RESEARCH PAPER

NETWORK PHARMACOLOGY AND MOLECULAR DOCKING-DRIVEN IDENTIFICATION OF HUB TARGETS REGULATED BY *Barleria lupulina* IN BREAST CANCER THERAPY

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ABSTRACT
Breast cancer remains a major cause of cancer-related deaths among women worldwide, with more than 2.3 million new cases and approximately 6,85,000 deaths annually. This study evaluated the anticancer potential of *Barleria lupulina* Lindl. (Acanthaceae) leaf extracts against MCF-7 breast cancer cells, supported by phytochemical profiling and computational analyses. The ethanolic extract was prepared by cold maceration and subjected to physicochemical evaluation, preliminary phytochemical screening, and quantitative estimation of total phenolics, flavonoids, and tannins. LC-MS analysis tentatively identified 14 phytoconstituents. Network pharmacology using SwissADME, SwissTargetPrediction, and STITCH identified 2,060 compound-associated targets, with 663 overlapping breast cancer-associated genes retrieved from GeneCards and OMIM. Hub targets included BDKRB1, NMUR2, HCAR2, and GPR17, while poncirin, dukunolide B, 4-caffeoyl-1,5-quinolactone, morroniside, and leonuride A were prioritised as hub compounds. Molecular docking against BRCA1 (4IGK), RAD51 (8C3N), bradykinin B1 receptor (7EIB), GPR17 (7Y89), and neuromedin U receptor 2 (7XK8) revealed protein-dependent binding, with poncirin showing the strongest affinity for 4IGK (−122.2), 7Y89 (−92.7), and 7XK8 (−116.6). The extract exhibited concentration-dependent DPPH (44.8–87.8%) and nitric oxide (43.8–91.1%) scavenging activity and reduced MCF-7 viability from 96.47% to 25.29% at 1–512 µg/mL, with an IC_{50} of 89.036 µg/mL. These findings suggest *B. lupulina* possesses promising antioxidant and cytotoxic activity, warranting further mechanistic and *in vivo* validation.

Keywords: *Barleria lupulina*; breast cancer; MCF-7; LC-MS; network pharmacology; molecular docking; antioxidant; cytotoxicity
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INTRODUCTION

Breast cancer is one of the most common cancers among women and it remains a major global health concern. Breast cancer incidence and mortality are influenced by genetic, hormonal, reproductive, lifestyle, and environmental factors [1,2]. Breast cancer (BC) is a major global public health concern, accounting for over 2.3 million new cases and approximately 685,000 deaths annually [1]. Its incidence varies considerably across regions, with higher age-standardised incidence rates reported in high-income regions, including Belgium, Australia, and Western Europe, according to GLOBOCAN 2020 [1,2]. Although low- and middle-income countries (LMICs) generally report lower incidence rates, they experience disproportionately high mortality due to late-stage diagnosis and limited access to healthcare. The global mortality-to-incidence ratio (MIR) is approximately 0.30, with higher ratios observed in resource-limited settings [2]. These disparities highlight the need to strengthen early detection, screening, healthcare infrastructure, treatment accessibility, and public awareness. Developing affordable diagnostic tools and effective therapeutic approaches is essential to improving breast cancer outcomes worldwide. Although conventional treatments like surgery, chemotherapy, radiotherapy, hormone therapy, and targeted therapy, have improved patient results, adverse effects and drug resistance remain important challenges [3].

Breast cancer is classified into different subtypes based on hormone receptor and HER2 status, including hormone receptor-positive, HER2-positive, and triple-negative breast cancer. These subtypes differ in their molecular characteristics, prognosis, and response to treatment [4]. Anticancer agents act through mechanisms such as cell cycle arrest, apoptosis induction, and inhibition of tumour

related signalling pathways. Antioxidants may also influence oxidative stress and cellular responses, although their effects depend on the compound and treatment context [5,6].

Natural products have gained attention as potential sources of anticancer compounds due to their structural diversity and biological activities. Several medicinal plants, including turmeric, green tea, garlic, ginseng, and *Withania somnifera*, have been investigated for their potential anticancer properties [7,8]. However, further experimental and computational validation is required to establish their therapeutic relevance.

Network-based techniques and molecular docking can aid in the discovery of possible bioactive chemicals and their interactions with cancer-related biological targets. Their eligibility for drug development is further evaluated by drug-likeness and ADMET evaluation [9,10]. Recent developments in artificial intelligence have made it possible to anticipate and rank promising applicants, which has increased computational screening.

The anticancer activity of leaf extracts from Barleria lupulina was assessed in this work using MCF-7 breast cancer cells. AI-assisted prediction, drug-likeness and ADMET evaluation, and molecular docking were among the computational methods used to further examine the identified phytochemical ingredients. The goal of this integrative method is to find possible anticancer candidates for more research.

Barleria lupulina Lindl. is an evergreen shrub with branches that is part of the Acanthaceae family. It has spiky branches, yellow to orange-yellow blooms, and narrow leaves with noticeable reddish midribs. Originally indigenous to Mauritius and portions of eastern India, it is now found in many tropical and subtropical areas, including as Africa and Southeast Asia. It can be found in Tamil Nadu and other western and southern parts of India [11,12].

Traditionally, different parts of *B. lupulina* have been used for medicinal purposes. Its leaves are applied to manage inflammation, insect stings, and snake bites, while the roots are also traditionally associated with anti-inflammatory uses. Phytochemical investigations have reported several classes of constituents, including iridoid glycosides, phenylethanoid glycosides, lignan glycosides, terpenoids, and phytosterols. Reported compounds include barlerin, shanzhiside methyl ester, forsythoside, oleanolic acid, lupeol, and β-sitosterol. Because of its unique foliage and lovely flowers, B. lupulina is grown as an ornamental plant in addition to its traditional therapeutic uses [13]. Further research into its biological activities is justified by its documented phytochemical variety and traditional usage. Therefore, using phytochemical identification and computational analyzes, the current study assesses the anticancer potential of B. lupulina leaf extracts against MCF-7 breast cancer cells.

