A1, CDK5, PIK3R1, CAPN1, SLC6A5, ADK, FPR2, FPR1, S1PR2, PIK3CB*) against 5544 background genes at a strength of 0.32. Figure S3 illustrates the top 15 biological processes, molecular functions, and cellular components. Additionally, network analysis identified Lupeol as the bioactive regulating the highest number of proteins, with NFKB1 being the primary target protein and the PI3K-Akt signalling pathway as the most modulated pathway. Similarly, Figure S4 presents the interactions between individual bio-actives, their respective modulated proteins, and the regulated pathways.

***In silico* Molecular Docking**

A molecular docking study was conducted using *Nyctanthes arbor-tristis* Linn. structures as ligand groups and EGFR kinase as the target macromolecule. The docking analysis was performed using Molsoft ICM Pro. The downloaded structures of *Nyctanthes arbor-tristis* Linn. and reference standards were docked against EGFR kinase to determine binding conformations and binding affinity. A total of 57 structures of *Nyctanthes arbor-tristis* Linn., along with three standard inhibitors (Aderbasib, Erlotinib, and Gefitinib), were docked, and their binding affinities were compared. The molecular docking study revealed that among all docked *Nyctanthes arbor-tristis* Linn. compounds, Caffeic acid exhibited the highest binding affinity of −27.44 kcal/mol with the EGFR kinase domain complexed with a quinazoline inhibitor (GW572016, PDB: 1XKK). The binding affinities of the standard inhibitors were as follows: Gefitinib (STD1) at -30.7 kcal/mol, Erlotinib (STD2) at -19.94 kcal/mol, and Aderbasib (STD3) at -10.17 kcal/mol. Additionally, Caffeic acid, Naringetol, Crocetin, Astragalin, Linoleic acid, Pelargonic acid, Melanin, Curlone, Gallic acid, and Calceolarioside A demonstrated lower binding affinities compared to the reference standards. The visualized interactions of ligands within the binding pocket of PDB: 1XKK confirmed that all docked ligands were successfully bound to the identified binding site (Figure S5.). Figure S6 presents a 3D visualization of the *Nyctanthes arbor-tristis* Linn. compound interacting within the binding pocket of PDB: 1XKK. Molecular docking results revealed that 53 out of 57 docked *Nyctanthes arbor-tristis* Linn. compounds exhibited binding affinity with the EGFR kinase domain complexed with a quinazoline inhibitor (GW572016, PDB: 1XKK). Table S5 provides a summary of these docked compounds' specific interactions and binding affinities.

The integration of network pharmacology and molecular docking in this study provides translational insights into how the bioactive compounds of *Nyctanthes arbor-tristis* may contribute to breast cancer therapy. The identification of Lupeol and Caffeic acid as major regulators of the PI3K-Akt and EGFR signaling pathways underscores their potential as lead molecules for targeted anticancer drug development. These findings align with the current shift toward multi-target therapeutic strategies, emphasizing the relevance of phytochemicals in overcoming resistance mechanisms associated with single-target drugs. Moreover, the favourable ADMET and drug-likeness profiles of several bio-actives support their potential for oral formulations or adjuvant roles alongside standard chemotherapeutics. Collectively, *the in-silico* results form a mechanistic foundation for subsequent

in vitro and *in vivo* validations, bridging traditional medicinal knowledge of *Nyctanthes arbor-tristis* with modern translational oncology.

DISCUSSION

Traditional medicinal plants have long served as a cornerstone for modern drug discovery, offering a vast repository of bioactive compounds that often resemble pharmacologically relevant scaffolds or secondary metabolites (Tolstikova, Khvostov, & Bryzgalov, 2009). *Nyctanthes arbor-tristis* Linn. has been traditionally recognized for its diverse therapeutic potential, including demonstrated anticancer activity against HeLa, HepG2, and normal human peripheral lymphocyte cells (Bibechana Timsina, 2016). In contemporary research, the integration of *in silico* tools such as molecular modelling, structure–activity relationship analysis, pharmacophore identification, and network pharmacology has accelerated the exploration of complex herbal systems. These computational approaches, supported by machine learning and data mining, have become essential in identifying multi-target interactions and predicting pharmacokinetic behavior (Ekins, Mestres, & Testa, 2007).

The present study applied a network pharmacology and molecular docking framework to elucidate the mechanistic landscape of *Nyctanthes arbor-tristis* Linn. in breast cancer. Instead of focusing on isolated outcomes, the findings reveal a holistic, multi-target pharmacological profile consistent with the complex etiology of breast cancer. The identification of 57 bioactive compounds that collectively regulate 144 breast cancer-associated proteins underscores the plant's polypharmacology potential. Such multitarget interactions are particularly relevant in breast cancer, where overlapping pathways often contribute to drug resistance and tumor progression.

