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Exploring the multi target mechanisms of Auxarthrol F in TNBC treatment using network pharmacology and molecular docking.

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List of Abbreviation:

TNBC: Triple-Negative Breast Cancer.

ER: Estrogen Receptor.

PR: Progesterone Receptor.

HER2: Human Epidermal Growth Factor Receptor 2.

AR: Androgen Receptor.

PARP: Poly (ADP-ribose) Polymerase.

PD-L1: Programmed Death Ligand 1

PI3K/AKT/mTOR: Phosphatidylinositol 3-Kinase / Protein Kinase B / Mammalian Target of Rapamycin

KEGG: Kyoto Encyclopedia of Genes and Genomes.

PPI: Protein–Protein Interaction.

BP: Biological Process.

CC: Cellular Component.

MF: Molecular Function.

MNC: Maximum Neighborhood Component.

MCC: Maximum Clique Centrality.

SEA: Similarity Ensemble Approach.

SMILES: Simplified Molecular Input Line Entry System.

EGFR: Epidermal Growth Factor Receptor.

GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase.

HDAC1: Histone Deacetylase 1.

CASP3: cysteinyl aspartate-specific proteinases.

PDB: Protein Data Bank.

ΔG: Binding Energy.

NMR: Nuclear Magnetic Resonance.

IC50: Half Maximal Inhibitory Concentration

Abstract

Auxarthrol F is anthraquinone derivative known for its anticancer effects. Its cytotoxic activity against triple negative breast cancer (TNBC) has been demonstrated, however, the specific molecular mechanisms underlying its efficacy have remained elusive. This study, therefore, aimed to investigate the key targets and the molecular mechanisms of Auxarthrol F in TNBC treatment by employing network pharmacology and molecular docking approaches. Auxarthrol F targets and TNBC targets were determined using Swiss target prediction, SEA and Gene cards databases. Venn diagram analysis was utilized to determine the overlapped targets. Subsequently, protein-protein interaction (PPI) network was constructed using STRING database, where as Cytoscape software was used to identify the hub genes. GO enrichment and KEGG pathway analyses were performed using Shiny Go database. Molecular docking analysis was conducted to validate the binding conformation between Auxarthrol F and the putative Target genes. The findings revealed 90 anti TNBC targets which were implicated in various biological processes. The KEGG study demonstrated several pathways linked to TNBC including pathways in cancer, metabolic pathways, lipid and atherosclerosis, Rap1 signaling pathway and MAPK signaling pathway .PPI network suggested that Auxarthrol F may alleviate TNBC by modulating key genes, including EGFR, GAPDH, HDAC1 and CASP3, which further showed stable binding with Auxarthrol F, as demonstrated by molecular docking. In summary this investigation provides valuable insights into the potential targets and molecular processes and pathways through which Auxarthrol F exerts its anti-cancer effects. These findings not only enhance our understanding of Auxarthrol F pharmacological actions in TNBC but also lay theoretical ground work for future *in vitro* and *in vivo* investigations.

Keywords: Triple negative breast cancer, Auxarthrol F, Network pharmacology, Molecular docking, KEGG, signaling pathways.

Résumé

Auxarthrol F est un dérivé de l'anthraquinone connu pour ses effets anticancéreux. Son activité cytotoxique contre le cancer du sein triple négatif (TNBC) a été démontrée, cependant les mécanismes moléculaires spécifiques liés à son efficacité restent encore mal compris. Cette étude avait donc pour objectif d'identifier les cibles clés et les mécanismes moléculaires impliqués dans le traitement du TNBC par l'Auxarthrol F en utilisant des approches de pharmacologie de réseaux et de docking moléculaire. Les cibles de l'Auxarthrol F et celles du TNBC ont été identifiées à partir des bases de données Swiss Target Prediction, SEA et Gene Cards, puis une analyse par diagramme de Venn a permis de déterminer les cibles communes. Un réseau d'interactions protéine protéine (PPI) a ensuite été construit à l'aide de la base de données STRING, tandis que le logiciel Cytoscape a servi à identifier les gènes centraux. Les analyses d'enrichissement GO et des voies KEGG ont été réalisées à l'aide de la base de données Shiny GO, et une analyse de docking moléculaire a été effectuée afin de valider les liaisons entre l'Auxarthrol F et les gènes cibles potentiels. Les résultats ont révélé 90 cibles anti TNBC impliquées dans divers processus biologiques. L'analyse KEGG a mis en évidence plusieurs voies associées au TNBC, notamment les voies du cancer, les voies métaboliques, les voies liées aux lipides et à l'athérosclérose, la voie de signalisation Rap1 et la voie de signalisation MAPK. Le réseau PPI a suggéré que l'Auxarthrol F pourrait agir contre le TNBC en modulant des gènes clés, notamment EGFR, GAPDH, HDAC1 et CASP3, lesquels ont également montré une liaison stable avec l'Auxarthrol F selon les résultats du docking moléculaire. En résumé, cette étude apporte des informations précieuses sur les cibles potentielles ainsi que sur les processus et voies moléculaires par lesquels l'Auxarthrol F exerce ses effets anticancéreux. Ces résultats améliorent non seulement la compréhension des actions pharmacologiques de l'Auxarthrol F dans le TNBC, mais fournissent également une base théorique pour de futures études *in vitro* et *in vivo*.

Mots clés : Cancer du sein triple négatif, Auxarthrol F, pharmacologie des réseaux, docking moléculaire, KEGG, voies de signalisation.

ملخص

هدفت هذه الدراسة إلى استكشاف الأهداف الجزيئية والآليات البيولوجية المحتملة لمركب Auxarthrol F في علاج سرطان الثدي الثلاثي السلبي (TNBC) باستخدام منهجيات الصيدلة الشبكية والإرساء الجزيئي. تم تحديد الأهداف المشتركة بين المركب والمرض بالاعتماد على قواعد بيانات متخصصة، ثم تحليل شبكات تفاعل البروتينات وتحديد الجينات المحورية والمسارات البيولوجية المرتبطة بها. أظهرت النتائج وجود 90 هدفًا محتملاً مضادًا للسرطان،كما كشفت تحليلات KEGG عن ارتباط المركب بعدة مسارات مهمة، من بينها مسارات السرطان، والتمثيل الغذائي، ومسارRap1 ، ومسار .MAPK كما بينت الدراسة أن Auxarthrol F قد يمارس تأثيره المضاد للسرطان من خلال تنظيم جينات رئيسية مثل EGFR و GAPDHو HDAC1وCASP3، مع إظهار ارتباط جزيئي مستقر بهذه الأهداف. وتشير هذه النتائج إلى أن Auxarthrol Fيمتلك إمكانات واعدة في علاج سرطان الثدي الثلاثي السلبي، مما يوفر أساسًا نظريًا لدراسات مستقبلية مخبرية وحيوية للتحقق من فعاليته وآلية عمله.

الكلمات المفتاحية: سرطان الثدي الثلاثي السلبي(TNBC) ، أوكسارثرول F ، الصيدلة الشبكية، الإرساء الجزيئي، KEGG، مسارات الإشارة الخلوية.

INTRODUCTION

Introduction

Breast cancer is the most common malignant tumor found in women throughout the world. Triple negative breast cancer (TNBC), which is the most aggressive subtype of breast cancer without an expression of the progesterone receptor, estrogen receptor, and human epidermal growth factor receptor 2, accounts for 10–20% of all breast cancers (Zhang et al., 2021).This type of breast cancer is associated with three key characteristic occurrence at a younger age, a high recurrence rate, and strong invasiveness (Z. Wu et al., 2024).This particular form of breast cancer is widely regarded as one of the most hostile (Z. Wu et al., 2024). Chemotherapy remains the mainstay of treatment; however, the efficacy is often limited due to drug resistance in TNBC cells (Zhao et al., 2025).Traditional treatment methods have now developed resistance and various adverse effects, highlighting an urgent need for attention. Therefore, it is advisable to substitute these conventional therapies with innovative medications (Singh et al., 2025).

Natural products have long attracted attention due to their therapeutic potential, favorable safety profile, and relatively low side effects, making them promising candidates in cancer treatment. Therefore, the development of novel natural compounds as complementary and alternative strategies for TNBC is of considerable importance (Z. Wu et al., 2024).Auxarthrol F is natural compound belongs to the anthraquinone family, which are aromatic compounds. It is primarily found in marine fungi such as *Sporendonema casei*. Auxarthrol F has been shown to exhibit potential anti-cancer activity against TNBC cells among other cell lines (Ge et al., 2019; Hafez Ghoran et al., 2022).However, no study has so far been done on its underlying molecular mechanisms. Since cancer is a complex disease involving various processes, targets, and mechanisms, a deeper analysis by which Auxarthrol F exhibits its anticancer effect should be done.

Network pharmacology, an innovative method in drug exploration, has been developed by combining bioinformatics, biological systems, and pharmacology. This approach employs systems of biology and bioinformatics techniques to thoroughly examine the interplay among diseases, target genes, and medications. In contrast to conventional single target drug research techniques, network pharmacology can uncover the collaborative effects of drugs on various targets and pathways, thereby offering a more complete insight into their functioning. This technique is especially effective for analyzing the therapeutic mechanisms associated with intricate diseases like TNBC (Zhao et al., 2025).

Molecular docking is a fundamental computational technique for structure based drug discovery and target evaluation. This methodology simulates and predicts the non-covalent interactions between a small molecule (ligand) and the active binding site of a target protein at

an atomic resolution. The core importance of molecular docking lies in its ability to efficiently sample ligand conformations and rank them using advanced scoring functions, thereby providing critical estimates of binding affinities and structural orientations. By utilizing this dry lab approach, researchers can perform high throughput virtual screening to filter prospective candidates with high specificity and precision. Ultimately, this computer aided evaluation offers a distinct advantage over traditional *in vivo* strategies by significantly minimizing the investment of resource and time, while offering meticulous structural insights that guide and rationalize subsequent experimental validation (Agu et al., 2023).

In the present study, we employed a network pharmacology approach integrated with molecular docking to investigate the potential molecular mechanisms of Auxarthrol F in TNBC treatment. We first identified the target genes associated with Auxarthrol F and TNBC from various databases and constructed protein protein interaction (PPI) network. Then we identified the key targets of Auxarthrol F against TNBC. Thereafter, Gene Ontology (GO) and KEGG pathway enrichment analyses were conducted to gain insights into the biological significance of these targets and the relevant pathways. Finally, molecular docking studies were performed to evaluate the interactions of Auxarthrol F with the hub genes, shedding light on its potential therapeutic impact in TNBC.