2. MATERIALS AND METHODS

2.1. Collection and Authentication of Plant Material

In September 2025, *Barleria lupulina* Lindl. leaves were collected in Sengottai, Tirunelveli, Tamil Nadu, India. Mr. V. Chelladurai, Retired Research Officer in Botany, C.C.R.A.S., Government of India, Tirunelveli, authenticated the plant (Authentication No.: XCH-40750). Disease-free leaves without visible contamination were selected for the study.

2.2. Physicochemical Evaluation

Total ash, water-soluble ash, and acid-insoluble ash values were measured in accordance with Indian Pharmacopoeia (IP) procedures [14-17]. Water-soluble and ethanol-soluble extractive values were obtained using the maceration method with chloroform water and 90% ethanol, respectively, as per standard protocols [18,19]. Loss on drying was assessed by drying powdered leaves at 105°C for 5 hours, followed by calculation of percentage weight loss [20].

2.3. Preparation of Ethanolic Extract

250 grams of Powdered *B. lupulina* leaves were macerated with 1 L of ethanol. After filtration, the filtrate was concentrated under reduced pressure and stored appropriately for further analysis.

2.4. Preliminary Phytochemical Screening

Standard qualitative assays were used to screen the ethanolic extract of Barleria lupulina for main phytochemical components [21,22]. Alkaloids, steroids, coumarins, tannins, saponins, flavonoids, phenols, proteins, carbohydrates, and terpenoids were identified using their respective characteristic chemical reactions, including colour changes, precipitate formation, and foam formation.

2.5. Quantitative Estimation of Phytochemicals

2.5.1. Total Phenolic Content

Gallic acid was used as the reference standard in the Folin-Ciocalteu method to calculate the total phenolic content [23]. After 30 minutes of incubation at 40°C, the reaction mixture's absorbance was measured at 765 nm. The gallic acid calibration curve was used to determine the phenolic concentration.

2.5.2. Total Flavonoid Content

Using quercetin as the reference standard, the aluminum chloride colorimetric method was used to quantify the total flavonoid concentration [24]. After the reaction with sodium nitrite, sodium hydroxide, and aluminum chloride, absorbance was measured. The quercetin calibration curve was used to determine the flavonoid concentration.

2.5.3. Total Tannin Content

Total tannin content was determined using a Folin–Ciocalteu-based colorimetric method [25]. After incubation with the reagents at room temperature for 30 min, absorbance was measured at 725 nm, and

tannin content was calculated using the appropriate reference-standard calibration curve.

2.6. Liquid Chromatography–Mass Spectrometry (LC–MS) Analysis

An Agilent 6550 Q-TOF mass spectrometer with a Dual AJS electrospray ionization source was used to analyze the ethanolic leaf extract of *Barleria lupulina*. AutoMS2 acquisition was used to conduct analysis in positive-ion mode over a m/z range of 100–1200. Water containing 0.1% formic acid (mobile phase A) and acetonitrile (mobile phase B) was used for chromatographic separation at a flow rate of 0.3 mL/min. The column temperature was kept at 35 °C, and the injection volume was 5 µL. The chemical components of the extract were described using the resulting mass spectra.

2.7. Network Pharmacology

2.7.1. Phytochemical Identification and ADME Screening

Compounds reported in published investigations of *B. lupulina* were added to phytochemicals discovered by LC-MS. Scopus, Web of Science, PubMed, and Google Scholar were used to search for pertinent material. For computational study, PubChem provided canonical SMILES representations of a few chosen chemicals.

SwissADME and Molsoft were used to assess physicochemical properties, drug-likeness, predicted gastrointestinal absorption, and other relevant pharmacokinetic characteristics. Compounds were prioritised using the reported cut-offs of predicted oral bioavailability ≥0.30 and drug-likeness score ≥0.18 [26].

2.7.2. Prediction of Protein Targets

Potential human protein targets of the selected phytochemicals were predicted using SwissTargetPrediction and STITCH [27,28]. The predicted targets were restricted to *Homo sapiens*, compiled, and deduplicated before comparison with breast cancer-associated genes.

2.7.3. Identification of Breast Cancer-Associated Genes

Breast cancer-associated genes were retrieved from GeneCards and the Online Mendelian Inheritance in Man (OMIM) database using breast cancer-related search terms [29]. The predicted phytochemical targets were compared with the disease-associated gene set, and overlapping genes were identified using a Venn diagram tool. The overlapping genes were selected as candidate targets for subsequent interaction-network analysis.

2.7.4. Protein–Protein Interaction Network

A protein–protein interaction (PPI) network was created by submitting the overlapping potential targets to STRING. A minimum interaction score of 0.7 was used, and the organism was limited to *Homo sapiens*. For network analysis and visualization, the resulting interaction data were exported.

2.7.5. Compound–Target Network Analysis

Cytoscape version 3.10.3 was used to create a network of compound–target interactions [28]. The CytoHubba plugin was used to analyze network topology. Highly related molecules and prospects for additional research were identified using degree, betweenness centrality, and closeness centrality.

2.8. Molecular Docking

2.8.1. Protein Preparation

Three-dimensional protein structures were retrieved from the RCSB Protein Data Bank. The selected structures were BRCA1 (PDB ID: 4IGK), RAD51 recombinase (8C3N), bradykinin B1 receptor (7EIB), GPR17–Gi complex (7Y89), and neuromedin U receptor 2 (7XK8). BRCA1 was included as an additional breast cancer-related target, although it was not identified through the network-pharmacology overlap.