Among the identified compounds, Lupeol emerged as a central modulator, with NFKB1 and the PI3K–Akt signaling pathway serving as pivotal molecular nodes. Modulation of the PI3K–Akt cascade, a pathway frequently dysregulated in breast carcinogenesis, suggests that *Nyctanthes arbor-tristis* Linn. may restore cellular equilibrium by inhibiting aberrant proliferation and promoting apoptosis. This aligns with prior evidence indicating that Lupeol and related triterpenoids suppress Akt phosphorylation and NF-kB activation, thus impeding tumor growth and metastasis. Consequently, the synergistic interplay among NAT's constituents may offer therapeutic advantages over conventional single-target strategies.

ADMET and drug-likeness assessments further revealed that compounds such as Nicotiflorin and Lupeol possess favourable pharmacokinetic and safety profiles, indicating potential for oral bioavailability and systemic tolerability. Nonetheless, it is important to acknowledge that these computational findings serve as predictive indicators rather than conclusive evidence. The reliability of such analyses is constrained by database accuracy and algorithmic limitations; therefore, empirical validation through *in vitro* and *in vivo* studies remains essential to confirm bioavailability, metabolism, and toxicity outcomes.

Notably, Caffeic acid displayed a strong binding affinity (−27.44 kcal/mol) toward the EGFR kinase domain, suggesting a potential mechanism comparable to that of established EGFR inhibitors such as gefitinib. Since EGFR overexpression is implicated in aggressive breast cancer subtypes, particularly triple-negative breast cancer, this observation supports the therapeutic promise of *Nyctanthes arbor-tristis* Linn. bioactives as adjunctive or combinatorial agents rather than standalone drugs.

Despite these promising findings, the study has certain limitations that must be acknowledged. While this study provides valuable mechanistic insights into the anticancer potential of *Nyctanthes arbor-tristis* Linn., several limitations should be noted. The results are based solely on *in silico* analyses, which, despite their predictive value, cannot fully represent biological complexity. The accuracy of these findings depends on database quality and computational assumptions. Notably, the biological significance of mo-

lecular docking scores (e.g., −27 kcal/mol) remains uncertain, as such values do not directly translate to *in vivo* efficacy. The absence of *in vitro* and *in vivo* validation restricts confirmation of the predicted targets, pathways, and interactions. Importantly, while docking results indicated strong binding affinities (e.g., −27 kcal/mol for Caffeic acid), the biological significance of such scores remains uncertain. Docking energy values are computational estimates and do not necessarily reflect the true binding strength or pharmacological activity in biological systems. Thus, the predicted interactions should be interpreted cautiously and validated experimentally to determine their real therapeutic relevance. Therefore, future studies should include molecular assays, cell-based models, and animal experiments to validate these predictions and assess pharmacokinetics, bioavailability, and toxicity, ensuring the translational reliability of *Nyctanthes arbor-tristis* in cancer therapy.

CONCLUSION

The present study systematically explored the therapeutic potential of *Nyctanthes arbor-tristis* Linn. in breast cancer using a comprehensive network pharmacology and molecular docking approach. A total of 57 bioactive compounds were identified, among which Lupeol and Caffeic acid emerged as key regulators of multiple breast cancer-associated targets, particularly through the PI3K-Akt and EGFR signaling pathways. Gene Ontology and pathway enrichment analyses further revealed the plant's multi-target, multi-pathway mode of action, suggesting synergistic regulation of cellular proliferation, apoptosis, and immune modulation. The strong binding affinities of several bioactives with EGFR kinase underscore their potential as natural inhibitors capable of modulating tumor progression mechanisms. From a clinical perspective, these findings highlight *Nyctanthes arbor-tristis* as a promising candidate for adjunctive or complementary breast cancer therapy. The plant's bioactives, particularly Lupeol, Nicotiflorin, and Caffeic acid, display favorable drug-likeness and ADMET profiles, supporting their potential for oral bioavailability and minimal toxicity, an important advantage over conventional chemotherapeutics that often cause severe adverse effects. Furthermore, the modulation of multiple signaling pathways indicates that *Nyctanthes arbor-tristis* could contribute to overcoming drug resistance and enhancing therapeutic efficacy when integrated with existing treatment regimens. Overall, this study establishes a scientific foundation for the traditional use of *Nyctanthes arbor-tristis* and supports its potential translation into clinically relevant phytopharmaceutical formulations. However, rigorous *in vitro, in vivo*, and clinical investigations are essential to validate these computational findings, optimize dosage strategies, and confirm their safety and therapeutic effectiveness in breast cancer management.

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AUTHOR CONTRIBUTION STATEMENT

O.T. and J.S. conceptualized and designed the study, with O.T. handling data collection. J.S. conducted data analysis and prepared the initial draft of the article. N.J. supervised the study, contributed to data analysis and interpretation, and provided essential revisions. All authors have reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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