Overall, our work is divided into two parts: The first part is devoted to a literature review. It consists of three chapters: Chapter 1 presents triple negative breast cancer (TNBC), Chapter 2 describes the bioactive compound Auxarthrol F, and Chapter 3 focuses on network pharmacology and *in silico* approaches. The second part concerns the experimental, which is composed of two chapters: chapter 1 describes the materials used and the *in silico* analysis methods adopted, whereas chapter 2 presents the obtained results and their discussion. Finally, this manuscript is concluded with a conclusion and some future perspectives.

BIBLIOGRAPHIC PART

CHAPTER 1:

TRIPLE NEGATIVE BREAST CANCER

1. Introduction to Triple Negative Breast Cancer

1.1 Definition of Triple Negative Breast Cancer

Triple negative breast cancer (TNBC) is a specific form of breast cancer that is identified by the absence of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2). The absence of these receptors leads to fewer available treatment choices, since standard hormonal therapies and HER2 focused treatments do not work effectively. As a result, TNBC tends to be linked with a more severe clinical trajectory, a worse outlook, and increased occurrences of metastasis and recurrence in comparison to other breast cancer types. Epidemiological data indicates that TNBC represents about 10 to 20 percent of all breast cancer (Jie et al., 2025).

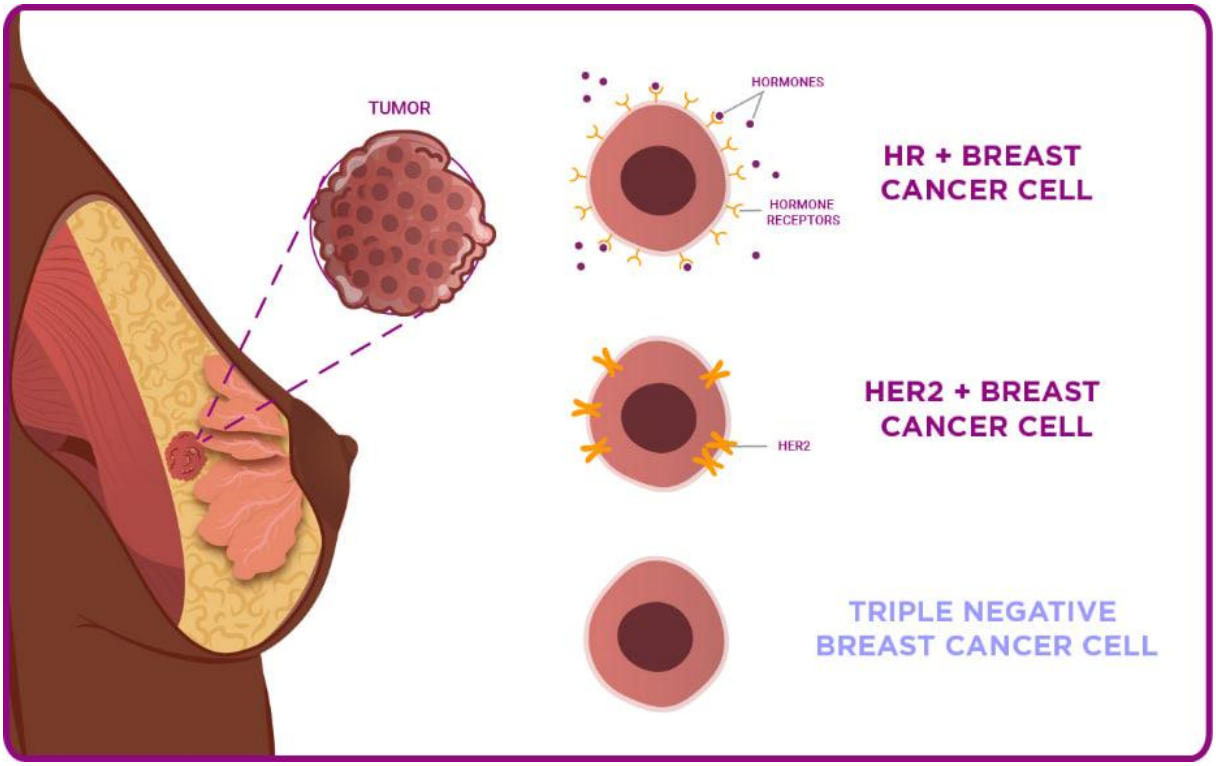


Figure 1 : Representation of triple negative breast cancer (*Black tnbc*, n.d.)

1.2 Epidemiology and Risk factor

1.2.1 Age

Advanced age serves as a significant risk factor for the development of breast cancer. Approximately 80% of diagnosed cases are found in people older than 50 years, while over 40% occur in individuals aged 65 and above. The likelihood of developing the condition escalates as one gets older, moving from 1.5% at the age of 40 to more than 4% by the time one reaches 70. Different types of breast cancer also differ based on age, triple negative breast cancer is predominantly seen in women younger than 40 (Łukasiewicz et al., 2021).

1.2.2 Sex

Due to variations in hormonal influences related to sex, females are identified as having a greater risk for triple negative breast cancer when compared to males. The breast tissues in women are particularly affected by hormones such as estrogen and progesterone, along with various hormonal imbalances. The presence of estrogens and androgens in the body is linked to an elevated likelihood of developing breast cancer. For women both before and after menopause, changes in natural sex hormone levels contribute to a heightened risk of breast cancer. In men, the occurrence of breast cancer stands at 1%. Key elements that elevate the risk of breast cancer in men include advanced age, mutations in the "BRCA2/BRCA1" genes, higher levels of estrogen, family history of the disease, and substantial exposure to radiation (Almansour, 2022).

1.2.3 Genetic mutation

Genetic mutations are changes in DNA. Some types of cancer, including breast cancer, can be linked to these mutations. Only a small percentage of breast cancer cases (about 5%–10%) are caused by inherited mutations. BRCA1 and BRCA2 genes are normal genes in the body that help control cell growth and act as tumor suppressor genes. When mutations occur in these genes, they may lose their normal function, which increases the risk of developing cancer (Łukasiewicz et al., 2021).

1.2.4 Genetic History

A person's family background serves as a significant risk element for breast cancer. Approximately 13 to 19% of those diagnosed with breast cancer have a close family member who has also experienced this illness. The probability rises when additional immediate family members are impacted, particularly if they received their diagnosis at a young age, specifically below 50 years old. In general, those with a familial background of breast cancer show a greater

occurrence of the disease, irrespective of their age. This heightened risk may be linked to changes in gene expression and factors from the surrounding environment. Additionally, a history of ovarian cancer in the family, notably instances connected to mutations in the BRCA1 and BRCA2 genes, can further elevate the likelihood of developing breast cancer (Obeagu & Obeagu, 2024).

1.2.5 Breast Tissue Density

Breast tissue density is categorized into low density, fatty breast tissue, and high density breast tissue. It has been observed that women undergoing hormone replacement therapy tend to have denser breast tissue earlier in life, during pregnancy, and while breastfeeding, even if they maintain a lower body mass index. In women who are either premenopausal or post-menopausal, the density of breast tissue plays a role in determining cancer risk, higher density levels correspond to an increased risk (Almansour, 2022).

1.2.6 Radiation therapy

The risk of secondary cancers after radiotherapy depends on individual patient factors. It is higher in patients who receive radiotherapy at a young age, especially before 30 years old. The type of radiotherapy technique also affects the risk, with some methods being associated with a lower risk than others. In addition, a family history of breast cancer may further increase the risk. However, adding an extra radiation dose to the tumor are a may help reduce the risk of local recurrence (Lukasiewicz et al., 2021).

1.2.7 Drugs

Diethylstilbestrol is seen as a possible contributing factor to breast cancer in expectant mothers. Consuming this medication during pregnancy might elevate the likelihood of breast cancer for both the mother and her offspring. This influence can take place independent of the presence of estrogen and progesterone receptors, and the danger heightens with larger amounts taken (Almansour, 2022).

1.3 Clinical Manifestation and Symptomology

The possible symptoms include:

- The development of a mass or bulge in the breast or a region of the breast near/on the armpit.
- Any enlargement or alteration in size or shape of the breast.

- Changes (such as puckering/dimpling) to the outer skin of the breast (such as rashes or redness) from what would normally be expected.
- Leakage of some kind of fluid (not milk) from a woman's nipple that is not pregnant; this type of leakage/use can occur with both male and female nipples.
- Movement (up, down, left, right) of a woman's nipple from its usual position on the breast compared to before diagnose (*Triple NegativeBreast Cancer*, n.d.).

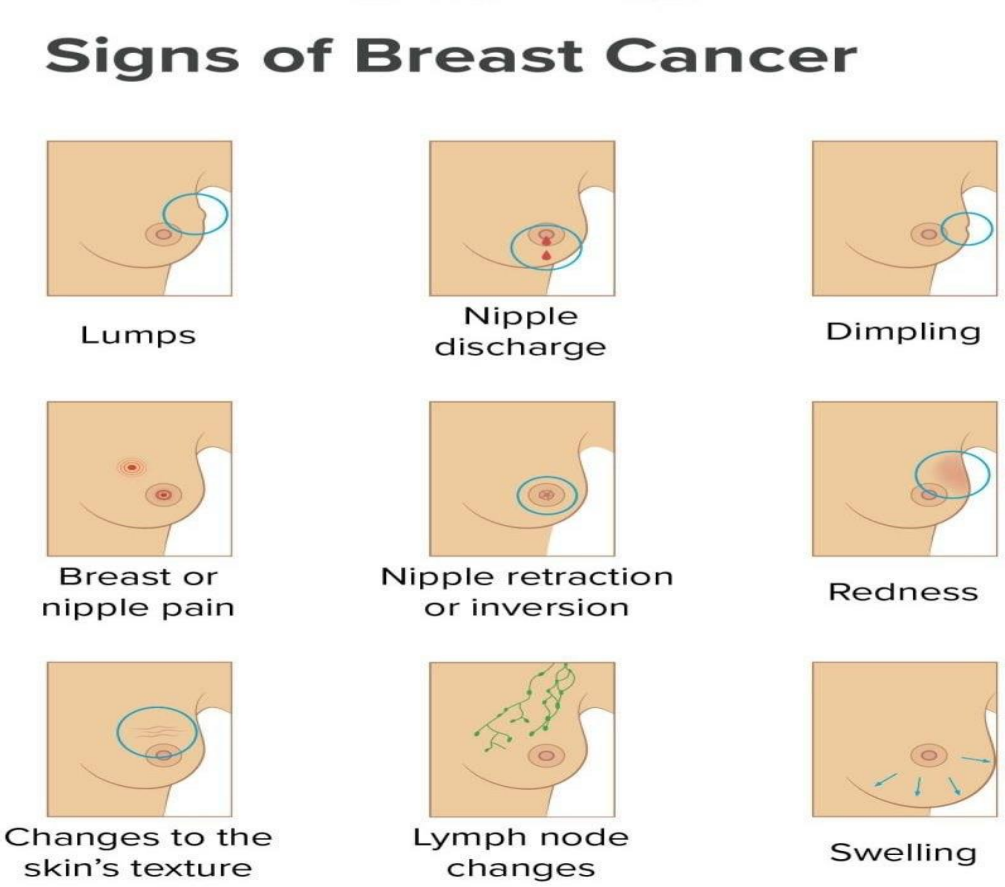


Figure 2: Signs of triple negative breast cancer.(*Cancer cytecare Hospitals*, n.d.)