Protein preparation was performed using Molegro Virtual Docker (MVD), version 6.0. Non-essential water molecules and heteroatoms were removed, and missing hydrogen atoms were added. Binding cavities were identified using the software's cavity-detection algorithm, and the selected cavity for each protein was used to define the docking search region [30].

2.8.2. Ligand Preparation

Five phytochemicals-Leonuride A, morroniside, dukunolide, 4-caffeoyl-1,5-quinolactone, and poncirin were selected for molecular docking based on the reported selection criteria. Their three-dimensional structures were obtained from PubChem in SDF format. Ligand geometry was optimised using Chem3D with the MMFF94 force field before importing the structures into MVD [30-32].

2.8.3. Docking Procedure

Molecular docking was performed using the MolDock SE algorithm in MVD. Binding cavities were defined individually for each protein, and multiple ligand poses were generated and ranked using the MolDock Score and Re-Rank Score. The most favourable poses were selected based on docking scores and plausible binding orientations. The reported pose-clustering energy threshold was 100 [31].

2.8.4. Protein–Ligand Interaction Analysis

The top-ranked protein-ligand complexes were analysed using MVD to identify hydrogen bonds, hydrophobic contacts, and other predicted intermolecular interactions. Two-dimensional and three-dimensional interaction diagrams were generated to visualise ligand-binding orientations and interacting residues. Docking scores and interaction patterns were used to compare the predicted binding behaviour of the selected phytochemicals across the investigated targets [32].

2.9. In Vitro Antioxidant Activity

2.9.1. DPPH Radical Scavenging Assay

The reported approach was used to assess DPPH radical scavenging activity [33]. Different amounts of

the ethanolic extract and ascorbic acid standard were made and combined with the DPPH solution. After 30 minutes of dark room temperature incubation, absorbance at 517 nm was measured in comparison to the proper control. The concentration–inhibition relationship was used to calculate the IC_{50} values and the percentage of radical scavenging activity.

2.9.2. Nitric Oxide Scavenging Assay

Griess reagent and sodium nitroprusside were used to assess nitric oxide scavenging activity [34]. Phosphate-buffered saline (pH 7.4) containing 10 mM sodium nitroprusside was incubated with extract samples at various concentrations for three hours at 30°C. After adding the Griess reagent, absorbance was measured at 550 nm. The reference standard was ascorbic acid. The experimental results were used to calculate IC_{50} values and percentage inhibition.

2.10. In Vitro Cytotoxicity Against MCF-7 Cells

According to Mosmann (1983), the MTT assay was used to assess the cytotoxic activity of the *B. lupulina* leaf extract against MCF-7 breast cancer cells. Before being treated with extract concentrations ranging from 1 to 512 µg/mL for 24 to 48 hours, cells were seeded in 96-well plates and incubated at 37 °C for 48 hours. After treatment, the medium was taken out and 5 mg/mL of MTT solution was added. The formazan crystals were dissolved in 100 µL of DMSO following a 4-hour incubation period at room temperature. Cell viability was evaluated by measuring absorbance at 570 nm. The extract's IC_{50} value against MCF-7 cells was reported to be 89.036 µg/mL. Three separate measurements were made.

3.RESULTS AND DISCUSSION

3.1. Physicochemical Evaluation of *Barleria lupulina* Leaves

The physicochemical parameters of *B. lupulina* leaves were assessed to establish preliminary quality-control characteristics. The total ash, water-soluble ash, and acid-insoluble ash values were 10.45 ± 0.12%, 4.23 ± 0.08%, and 1.12 ± 0.04% w/w, respectively. The moisture content and loss on drying were 8.25 ± 0.78% and 7.34 ± 0.15%, respectively, while the water-soluble extractive value was 18.60 ± 1.42% w/w. These parameters provide baseline information on the inorganic constituents, moisture status, and water-extractable components of the plant material and may support its preliminary quality assessment.

Values are expressed as mean ± SD (n = 3).

3.2. Extraction of *Barleria lupulina*

Extraction of 250 g of *B. lupulina* leaf material using 1 L of solvent yielded 18.56 g of crude extract, corresponding to a percentage yield of 7.42%. The recovered extract was subsequently used for phytochemical screening and biological evaluation.

3.3. Phytochemical Screening of the Ethanolic Extract

Preliminary screening of the ethanolic extract of *B. lupulina* revealed the presence of terpenoids, flavonoids, anthraquinones, carbohydrates, alkaloids, phenols, tannins, saponins, and proteins. Steroids and glycosides were not detected under the conditions employed. These findings indicate the presence of diverse phytochemical constituents in the extract, although their individual contributions to the observed biological activities require further investigation.

3.4. Quantitative Estimation of Total Phenolic, Flavonoid, and Tannin Contents

At a test concentration of 1000 µg/mL, the ethanolic extract exhibited total phenolic, flavonoid, and tannin content values of 3.580 ± 0.41, 2.646 ± 0.30, and 2.556 ± 0.30, respectively. Phenolic content was the highest among the three measurements, followed by flavonoid and tannin content. The presence of these phytochemical groups may contribute to the antioxidant potential of the extract. However, the relationship between their measured levels and biological activity requires further validation.

3.5. LC–MS Analysis and Phytochemical Identification

Base peak chromatograms obtained in positive and negative electrospray ionisation modes showed distinct ionisation profiles. In positive mode, prominent signals occurred at approximately 1.2, 6.5, 8.0, and 26.0 min, with a maximum intensity of approximately 1.9×10^6 counts. Negative mode showed its most intense signal at approximately 8.0 min, reaching 3.1×10^6 counts, together with additional peaks at approximately 7.0, 10.5, 11.0, and 12.5 min. The differing profiles indicate complementary detection of ionisable constituents across the two modes.