1.4 Current and Emerging Therapeutic Strategies

1.4.1 Surgery

Triple negative breast cancer is addressed through surgical means. Depending on the stage of the cancer, a lumpectomy may be conducted, where the tumor and a small section of adjacent tissue are excised, or a mastectomy, which entails the complete removal of the breast, may be carried out (Obidiro et al., 2023).

1.4.2 Chemotherapy

Chemotherapy is a key treatment for triple negative breast cancer (TNBC) because of its aggressive nature and lack of targeted therapies. Standard treatments include anthracyclines such as doxorubicin and taxanes like paclitaxel. Treatment selection may depend on tumor biology and genetic markers linked to therapy resistance, including PIM-1 and PLAC8. Combining chemotherapy with immunotherapy, especially in PD-L1 positive tumors, may improve response rates. New therapies, such as antibody drug conjugates, are being investigated for patients resistant to conventional treatments. Chemotherapy can cause significant side effects, including neutropenia, anemia, and gastrointestinal toxicity, which may require dose modifications or treatment delays. Supportive therapies like granulocyte colony stimulating factors can reduce these adverse effects. Neoadjuvant chemotherapy is commonly used in locally advanced TNBC to reduce tumor size before surgery and increase the chances of breast conserving surgery. It may achieve pathological complete response (PCR), which is associated with better outcomes. However, treatment responses vary among patients, leading to studies on biomarkers such as circulating tumor cells and genetic profiling to predict outcomes (Jie et al., 2025).

1.4.3 Radiation

Radiation treatment is frequently administered following a mastectomy to eradicate any leftover cancer cells in the breast and lymph nodes and is also utilized for metastatic cancer management. Nevertheless, radiation treatment has the potential to lead to a recurrence in 7 to 12.6% of individuals within a five year timeframe, and there might be the emergence of resistance as well. Present research is assessing intraoperative radiation therapy (IORT) among patients with triple negative breast cancer to enhance therapeutic result (Obidiro et al., 2023).

1.4.3 Immunotherapy

Immunotherapy has emerged as a promising treatment strategy for triple negative breast cancer (TNBC), especially after the development of immune checkpoint inhibitors such as PD-(L)1 inhibitors. TNBC shows higher expression of PD-L1 and tumor infiltrating lymphocytes compared to other breast cancer subtypes, making it more likely to respond to immunotherapy. However, the efficacy of PD-(L) 1 inhibitor monotherapy remains limited, benefiting only a small proportion of patients. Therefore, several combination strategies have been developed to improve treatment outcomes, including combining immunotherapy with chemotherapy, radiotherapy, targeted therapy, antibody drug conjugates, and PARP inhibitors. Clinical trials demonstrated that combinations such as pembrolizumab with chemotherapy significantly improved pathological complete response, progression free survival, and overall survival, particularly in PD-L1 positive patients. Immunotherapy has therefore become an important therapeutic approach for improving outcomes in TNBC patients (Li et al., 2023).

1.4.4 Targeted Therapy for TNBC

1.4.4.1 Research Progress on PARP Inhibitors

PARP inhibitors have surfaced as a major advance in managing TNBC, particularly for individuals possessing BRCA1/2 mutations. These agents function by leveraging the principle of synthetic lethality, which allows for the selective eradication of cancer cells that lack effective homologous recombination repair, ultimately resulting in cell death. Clinical trials have shown the effectiveness of PARP inhibitors like Olaparib and Talazoparib in extending progression free survival for patients carrying germline BRCA mutations. Additionally, current research is exploring the viability of PARP inhibitors in cases of TNBC without BRCA mutations, as well as their combined use with other treatment modalities, such as immune checkpoint inhibitors and chemotherapy, to boost treatment efficacy and counteract resistance. The accumulating evidence endorses the incorporation of PARP inhibitors within the treatment framework for TNBC, emphasizing their importance in personalized medicine and targeted therapeutic approaches (Jie et al., 2025)**.**

1.4.4.2 Combination with ADCs

ADCs are composed of a monoclonal antibody, a linker, and a cytotoxic payload. Besides delivering the payload to tumor cells, ADCs can induce ADCC, ADCP, CDC, and bystander killing effects. Their payloads also have immune modulatory effects and may activate dendritic cells, enhancing the activity of PD-(L) 1 inhibitors. Sacituzumab govite can, targeting Trop 2

with SN-38 payload, has been approved for advanced TNBC after at least two prior treatments, and is being evaluated in combination with Pembrolizumab for metastatic TNBC. Ladiratuzumab vedotin targets LIV-1 with MMAE payload and showed manageable safety and an ORR of 54% in combination with Pembrolizumab in advanced TNBC. Several ongoing trials are evaluating PD-(L)1 inhibitors combined with ADCs in early and advanced TNBC (Li et al., 2023).

1.4.4.3 PI3k/AKT/mTOR Inhibitors

P13/AKT/mTOR inhibitors are considered potential treatments for TNBC with altered molecular status. These pathways regulate metabolism, proliferation, survival, growth, and angiogenesis. AKT inhibitors such as Ipatasertib combined with PTX improved progression free survival in studies. Although PI3K/AKT/mTOR inhibitors have not yet been approved for TNBC, several agents are under investigation. Everolimus, an mTOR inhibitor approved for hormone receptor positive HER2 positive breast cancer, is currently being studied for advanced TNBC. Other inhibitors under study include PI3K inhibitors (Buparlisib, Taselisib), AKT inhibitors (Capivasertib, Ipatasertib), and mTOR inhibitors (Obidiro et al., 2023).

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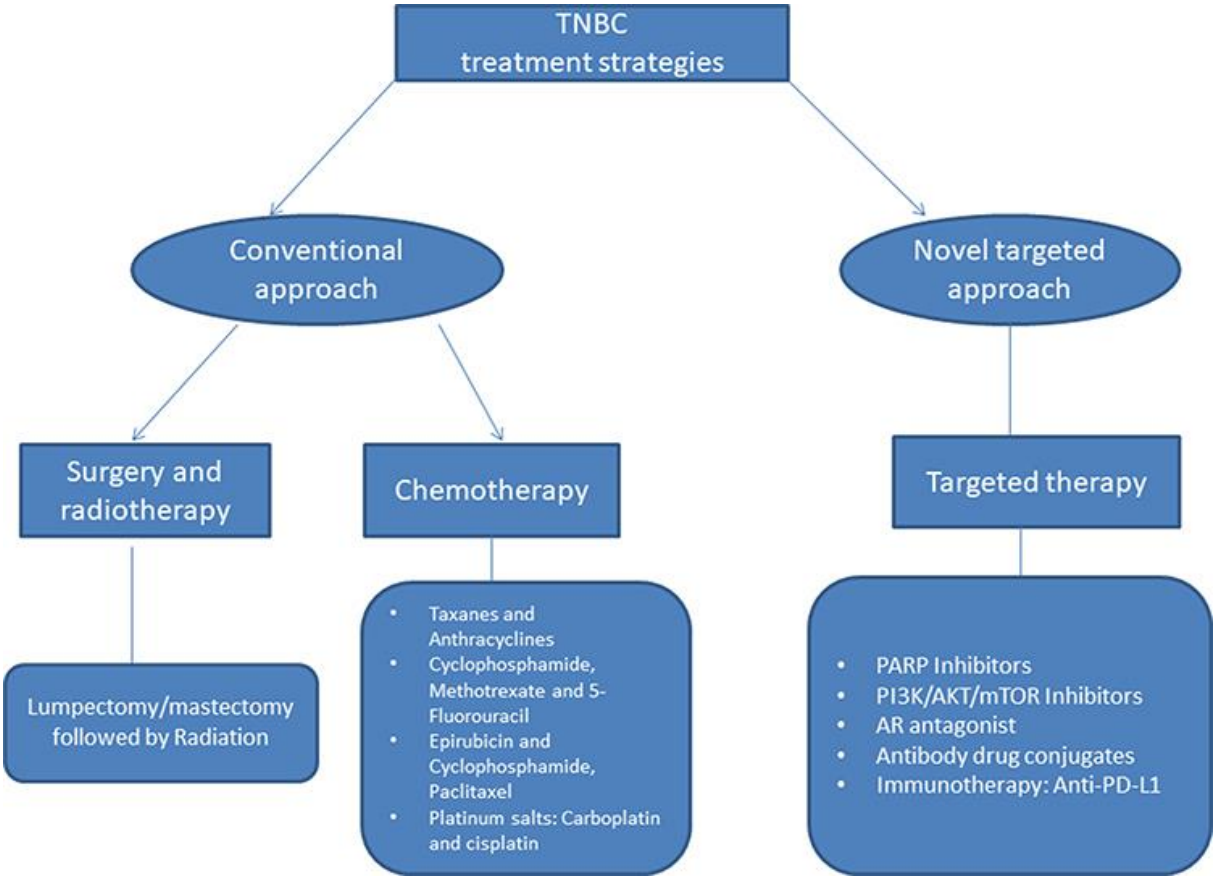


Figure 3:Traditional and innovative targeted methods for triple-negative breast cancer treatment (Maqbool et al., 2022).

CHAPTER 2:

AUXARTHROL F

2.1 Anthraquinone

The anthraquinone family are a group of aromatic compounds that are widely distributed in nature, especially in fungi, plants, and lichens. Their structure is based on anthracene and includes two carbonyl groups.

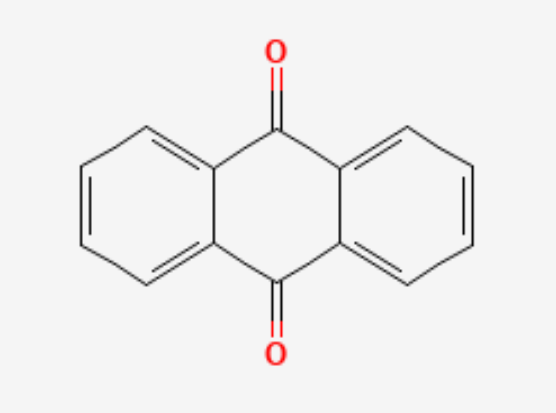


Figure 4 : Anthraquinone (*Pub hem*).

Anthraquinones are biosynthesized through the polyketide pathways acetyl CoA and malonyl CoA units. Their structural diversity is generated through enzymatic modification during biosynthesis (Hertweck, 2009).

Anthraquinones are also considered important scaffolds in drug discovery and development. They possess many biological activities including antibacterial, antifungal, and anticancer and enzyme inhibition effects. Their biological activities are influenced by their chemical structure. These compounds are important in cancer research due to their cytotoxicity against different cancer cells, which is mainly related to interaction with biological targets such as enzyme and DNA.Some anthraquinone compounds can inhibit topoisomerase and affect cell cycle progression (Greco et al., 2021; Hafez Ghoran et al., 2022).

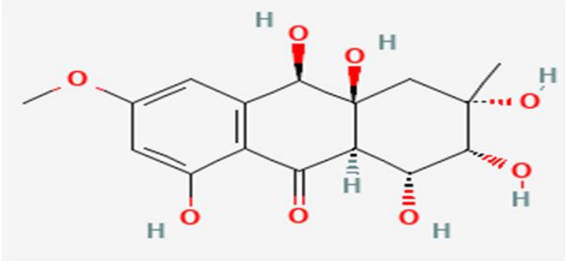
2.2 Auxarthrol F

2.2.1 Physico chemical properties

Auxarthrol F is a marine derived anthraquinone that was first reported by Ge et al. (2019) from the fungus *Sporendonema casei* HDN16-802, as part of a group of compounds named Auxarthrol A–H. Its structure was elucidated using spectroscopic techniques including 1D and 2D NMR (^{1}H, ^{13}C, COSY, HSQC, and HMBC) and high resolution mass spectrometry. The

molecular formula of Auxarthrol F was determined as $C_{16}H_{20}O_8$ (Ge et al., 2019; Hafez Ghoran et al., 2022).

Table 1: Key physic chemical properties of Auxarthrol F (NCBI, 2026).

Property	Description
Chemical Structure	
Compound Name	Auxarthrol F
Chemical Class	Hydroanthraquinone (anthraquinone derivative)
Source	Marine-derived fungus." *Sporendonema casei*"
Molecular Formula	$C_{16}H_{20}O_8$
Molecular Weight	340.32 g/mol
Exact Mass	340.11581759 Da
Hydrogen Bond Acceptor Count	8
Hydrogen Bond Donor Count	6
Appearance	Pale yellow powder
Water Solubility	Low aqueous solubility (predicted)
Solubility in Organic Solvents	Soluble in polar organic solvents such as methanol
Polarity	Moderate to high due to multiple hydroxyl groups
UV Absorption (λmax)	Approximately 280 nm and 340 nm
IR Functional Groups	Hydroxyl (O–H), carbonyl (C=O), and aromatic ring vibrations
Optical Rotation	[α]D ≈ −65.0 (MeOH)
Mass Spectrometry (HRESIMS)	m/z 341 [M+H] +
Lipophilicity (Log P)	Expected to be low to moderate
Hydrogen Bonding Capacity	High
Structural Features	Polyoxygenated hydroanthraquinone skeleton with multiple stereocenters

2.2.2 Biological activities

Auxarthrol F, an anthraquinone derivative isolated from the marine derived fungus *Sporendonema casei*, was evaluated for several biological activities, including cytotoxic, antimicrobial, anticoagulant, and antitubercular effects. Cytotoxic activity was assessed against

eleven human cell lines, namely HL-60 (human promyelocyticleukemia), HeLa (cervical cancer), HCT-116 (colon cancer), MGC-803 (gastric cancer), HO8910 (ovarian cancer), MDA-MB-231 (triple-negative breast cancer), SH-SY5Y (neuroblastoma), PC-3 (prostate cancer), BEL-7402 (hepatocellular carcinoma), K562 (chronic myeloid leukemia), and L-02 (normal human liver cells). Auxarthrol F demonstrated moderate cytotoxic activity against several of these cancer cell lines, highlighting its potential as an anticancer agent (Ge et al., 2019).

The antimicrobial activity of Auxarthrol F was evaluated against seven microorganisms, including *Mycobacterium phlei*, *Proteus* species, *Bacillus subtilis*, *Candida albicans*, *Vibrio parahaemolyticus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The compound inhibited the growth of the tested microorganisms, indicating a promising antimicrobial potential (Ge et al., 2019).

In addition, Auxarthrol F was investigated for anticoagulant activity and exhibited an inhibition ratio of 14.4%. The compound was also evaluated for antitubercular activity against the AlRa strain; however, no significant antitubercular effect was observed under the experimental conditions. Overall, these findings suggest that Auxarthrol F possesses diverse biological properties, particularly cytotoxic and antimicrobial activities, making it a promising lead compound for the development of novel therapeutic agents (Ge et al., 2019; Greco et al., 2021).

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CHAPTER 3:

NETWORK PHARMACOLOGY AND COMPUTATIONAL DRUG DISCOVERY

3.1 *In silico* methods

The term *in silico* refers to scientific studies, tests, or simulations carried out solely through computer systems and sophisticated computational models. This contemporary method combines computer science with molecular biology to create accurate data representations, enabling the analysis and assessment of chemical compounds without requiring prompt physical experimentation (Al-Mohaya et al., 2024).

Due to various factors, computer based research plays a crucial role in the pharmaceutical development process. Methods like virtual ligand screening, molecular modeling, and docking based virtual screening serve as computational strategies to identify novel drug candidates, predict how these potential drugs will bind, and analyze their interactions. The combination of these methods with traditional *in vitro* and *in vivo* studies can accelerate the drug development timeline while reducing resource expenditure. Furthermore, in the initial stages of drug development, *in silico* methods assist in predicting toxicity, optimizing processes, and identifying promising compounds, ultimately leading to time and cost savings (Roney & MohdAluwi, 2024)**.**

3.1.1 Network Pharmacology

3.1.1.1 Principle of network pharmacology

Network pharmacology (NP) is an emerging field that seeks to comprehend how drugs function and interact with various targets. This discipline employs computational techniques to methodically examine the molecular interactions of medicinal compounds within biological cells (Chandran et al., 2017).

3.1.1.2 Importance of Network pharmacology

Network pharmacology aids in comprehending the intricate connections between herbal remedies and the entire organism (Chandran et al., 2017).On the other hand, it offers a broader insight into how drugs function and the mechanisms of diseases, emphasizing its importance in contemporary healthcare and its capability to transform drug development (Martins, 2024).Unlike the standard method of targeting one drug to a single target (Chandran et al., 2017),this comprehensive strategy examines the complex relationships between genes, proteins, metabolites, and signaling pathways (Martins, 2024).Within a complicated biological network, a single drug may interact with multiple targets, or various drugs may influence the same target (Chandran et al., 2017) Consequently, this holistic approach facilitates the

identification of various therapeutic targets at once, thereby enhancing the chances of finding more effective and safer medications for complex, multifactorial conditions (Martins, 2024).

Moreover, this approach expands therapeutic alternatives and facilitates the identification of novel drug targets and leads (Chandran et al., 2017).It is also highly effective in pinpointing opportunities for drug repositioning (Martins, 2024). By evaluating current medications within a network perspective, scientists can identify new applications, thereby speeding up the drug creation process and minimizing the expenses and hazards linked to novel drug development (Martins, 2024). Furthermore, network pharmacology assists in fore casting drug combinations, allowing for the creation of therapies that target several pathways to produce better therapeutic results while keeping side effects to a minimum (Martins, 2024). This directly supports the rational formulation of therapies and seeks to enhance the safety and effectiveness of current drugs (Chandran et al., 2017).

Additionally, it offers significant potential for investigating traditional practices to address modern issues in medication development (Chandran et al., 2017). In the area of traditional medicine, network pharmacology contributes to clarifying the molecular processes that under pin the effectiveness of herbal substances by charting their relations hips with in biological networks (Martins, 2024). This methodology delivers insights into how traditional medicine works based on its active components through the use of contemporary integrated methods (Chandran et al., 2017). As a result, it promotes the collaboration of traditional and contemporary medicine, laying the ground work for the development of evidence based herbal treatments (Martins, 2024).Despite some limitations, it continues to be a promising strategy as it reveals overlooked traditional medical insights (Chandran et al., 2017). Ultimately, with on-going technological progress, network pharmacology is poised to be instrumental in influencing the future of personalized medicine and innovations in treatment (Martins, 2024).

3.1.2 Molecular docking

3.1.2.1 Principle of molecular docking

Molecular docking serves as a crucial computational method in drug discovery and development, facilitating the analysis of interactions between small molecules (ligands) and their target proteins (Kulkarni et al, 2025).This technique predicts protein ligand interactions and provides valuable insights into molecular recognition, binding affinity, and reaction mechanisms (Krishna, 2026).

3.1.2.2 Importance of molecular docking

Molecular docking is considered a key technique in computer aided drug design, as it strengthens modern drug discovery workflows by supporting virtual screening, hit discovery, and lead optimization processes, which ultimately improves the overall efficiency of drug development (Kulkarni et al., 2025). This method allows the estimation of interactions between ligands and biological targets, including binding modes and affinities, which assists in selecting promising drug candidates and in the rational development of novel therapeutic agents (Agu et al., 2023; Kulkarni et al., 2025).Furthermore, it provides a rapid and cost efficient alternative to experimental screening by enabling the evaluation of large compound libraries, thereby reducing both time and experimental workload during early drug discovery stages (Kulkarni et al., 2025).