Fourteen phytochemical constituents were tentatively assigned based on chromatographic and mass-spectrometric data, including retention time, molecular formula, and molecular mass comparisons. The proposed identities represented diverse chemical groups, including phenolic acids, flavonoids and their glycosides, iridoid glycosides, and other specialised metabolites. These assignments provide a preliminary chemical profile of *B. lupulina*; confirmation of individual compounds would require appropriate diagnostic MS/MS fragmentation and, where available, comparison with authentic reference standards.

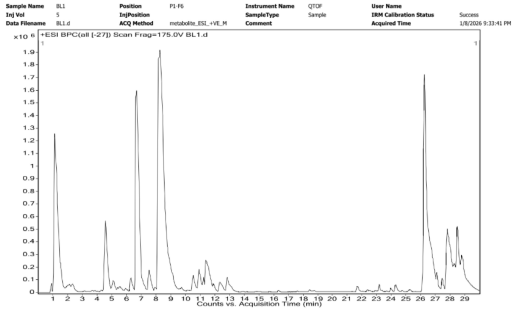


Figure 1: LC MS analysis for phytocostituent of *Barleria lupulina* (positive rotation peak)

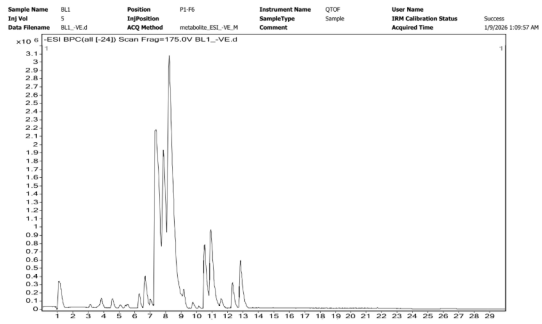


Figure 2; LC MS analysis for phytocostituent of *Barleria lupulina* (negative rotation peak)

3.6. Network pharmacology and ADMET screening

3.6.1. Phytochemical Library and ADMET Screening

GC-MS and LC-MS profiling generated an initial library of 21 phytoconstituents, comprising seven compounds identified by GC-MS from the literature and 14 tentatively identified by LC-MS. The compounds represented diverse chemical classes, including terpenoids, fatty acid derivatives, flavonoid glycosides, iridoid glycosides, and phenolic acid derivatives.

The compounds were evaluated using SwissADME and Molsoft for their physicochemical properties and predicted drug-likeness. According to the applied selection criteria of oral bioavailability ≥ 0.30 and drug-likeness score ≥ 0.18, all 21 compounds were retained for target prediction. These computational assessments provide preliminary information on the compounds' drug-like characteristics but do not establish their actual oral bioavailability, safety, or pharmacological efficacy.

3.6.2. Target Prediction and Breast Cancer Gene Overlap

SwissTargetPrediction was used to predict potential protein targets for the phytoconstituents. Following the application of the specified human-target and probability filters, 2,060 compound-associated targets were compiled for comparison with breast cancer-associated genes retrieved from GeneCards and OMIM. The intersection analysis identified 663 shared targets, representing potential links between the predicted molecular targets of *B. lupulina* constituents and breast cancer-associated biology. These overlapping targets were subsequently selected for protein–protein interaction analysis and functional prioritisation.

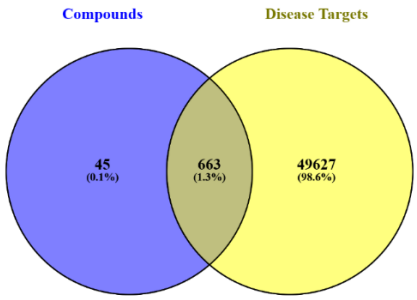


Figure 3: Venn diagram illustrating the intersection between 2,060 compound-predicted targets (SwissTargetPrediction) and 50,663 breast cancer disease genes (GeneCards and OMIM). The overlapping region contains 663 candidate therapeutic targets.

3.6.3. Protein–Protein Interaction Network and Hub Target Analysis

The 663 overlapping targets were analysed using STRING at a minimum interaction confidence score of 0.700, and the resulting network was visualised in Cytoscape 3.10.3. Network centrality measures, including degree, betweenness, and closeness, were used to prioritise highly connected nodes. The reported top five candidates were BDKRB1, NMUR2, HCAR2, GPR17, and glutamic acid.

These candidates require further biological and methodological validation before their roles in breast cancer can be interpreted. In particular, glutamic acid is a metabolite rather than a protein target and should not be presented as a protein–protein interaction hub without clarification of how it entered the network.

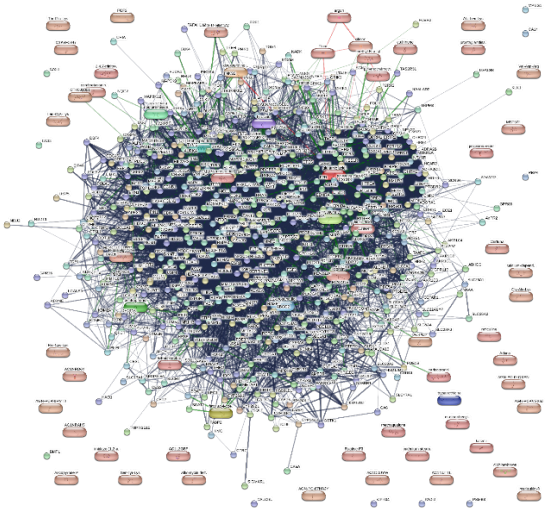


Figure 4: The STRING analysis generated a high-confidence PPI network comprising the 663 input proteins and their first-order interaction partners.