In addition, molecular docking plays an important role in lead optimization by guiding structural refinement of compounds to enhance their binding strength and pharmacological properties (Anusha & Govinda Rao, 2025).

Recent developments involving artificial intelligence and machine learning approaches have further improved the reliability and speed of docking based predictions (Anusha & Govinda Rao, 2025). Docking is also widely applied in drug repurposing strategies, where existing approved molecules are reassessed for new biological targets, offering advantages such as reduced development cost and shorter development timelines due to known safety profiles (Anusha & Govinda Rao, 2025).Moreover, when integrated with molecular dynamics simulations, docking provides additional insight into the conformational stability and dynamic interactions of protein ligand complexes (Agu et al., 2023).

EXPERIMENTAL PART

MATERIALS AND METHODS

4.1 Exploring Auxarthrol F associated targets

To identify the potential targets of Auxarthrol F, we imported its SMILES formula, which was obtained from PubChem online database (https://pubchem.ncbi.nlm.nih.gov), into Swiss Target Prediction (https://www.swisstargetprediction.ch/) and the Similarity Ensemble Approach database (SEA) (https://sea.bkslab.org/).

4.2 Screening of triple negative breast cancer related genes

Triple negative breast cancer (TNBC) associated genes were screened by retrieving TNBC targets from Gene Cards (5.25.0) (https://www.genecards.org/).

4.3 Identification of shared target genes between Auxarthrol F and TNBC

To determine the shared genes between Auxarthrol F and TNBC, we utilized an online Venn diagram tool (2.1) (http://bioinfogp.cnb.csic.es/tools/venny/) (Accessed:15/02/2026).The overlapping genes from both sets were considered as Auxarthrol F target genes implicated in TNBC treatment.

4.4 Protein- protein interaction network and hub genes identification

The overlapping genes were subsequently analyzed for protein protein interaction (PPI) using STRING database (12.0) (https://string-db.org/) (Accessed:15/02/2026). To identify key hub genes, multiple centrality algorithms, including degree, closeness centrality, betweenness centrality, Maximum neighborhood Component (MNC), and Maximum Clique Centrality (MCC), were calculated using the Cyto Hubba plugin. The top ranked genes identified by each algorithm were intersected using Venn diagram to determine the overlapping candidates. The overlapping genes of all to pological methods were considered the hub genes.

4.5 Pathways and functional enrichment via GO and KEGG

The core targets were subjected to functional enrichment analysis for gene ontology (GO) and KEGG pathways using Shiny GO database (0.82) (http://bioinformatics.sdstate.edu/go/) (Accessed: 15/02/2026). GO annotation encompassed molecular functions (MFs), cellular components (CCs), and biological processes (BPs).

4.6 Molecular Docking

4.6.1 Materials and software

In silico molecular docking study was conducted using the following resources:

4.6.1.1 Ligand: Auxarthrol F

4.6.1.2 Target protein

The crystal structures were retrieved from the RCSB protein data bank (V.5.0) (https://www.rcsb.org/). EGFR (PDB ID: 4EWH), GAPDH (PDB ID: 6M61), HDAC1 (PDB ID: 5ICN), and CASP3 (PDB ID: 6X8I).

4.6.1.3 PubChem database

To obtain the SMILES formula and the chemical structure of the studied ligand.

4.6.1.4 SWISS DOCK (V 2.0) (Accessed on March 16, 2026): based on Auto Dock Vina Engine (https://www.swissdock.ch/).

4.6.1.5 BIOVIA Discovery Studio Visualizer (v24.1.0.23298.)

Used for 2D interaction analysis and mapping.

4.6.1.6 PYMOL (4.6.0)

Used for 3D visualization and active site identification.

4.6.2 Methods

4.6.2.1 Ligand preparation

The ligand, Auxarthrol F, was submitted to the server by uploading its SMILES from PubChem.

C[C@@]1(C[C@@]2([C@@H]([C@H]([C@H]1O)O)C(=O)C3=C([C@H]2O)C=C(C=C3O)OC)O)O

The ligand structure was subsequently optimized using the "prepare ligand" feature.

4.6.2.2 Protein preparation

The X ray crystal structures of human proteins in complex with an inhibitor was retrieved form the protein data bank (https://www.rcsb.org/) in PDB format. The target proteins selected for this study are: EGFR (PDB ID: 4EWH); GAPDH (PDB ID: 6M61); HDAC1 (PDB ID: 5ICN); CASP3 (PDB ID: 6X8I). The target proteins were then prepared using the "prepare protein" tool, which removed heteroatom's, water molecules, and added polar hydrogens to stabilize the binding environment.

4.6.2.3 Identifying Binding Pockets

The PYMOL program was utilized to identify the specific binding pockets for each target protein. Upon obtaining the coordinates, they were manually entered into the Swiss dock panel as follows:

EGFR : X= 39.315, Y= -30.855, Z=-12.306.

HDAC1 : X= -48.150, Y= 34.806, Z=-5.504.

CASP3 : X= -7.177, Y= 7.467, Z=-15.630.

GAPDH : X= -16.798, Y= -10.675, Z= -15.518.

The grid box size for all simulations was set as 20 x 20 x 20 A°.

4.6.2.4 Selecting Parameters

Sampling Exhaustivity in Auto Dock Vina defines the thoroughness of the sampling process. The sampling exhaustivity in this experiment was set to 64 to ensure high precision sampling across different angular ranges.

4.6.2.5 Docking

After running the docking, the results were provided as Zip file. The docking poses were ranked based on their binding affinity (ΔG, kcal/mol).

RESULTS

5.1 Identification of shared target between Auxarthrol F and TNBC

A total of 113 potential targets of Auxarthrol F were identified from SWISS Target Prediction and SEA databases, while 9020 TNBC related targets were retrieved from Gene cards. The intersection analysis revealed 90 common targets (Figure 05) representing potential therapeutic targets of Auxarthrol F against TNBC.

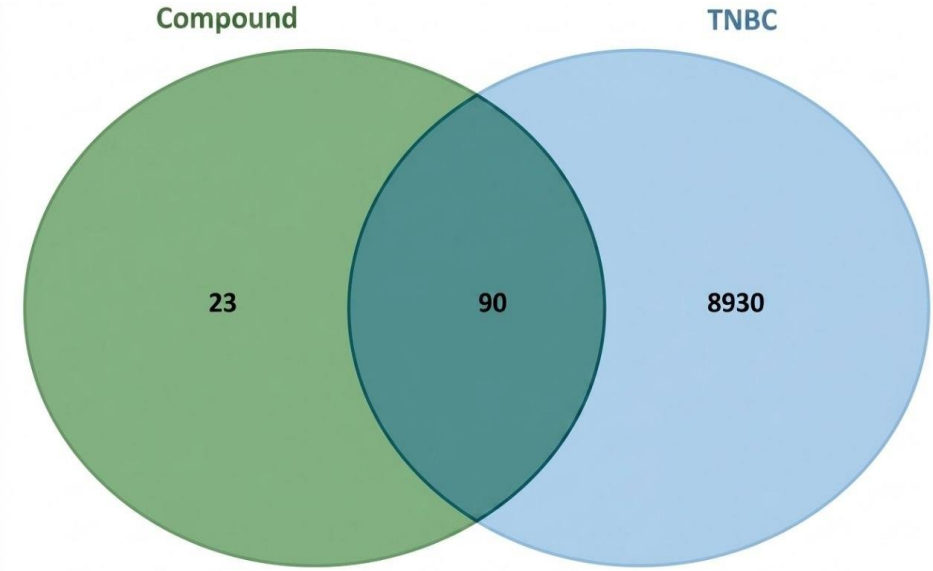


Figure 5: Venn diagram analysis showing the overlap between targets of Auxarthrol F and TNBC associated genes.

5.2 Protein –Protein interaction network

A PPI network was constructed using STRING database. The PPI network (Figure06) of the 90 shared targets consisted of 90 nodes and 402 edges. The average node degree was 8.98 and the average local clustering coefficients was 0.568. The expected number of edges was 165 and the PPI enrichment *p*-value was <1, 0e-16, indicating that the observed interactions significantly higher than expected and that the proteins were biologically connected as a functioning group.

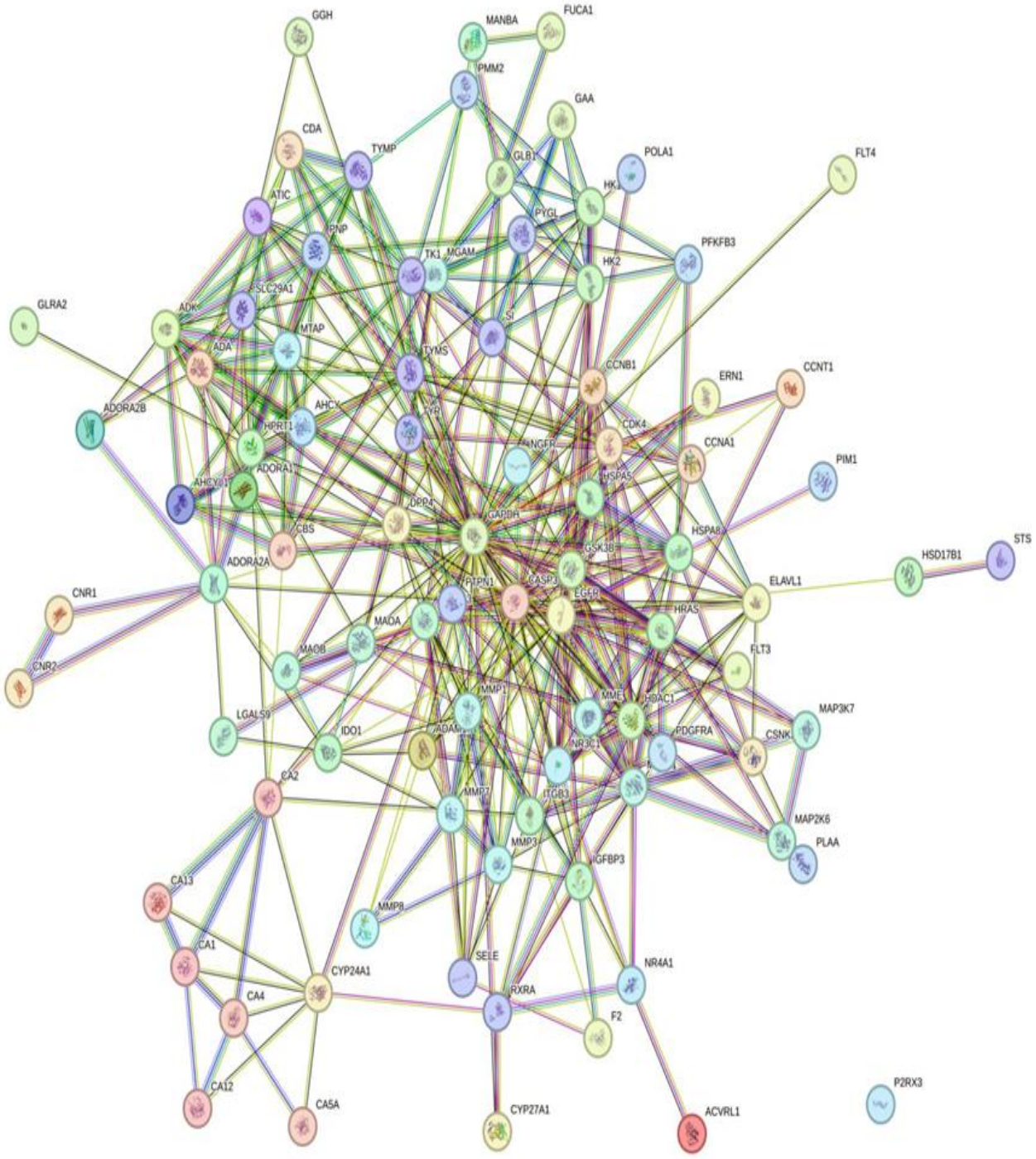


Figure 6: PPI network visualizing the interaction among the shard targets between Auxarthrol F and TNBC. Each one represents a protein while the edges indicate predicted interaction.

5.3 Identification of hub genes

The PPI network was imported into Cytoscape (version 3.10.3) for topological analysis. Hub genes were identified using the CytoHubba plugin based on five algorithms: Degree, Betweenness Centrality,Closeness Centrality,MCC,and MNC (Figure 07). The intersection of

the top ranked genes from these five methods was selected as the final core targets; hub genes, which were: **GAPDH, CASP3, EGFR, and HDAC1 (**Figure 08**).**

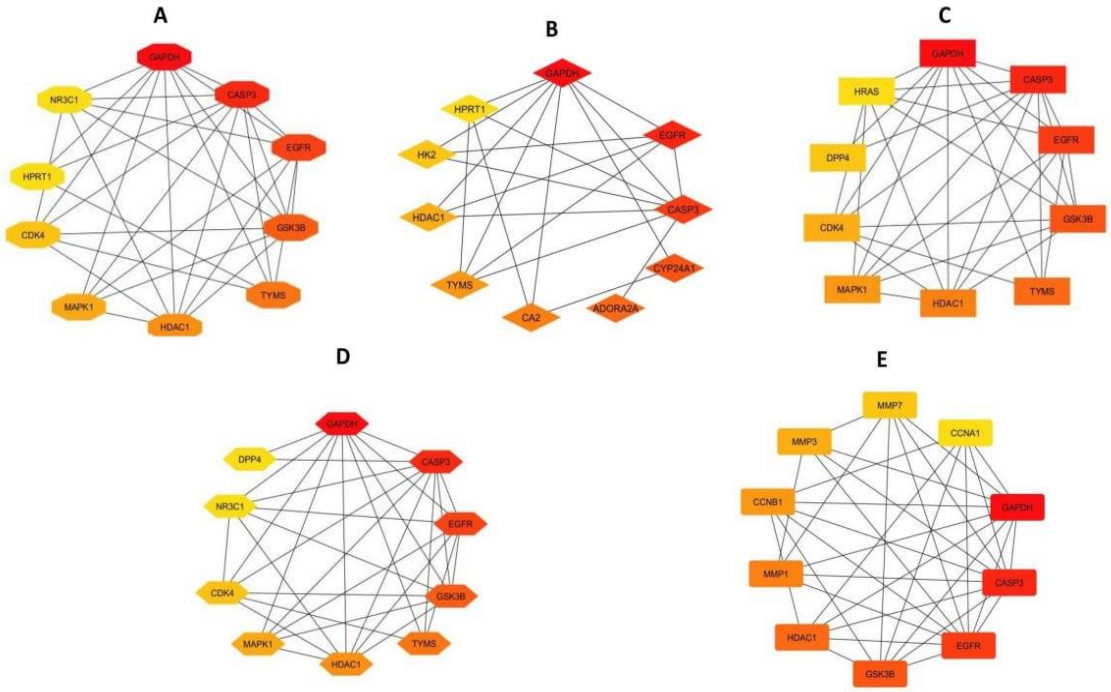


Figure 7: Hub genes using five algorithms in Cytoscape: A) Degree, B) Betweenness, C) Closeness, D) MNC, and E) MCC.

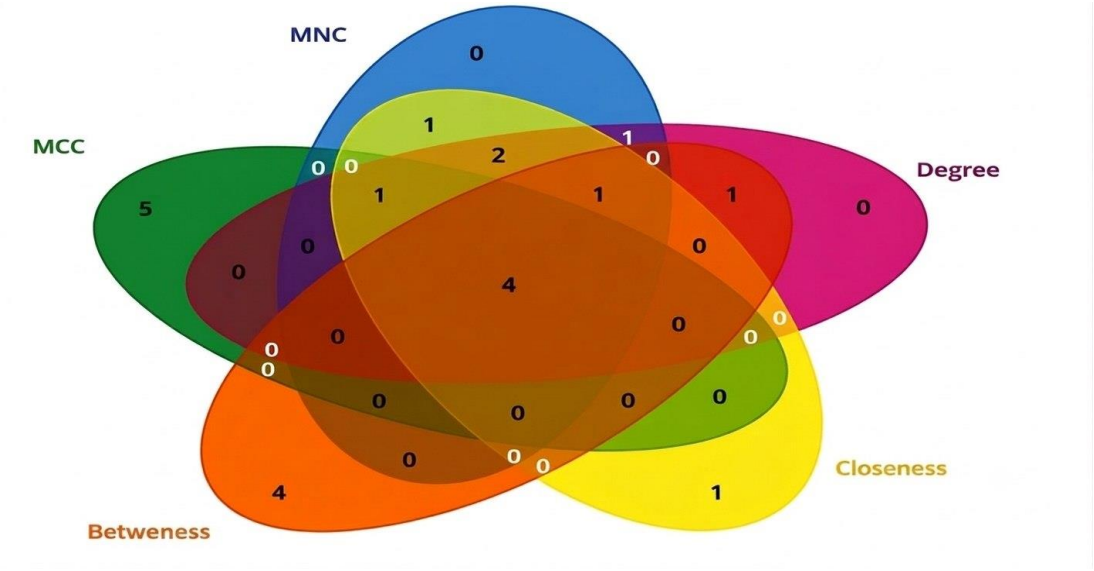


Figure 8: Venn diagram analysis showing the overlap between hub genes of the five topological methods. The shared genes are considered key hub genes

5.4 Gene ontogly (GO) enrichment analysis

To explore the biological roles of the shared targets, GO enrichment analysis was performed using the Shiny GO web tool. The top enriched terms were categorized into Biological Process (BP) Cellular Component (CC), and Molecular Function (MF). The most significantly enriched BP term included Response to chemical, Response to oxygen containing compound, small molecule processes, Response to lipid, one carbon metabolic process, Reg of cell migration and Locomotion. CC included Extracellular space Vesicle, Vesicle lumen, Extracellular organelle, Extracellular membrane bounded organelle, Extracellular exosomes, Filcon-1 rich granule. MF Carbonate dehydratase activity, heterocyclic compound binding, identical protein binding, Transition metal ion binding, Anion binding, Hydro lyase activity, Zinc ion binding. This finding suggests their involvement in the anti-cancer activity of Auxarthrol F (Figure 09).

5.5 KEGG pathway enrichment analysis

KEGG pathway enrichment analysis identified multiple significantly enriched pathways (FDR < 0.05). Among them, metabolic pathways showed the highest number of enriched genes. In addition, cancer related pathways such as pathways in cancer, metabolic pathways, lipid and atherosclerosis, Rap1 signaling pathway,MAPK signaling pathway, Galactose, starch and sucrose metabolism, Nitrogen metabolism also demonstrated strong enrichment based on −log10(FDR) values. These findings suggest that Auxarthrol F may exert its therapeutic effects through modulation of metabolic and oncogenic signaling networks (Figure 09).

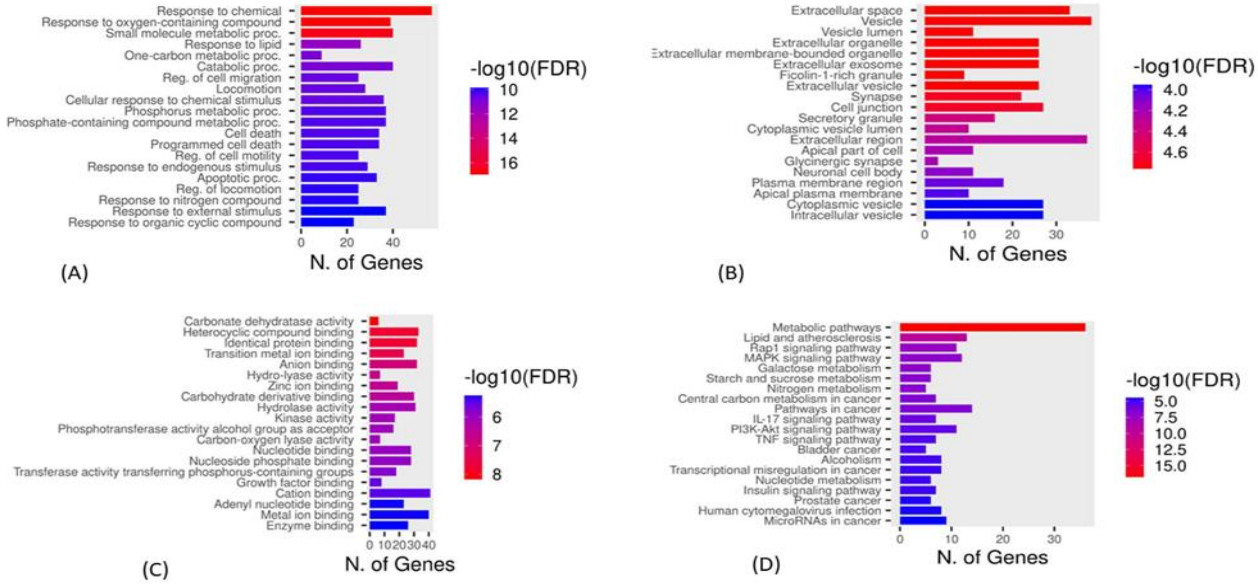


Figure 9: GO and pathway enrichment analysis of the identified genes. A) Biological processes, B) Cellular components, C) molecular function, D) KEGG pathways. The most significantly enriched terms are shown based on gene number and −log10 (FDR).