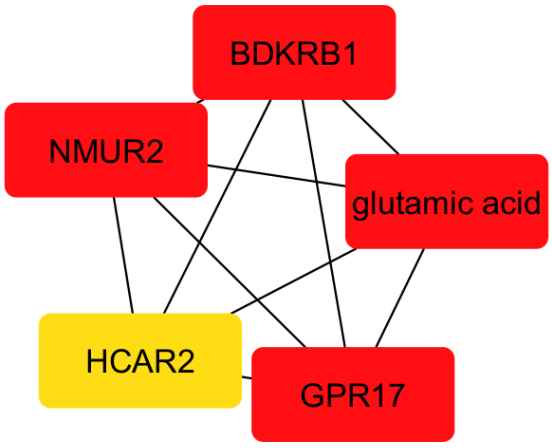


Figure 5: The top 5 hub targets (BDKRB1, NMUR2, HCAR2, GPR17, glutamic acid) identified by CytoHubba centrality analysis are highlighted in red

3.6.4 Compound–Target Network and Hub Compound Prioritisation

A compound–target network was constructed in Cytoscape to visualise the predicted relationships between the phytoconstituents and the overlapping breast cancer-associated targets. Centrality-based prioritisation identified poncirin, dukunolide B, 4-caffeoyl-1,5-quinolactone, morroniside, and leonuride A as the five leading compounds.

These compounds were prioritised for subsequent molecular docking based on their reported network positions. Their predicted interactions provide a basis for further investigation but do not, by themselves, establish direct target binding or synergistic activity.

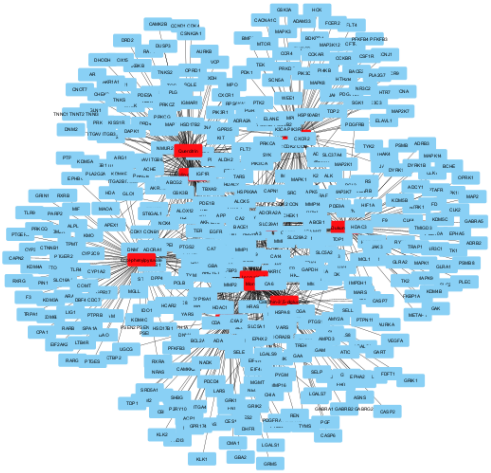


Figure 6: Compound Target Interaction of Barleria lupulina from Cytoscape

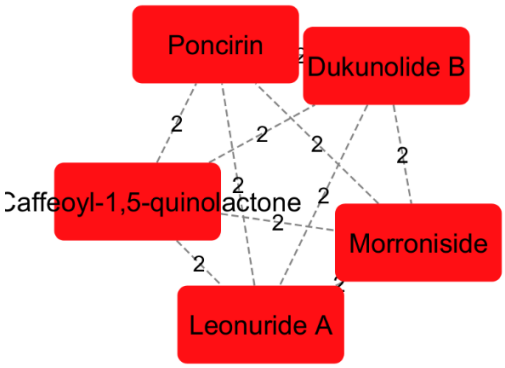


Figure 7 : Compound–Target Bipartite Network constructed in Cytoscape 3.10.3. Red rectangular nodes represent the top 5 hub compounds (Poncirin, Dukunolide B, 4-Caffeoyl-1,5-quinolactone, Morroniside, Leonuride A).

3.7. Molecular docking

3.7.1 Molecular Docking Analysis

Molecular docking was performed to evaluate the predicted binding poses of the five prioritised phytoconstituents against the selected protein structures, using olaparib as the reference ligand. The results varied according to the protein–ligand combination.

Against the structure designated PDB ID 4IGK, poncirin produced the most negative MolDock score (−122.2), followed by morroniside (−99.2) and leonuride A (−94.0). Against PDB ID 8C3N, 4-caffeoyl-1,5-quinolactone showed the most negative MolDock score (−107.9), followed by poncirin (−99.8) and leonuride A (−98.2). For PDB ID 7EIB, morroniside ranked first (−109.2), while poncirin showed the most negative score against PDB ID 7Y89 (−92.7) and PDB ID 7XK8 (−116.6).

The ranking differences indicate that the predicted binding preferences were protein-dependent. Several compounds also showed favourable hydrogen-bond score contributions, although these values should be interpreted alongside the overall docking scores and individual interaction patterns. The results identify candidates for further investigation; however, docking alone cannot establish binding affinity experimentally, target inhibition, or anticancer efficacy. These findings require validation through appropriate biochemical and cellular assays.

Table 1: Molecular Docking Scores of Selected Phytochemicals

Protein structure	Best-ranked compound	MolDock score	Olaparib score
4IGK	Poncirin	−122.2	−94.7
8C3N	4-Caffeoyl-1,5-quinolactone	−107.9	−72.9
7EIB	Morroniside	−109.2	−87.7

7Y89	Poncirin	−92.7	−77.3

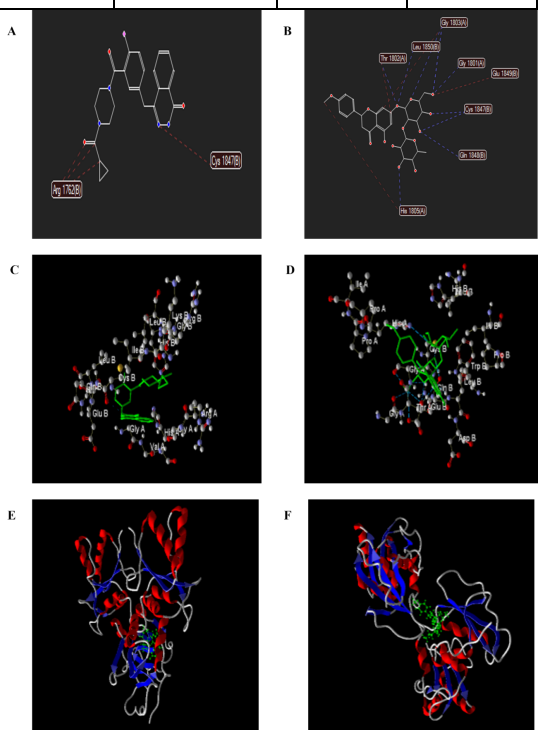


Figure 8 : Interaction of Olaparib and Poncirin with 4IGK Protein; 2D Structure of Olaparib (A) and Poncirin (B), 3D structure of Olaparib (C) and Poncirin (D), Secondary interaction of Olaparib (E) and Poncirin (F)