5.6 Docking results

5.6.1 Binding Affinity

The molecular docking results showed that Auxarthrol F exhibits varying binding affinities to ward TNBC related targets (EGFR, HDAC1, CASP3, and GAPDH).The binding energy (ΔG,kcal/mol) reflects the strength of interaction between the ligand and the protein, where more negative binding energy value indicates stronger and more thermodynamically favorable interactions. Among the studied targets, the highest binding affinity was observed with EGFR (-8.008 kcal/mol), followed by GAPDH (-7.167 kcal/mol), HDAC1 (-6.623 kcal/mol), and CASP3 (-6.176). All the values were < -5kcal/mol, which indicated that Auxarthrol F exhibited strong binding affinities towards the target proteins, suggesting their potential as a promising target in TNBC treatment.

5.6.2 Protein-ligand interaction analysis

The detailed interaction analysis of the docked complexes showed that Auxarthrol F is stabilized with in the active sites of the target proteins through multiple non covalent interactions.

The higher binding affinity was observed toward EGFR (-8.008kcal/mol), indicating a highly spontaneous and thermodynamically stable ligand protein complex. This stability is mainly driven by a strong network of conventional hydrogen bonds with key amino acid residues, including Ala208(EGFR), ARG 306(HDCA1), GLU248(CASP3), and ILE14 andSER122(GAPDH), where short interaction distance (<3A°) contributed to a firm anchoring of the ligand within the catalytic binding pockets.

In addition, the complex is further stabilized by hydrophobic interactions, involving residues such as LEU132 and PHE250, which enhance the stabilization of the ligand within hydrophobic regions. The main types of interaction involved include: Hydrogen bonds, van der walls, π–πstacking and π–alkyl interaction, and electrostatic interaction.

Collectively, these multi targets interaction suggests that Auxarthrol F can establish a stable ligand protein complex across all studied targets, highlighting its strong binding profile and structural complementarity within the catalytic sites.The following Table2 and Figure 10 summarize the docking results.

Table 2.Molecular docking results and interaction types of Auxarthrol F with target proteins.

Target Protein	Binding Energy (kcal/mol)	Type of interaction with amino acid residues of target Proteins		
		H-bonds	Van der Walls	Hydrophobic
EGFR	−8.008	Ala208	Arg142, Leu207, Ala156, Val140, Lys158, Leu259, Gly133	Leu132
GAPDH	−7.167	Ser122	Ile14, Thr99, Glu317, Val188, Gly12	Arg13, Ala183
HDAC1	−6.623	Gln338, Lys331	—	Arg306, Tyr 336
CASP3	−6.176	Glu248, Asp404	Phe247, Asn208, Asp211	Phe250, Trp214

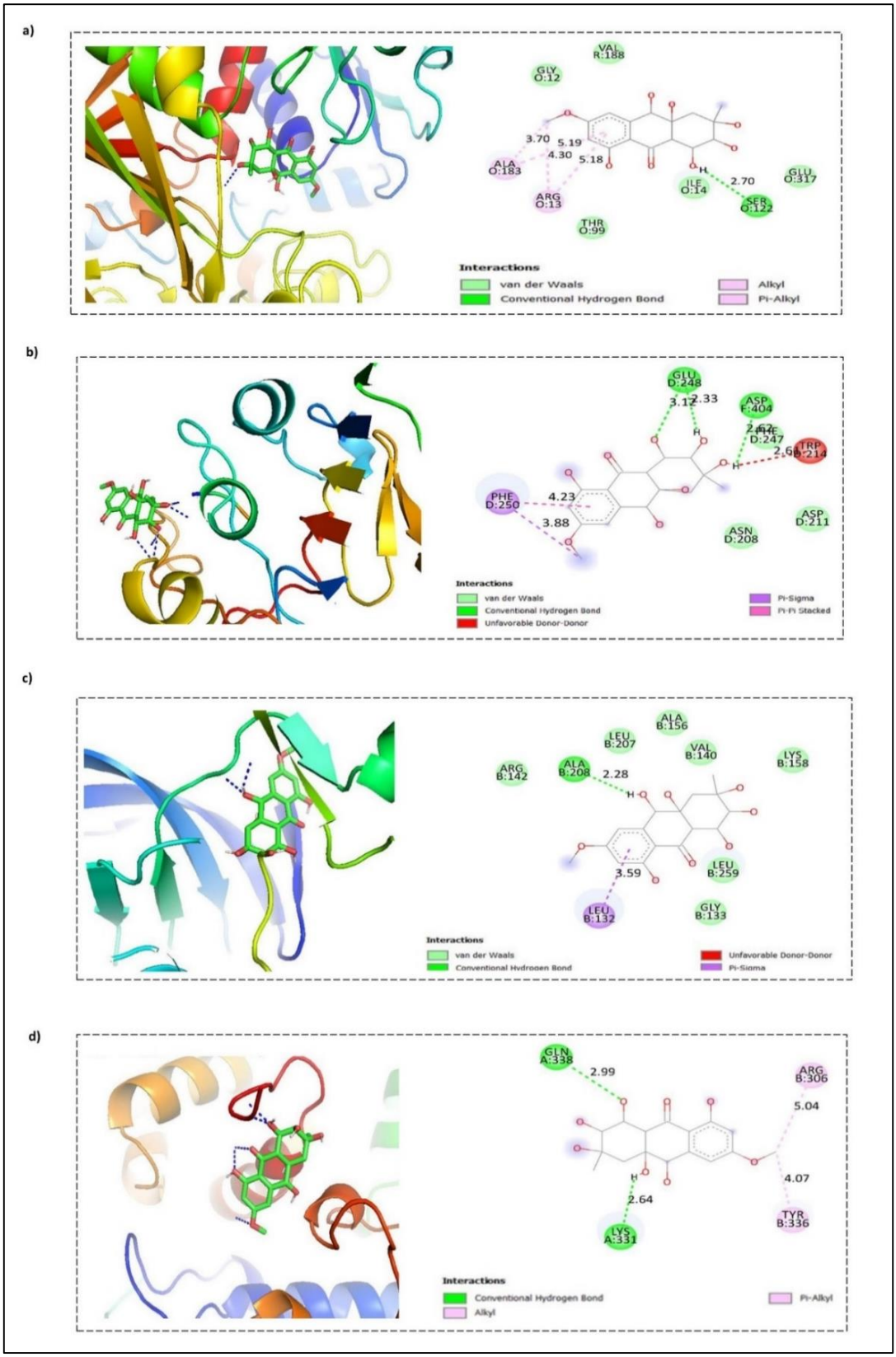


Figure 10: 3D and 2D visualizations of the interactions between Auxarthrol F and the target proteins. a) Auxarthrol F-GAPDH. b) Auxarthrol F-casp3. c) Auxarthrol F-EGFR. d) Auxarthrol F-HDAC1.

Through comparative analysis of each target protein, it became apparent that there are noticeable differences between the binding affinities and profiles of each candidate protein. The binding affinity of EGFR to the ligand "Auxarthrol F" best fit is -8.008 kcal/mol and is consistent with a strong hydrogen bond, as well as the presence of numerous stabilizing types of interactions. In comparison, the binding affinity of GAPDH is rated second best with a total binding energy score of -7.167 kcal/mol - relatively stable due to numerous van der Waals and hydrophobic interactions and having fewer hydrogen bonds than EGFR. The binding affinity of HDAC1 (-6.623 kcal/mol) is moderate, as there is considerable stability via hydrogen bond type interactions but has significantly reduced hydrophobic type interaction stability relative to EGFR. Caspase-3 has the lowest binding affinity (-6.176 kcal/mol), which may be attributed to an unfavourable interaction that affects the overall stability of the complex produced from the ligand protein.

DISCUSSION

Auxarthrol F is a natural compound that has attracted attention due to its multiple biological activities. It is considered a promising lead compound for development of new therapeutic agents. Its main importance is related to its biological effects including antibacterial, anticoagulant and anticancer activities (Ge et al., 2019).

Auxarthrol F has been shown to possess a promising anticancer activity by inhibiting the growth of several human cancer cell lines including leukemia (HL-60 and K562), colon (HCT-116), cervical (HeLa), breast (MDA-MB-231), gastric (MGC-803), ovarian (HO8910), prostate (PC-3), liver (BEL-7402) and neuroblastoma (SH-SY5Y) (Ge et al., 2019). However, no study has so far been done on its molecular mechanisms underlying its anticancer potential. Therefore, in the current study, we used network pharmacology and molecular docking approaches to determine the target genes and the mechanisms of Auxarthrol F for the treatment of TNBC.

Based on our network analysis, the complexity of the interaction suggests that Auxarthrol F may exert its effects on triple negative breast cancer TNBC through multiple genes and signalling pathways.

6.1 Functional Enrichment Analysis (GO)

Gene ontology enrichment analysis classifies into three main categories biological processes (BP), molecule functions (MF), and cellular component (CC). In this study, GO analysis revealed multiple enriched terms across these categories, providing a comprehensive understanding of gene functions in Triple negative breast cancer. A total of Top 20 enriched biological processes were identified through gene ontology enrichment analysis. However top processes were prioritized because they showed the highest enrichment scores and lowest p values, indicating a stronger association with the studied condition. Focusing on the most enriched terms allows a clearer interpretation of the key biological mechanisms involved (Ashburner et al., 2000).