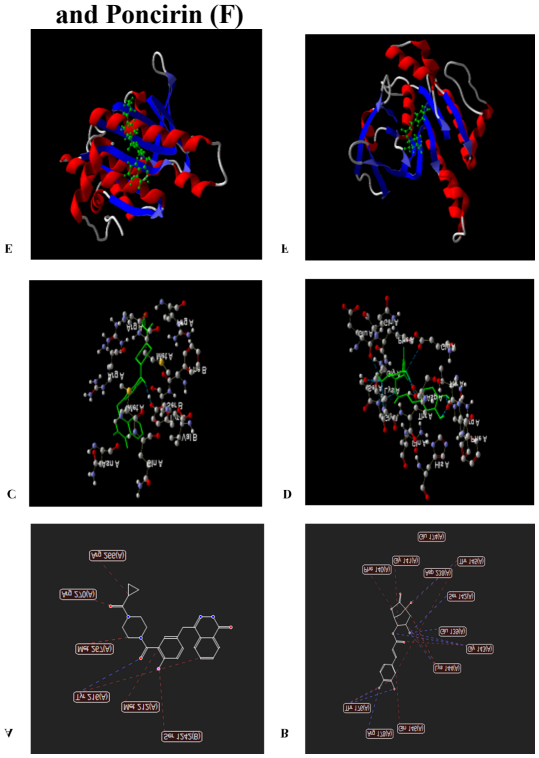


Figure 9 : Interaction of Olaparib and 4-Caffeoyl-1,5-quinolactone with 8C3N Protein; 2D Structure of Olaparib (A) and 4-Caffeoyl-1,5-quinolactone (B), 3D structure of Olaparib (C) and 4-Caffeoyl-1,5-quinolactone (D), Secondary interaction of Olaparib (E) and 4-Caffeoyl-1,5-quinolactone (F)

7XK8	Poncirin	−116.6	−87.0

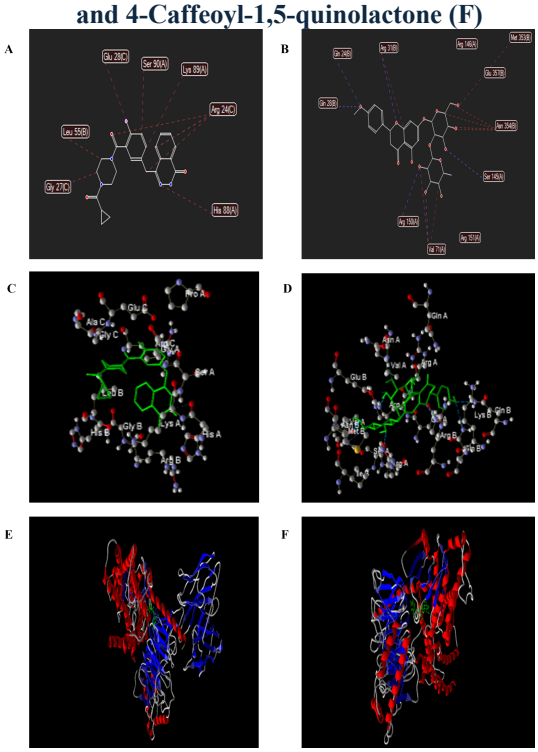


Figure 10: Interaction of Olaparib and Morroniside with 7EIB Protein; 2D Structure of Olaparib (A) and Morroniside (B), 3D structure of Olaparib (C) and Morroniside (D), Secondary interaction of Olaparib (E) and Morroniside (F)

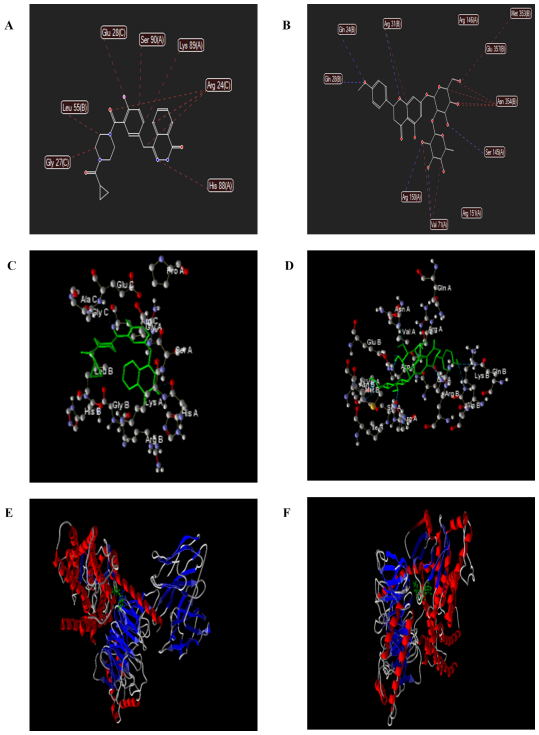


Figure 11 : Interaction of Olaparib and Poncirin with 7Y89 Protein; 2D Structure of Olaparib (A) and Poncirin (B), 3D structure of Olaparib (C) and Poncirin (D), Secondary interaction of Olaparib (E) and Poncirin (F)

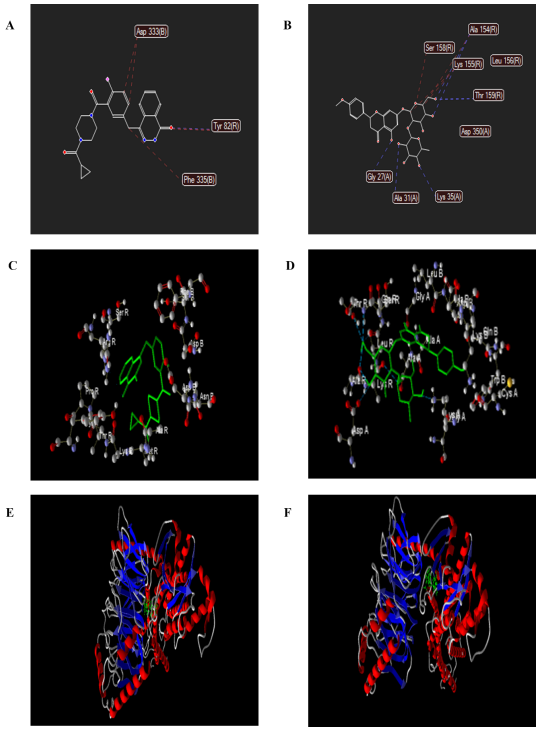


Figure 12: Interaction of Olaparib and Poncirin with 7XK8 Protein; 2D Structure of Olaparib (A) and Poncirin (B), 3D structure of Olaparib (C) and Poncirin (D), Secondary interaction of Olaparib (E) and Poncirin (F)

3.8. Antioxidant activity

3.8.1 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the ethanolic extract of *B. lupulina* increased with concentration, from 44.8 ± 4.6% at 200 µg/mL to 87.8 ± 3.6% at 1000 µg/mL. Under the same assay conditions, ascorbic acid showed inhibition values of 65.24 ± 3.42% and 96.45 ± 5.27% at these respective concentrations. Although the extract exhibited concentration-dependent radical scavenging activity, its inhibition remained lower than that of the reference standard at all tested concentrations. These findings indicate that the ethanolic extract possesses measurable in vitro antioxidant activity.

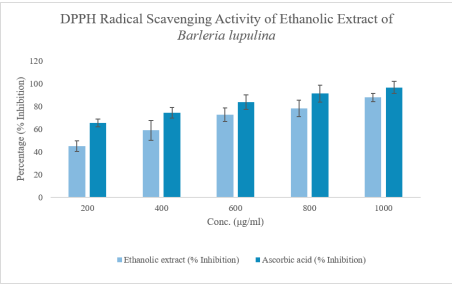


Figure 13: DPPH Radical Scavenging Activity of Ethanolic Extract of *Barleria lupulina*

3.8.2 Nitric Oxide Scavenging Activity

The ethanolic extract of *B. lupulina* exhibited concentration-dependent nitric oxide scavenging activity, increasing from 43.8 ± 3.1% at 200 µg/mL to 91.1 ± 6.8% at 1000 µg/mL. The corresponding inhibition values for ascorbic acid were 67 ± 4.6% and 97 ± 9.5%. The extract showed lower scavenging activity than the reference standard throughout the tested concentration range. These results suggest that the ethanolic extract can scavenge nitric oxide under the experimental conditions, although further studies are needed to establish the underlying mechanisms and biological relevance.

Figure 14: Nitric Oxide Scavenging Activity of *Barleria lupulina*

3.9. In vitro cytotoxicity against MCF-7 cells

Cytotoxic Activity of the Ethanolic Extract Against MCF-7 Cells

The cytotoxic activity of the ethanolic extract of *B. lupulina* was evaluated in MCF-7 breast cancer cells using the MTT assay. Cell viability decreased with increasing extract concentration, from 96.47 ± 0.65% at 1 µg/mL to 25.29 ± 0.67% at 512 µg/mL. At 128 µg/mL, cell viability was 46.02 ± 0.27%, indicating a concentration-dependent reduction in metabolic viability. The reported IC_{50} was 89.036 µg/mL.

These findings demonstrate that the ethanolic extract reduced MCF-7 cell viability under the tested conditions. However, the MTT assay measures cellular metabolic activity and cannot independently distinguish cytostatic effects from cell death. Additional studies, including apoptosis assays and evaluation of non-cancerous cells, would help

clarify the mechanism and selectivity of the observed cytotoxic effect.

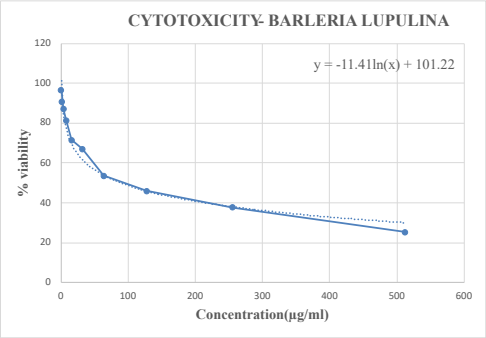


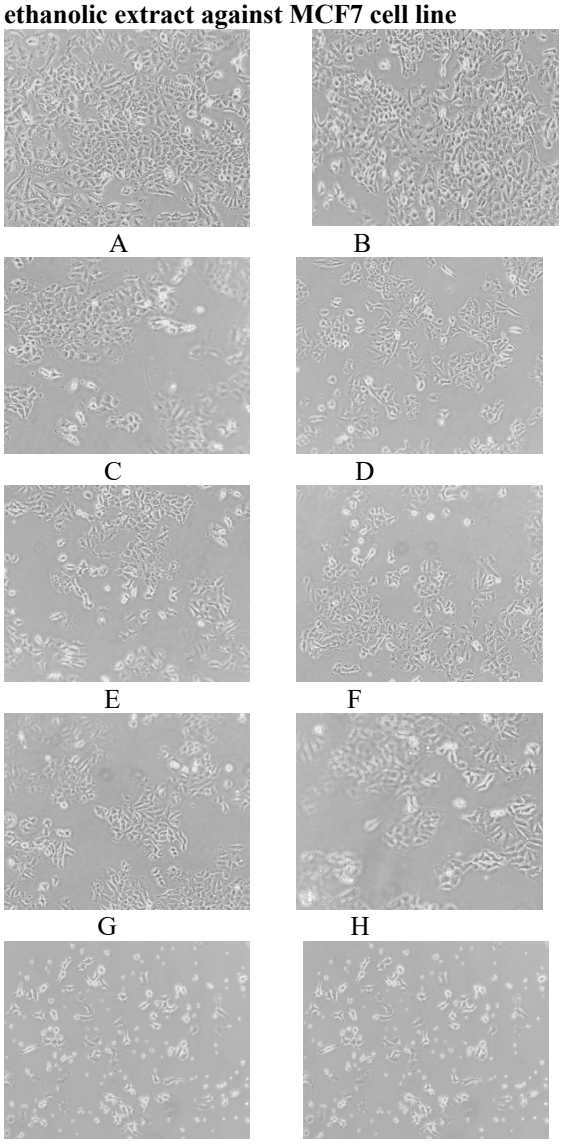
Figure 1 : Cytotoxicity of Barleria lupulina

IC $_{50}$ value against MCF-7 cell line

Table 2: IC_{50} value against MCF-7 cell line

SAMPLE	Concentration(µg/ml) IC_{50}
Barleria lupulina	89.036

Cytotoxicity activity of *Barleria lupulina* ethanolic extract against MCF7 cell line



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Figure 13: Cytotoxicity activity of *Barleria lupulina* ethanolic extract against MCF7 cell line (A -1 µg/ml; B - 2 µg/ml; C - 4 µg/ml; D - 8 µg/ml; E - 16 µg/ml; F -32 µg/ml; G- 64 µg/ml; H -128 µg/ml; I - 256 µg/ml; J -512 µg/ml)

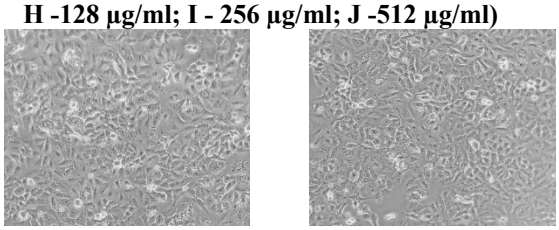


Figure 14: Control pictures of MCF7

4.DISCUSSION

Breast cancer is the most commonly diagnosed cancer among women worldwide, highlighting the need for safer and more effective treatment strategies. This study investigated the anticancer, antioxidant, and anti-inflammatory potential of *Barleria lupulina* leaf extract through phytochemical analysis, in vitro assays, and in silico network pharmacology.

Leaves were collected from Sengottai, Tirunelveli, Tamil Nadu, authenticated, and extracted using ethanol by cold maceration. Physicochemical evaluation, phytochemical screening, quantitative estimation of phenols, flavonoids, and tannins, and GC-MS and LC-MS analyses were performed. Antioxidant activity was assessed using DPPH and nitric oxide radical-scavenging assays, while anti-inflammatory activity was evaluated through HRBC membrane stabilization. Anticancer activity was determined using the MTT assay against MCF-7 breast cancer cells. Network pharmacology, protein–protein interaction analysis, and molecular docking were performed to explore potential therapeutic targets.

The results revealed a diverse phytochemical profile containing phenolic compounds, flavonoids, iridoids, and other bioactive constituents. GC-MS and LC-MS analyses identified 22 compounds. The extract demonstrated antioxidant and anti-inflammatory activities, along with concentration-dependent cytotoxicity against MCF-7 cells, reducing cell viability to 25.289% at 512 µg/mL. Network pharmacology identified 663 overlapping candidate targets associated with breast cancer. HIF1A and CASP8 emerged as important targets linked to cancer-related and apoptotic pathways, while molecular docking supported potential interactions between selected compounds and these targets.

Overall, the findings suggest that *Barleria lupulina* leaf extract has promising antioxidant, anti-inflammatory, and anticancer properties. Further studies involving active compound validation, animal models, and toxicity evaluation are required to establish its safety and therapeutic potential for future drug development.

5.CONCLUSION

This study validates the dual therapeutic potential of *Barleria lupulina* leaf extracts as a natural, multi-target anti-breast cancer and antioxidant agent through an integrated *in silico* and *in vitro* workflow. Advanced LC-MS and GC-MS profiling identified 22 primary active compounds, rich in phenolics and flavonoids, which exhibited potent, dose-dependent DPPH radical scavenging activity. Network pharmacology intersected these phytochemicals with 663 unique breast cancer target genes, while molecular docking highlighted outstanding binding affinities, particularly for Poncirin (MolDock score of -116.6 kcal/mol). Crucially, *in vitro* cell viability testing against human MCF-7 lines substantiated these mechanisms, demonstrating remarkable targeted cytotoxicity with a highly favorable value of 64 µg/ml. Ultimately, these findings establish the polypharmacological efficacy of *B. lupulina*, positioning its phytochemical scaffold as a biochemically sound lead candidate for modern targeted breast cancer therapies.

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