6.1.1 Biological Processes (BP)

In the current study, GO functional enrichment analysis showed that the target genes are mainly implicated in biological processes such as response to chemical, response to oxygen containing compound, small molecule metabolic process, response to lipid and one carbon metabolic process.

The “response to chemical” process plays a significant role in TNBC, as it regulates key cellular activities such as immune responses, inflammation, and tumor growth. According to previous studies, these effects are primarily mediated by cytokines and chemical signals present in the tumor microenvironment (Munkácsy et al., 2023).Responding to oxygen containing compounds plays also a crucial role in cancer progression, particularly in triple negative breast cancer (TNBC). It is well established that oxygen derived molecules, such as reactive oxygen species (ROS)can lead to DNA damage, thus, increasing cancer stem cell abundance (Munkácsy et al., 2023).

Moreover, small molecule metabolic process is also considered crucial for TNBC progression. It is well documented that amino acid metabolism catabolism is an important factor in supporting proliferation, survival, immune escape and aggressiveness in TNBC. For instance, TNBC tumors metabolize glutamine at increased levels. This amino acid is converted to glutamate and metabolized for energy and the creation of new cellular material, which helps TNBC tumors proliferate. In addition, glutaminolysis fuels the growth of TNBC tumors. The catabolism of serine and glycine provides cells with the necessary NADPH to regulate oxidative metabolism, which protects TNBC cells from oxidative damage. Tryptophan catabolism aids TNBC in escaping host immunity by inhibiting antitumor immune responses. In parallel, arginine metabolism produces nitric oxide (NO), which activates oncogenes and contributes to increased growth and resistance to chemotherapy of TNBC tumors (Munkácsy et al., 2023).

In the present study, the genes were also found to be implicated in “*response to lipid*” biological process. This latter reflects the ability of cancer cells to adapt to lipid availability in the tumor microenvironments. Importantly,it has been reported that fatty acid oxidation contributes to ATP production, allowing tumor cells to survive under metabolic stress conditions such as hypoxia and glucose deprivation. Thus, our findings highlight the critical role of fatty acid oxidation in supporting tumor growth, survival and metabolic adaptation in TNBC (Munkácsy et al., 2023).

One carbon metabolism was also found among the top ranked biological processes. Such process is involved in TNBC progression as it is important in nucleotide biosynthesis, redox equilibrium, and energy metabolism. Studies have shown that one carbon metabolism and glucose metabolism are linked together to help tumors grow (Coleman et al., 2021).

The enrichment of the "catabolic process" in our results suggests an increased breakdown of bio molecules to meet the high energy demands of TNBC cells. This finding is

supported by previous studies that indicated that glycolysis is significantly upregulated in TNBC, as evidenced by enhanced glucose uptake and lactate production, along with the up regulation of key glycolytic enzymes and transporters. This enhanced glycolytic activity reflects a shift toward energy producing catabolic pathways, enabling tumor cells to sustain rapid proliferation and survival (Munkácsy et al., 2023).

Our results also highlighted the involvement of Auxarthrol F target genes in the regulation of cell migration and thus could play a role in tumor advancement and spread. It has been reported that increased motility of triple negative breast cancer (TNBC) cells constitutes a pivotal step in their spread to distant locations. Interestingly, cell migration contributes to multiple phases of the metastatic cascade, including local tissue invasion, intravasation, extravasations, and colonization of secondary organs. Therefore, targeting these genes could be a promising approach to prevent TNBC cell migration and the development of metastasis (Koedoot et al., 2019).

Cell locomotion was also identified as one of the enriched biological processes in our gene ontology analysis.Cell locomotion is essential for the spread of metastatic breast cancer cells. Hence, modulating Auxarthrol F target genes could be beneficial in preventing metastatic spread and TNBC progression (Tagay et al., 2023).

6.1.2 Molecular Functions (MF)

Molecular Function describes the biochemical activity of a gene product at the molecular level, such as catalytic activity, binding activity, or transporter activity (Ashburner et al., 2000).

To better understand the metabolic and structural adaptations of these cells, our focus will be on the top seven molecular activities most closely associated with tumor survival and invasion: carbonate dehydratase activity, heterocyclic compound binding, identical protein binding, transition metal ion binding, and anion binding hydrolyase activity, these activities represent the fundamental functional mechanisms that allow the cells to thrive under challenging micro environmental conditions.

Carbonate dehydratase is the systematic classification name for the Carbonic Anhydrase (CA) enzyme family. It has been reported that Carbonic Anhydrase IX (CA IX) is highly active in TNBC, playing a crucial role in tumor survival, adaptation, and metastasis, particularly in hypoxic (low-oxygen) conditions. CA IX acts as a master regulator of pH in the tumor

microenvironment, enabling cancer cells to survive in highly acidic conditions and promoting metabolic (ArslanSolmaz et al., 2025).

Heterocyclic compound binding refers to the ability of molecules to interact with heterocyclic structures containing atoms such as nitrogen, oxygen, or sulfur (Bhavsar & Jain, 2024).The emergence of "heterocyclic compound binding" as a top MF highlights the intense molecular interaction between small molecules and DNA proteins in TNBC. This function is crucial for inducing the genomic instability and structural DNA alterations that drive tumor progression and adaptability (Bhavsar & Jain, 2024).

Identical protein binding refers to the capability of cellular proteins to interact selectively and non-covalently with an identical protein or proteins. In breast cancer, identical protein binding within the EB protein family, particularly EB1, serves as a fundamental mechanism for regulating microtubule dynamics. The selective, non-covalent interaction of EB1 with itself and its natural inhibitor, ATIP3 (Angiotensin II receptor interacting protein 3), highlights a critical regulatory axis in cellular architecture. While increased EB1 levels facilitate tumor aggressiveness and poor prognosis in breast cancer, direct interaction with ATIP3 acts as a vital check on these oncogenic properties by stabilizing microtubules and reducing turnover (Sasankan & Mohan, 2025).

The binding of transition metal ion's molecule function that involves the interaction of protein with metal and such as iron, zinc and copper these metal ions are essential for various cellular processes including enzymatic activity, DNA synthesis and cell proliferation (Carbon &Mungall, 2026).In triple negative breast cancer, regulations of metal iron binding, particularly iron metabolism, play a critical role in tumor progression. According to Wang et al. (2023), iron is essential to cell proliferation and energy metabolism and excess iron is considered to be a risk factor of carcinogenesis, tumor progression, and metastasis. Duan et al. (2026) stated that copper ion binding is a crucial molecular function in which copper acts as a cofactor to activate signaling pathways involved in tumor development and metastasis.

Anion Binding is a selective and non-covalent interaction of a protein with an anion, which is a negatively charged ion. These ions play a part in managing signaling pathways that promote cancer cell growth. A study by ten Kate et al. (2006), stated that reactive oxygen species ROS particularly superoxide anions, play a crucial role in increasing the adhesion of cancer cells to the microvascular endothelium. The exposure of the cells to these anions

stimulates the expression of adhesion molecules, which help the process of metastasis (Ten Kate et al., 2006).

Hydrolyase activity has a potential role in TNBC progression.Hydrolyases are enzymes that catalyze the cleavage of carbon oxygen bonds by elimination of water. For instance, fumarate hydratases (FH) is an enzyme that catalyzes the reversible hydration of fumarate to malate as part of the tricarboxylic acid cycle. It plays an important role in cellular metabolism. Earlier studies have reported that alterations in FH activity can lead to the accumulation of fumarate within the cells, activating oncogenic cascades and interfering with normal cellular processes. Fumarate can inhibit ketoglutarate dependent dioxygenases resulting in alterations of DNA methylation patterns and gene expression. Moreover, fumarate contributes to the activation of hypoxia inducible factor associated signalling pathways which are involved in cell growth angiogenesis survival migration and metastasis (Ten Kate et al., 2006).

Zinc (Zn) ion binding is also one of the contributing factors to the advancement of triple negative breast cancer (TNBC) through its impact on various biological processes and signalling pathways. According to (Qu et al., 2023),zinc binding proteins (ZBPs) may be implicated in cancer as a result of mutations and the amino acids with positive charges, including histidine, arginine, and lysine, as well as negatively charged aspartic acid and glutamic acid, along with the polar amino acid cysteine, have the ability to bind to Zn ions within cancer associated ZBPs.

6.1.3 Cellular Components (CC)

Cellular Component describes the location within the cell where a gene product performs its function, including cellular structures, organelles, and protein complexes (Ashburner et al., 2000).

The Gene Ontology (GO) analysis revealed a distinct pattern with a higher enrichment in the extracellular compartment followed by components related to intercellular communication, most notably vesicles and exosomes. This distribution indicates that the identified genes play a pivotal role in the biogenesis and secretion of membrane bound vesicles, a key mechanism employed by cancer cells to transport genetic and protein cargo, thereby modifying adjacent tissues. In this discussion, we will examine the five primary cellular components highlighted in the analysis and explore how these structures enhance tumor survival and metastatic potential.

The enrichment of extracellular space is a dominant mechanism by which cancer cells, through the secretion of unconventional proteins, remodel the tumor microenvironment from separative tissue environment into a pro invasive and metastatic niche, leading to tumor invasion and metastatic spread (Méndez et al., 2018).

According to GO cellular component databases defined vesicles intercellular or extracellular as any small fluid filled spherical organelle enclosed by a membrane.

Vesicles are among the top enrichment cellular components identified in the current study, where more than 30 genes have been involved. Vesicles play a crucial role in cellular communication and the transportation of bioactive molecules into the extracellular space during the modeling of the tumor microenvironment. Recent studies have identified extracellular vesicles as mediators of cell to cell communication, TNBC progression, immune regulation and transfer of active molecules such as protein, nucleic acid from parental to recipient (Wan et al., 2025).Micro ribonucleic acid carried by extracellular vesicles of TNBC taggers NF fibroblast to became cancer associated fibroblast (CAFs), leading to cancer growth and metastasis (Wan et al., 2025).

Another cellular component determined in the present study is vesicles lumen. It is defined as the volume enclosed by the membrane or protein that forms a vesicle. The internal volume of vesicles is important because it contains and transports molecules cargo such as proteins and RNAs to drive tumor growth, metastasis and therapy resistance (Wan et al., 2025).

Extracellular organelles or Membrane bound organelles of extracellular location are structures that are enclosed by a membrane of lipid bilayer type but which are localized outside the cell. Extracellular vesicles such as exosomes, microvesicles, and apoptotic bodies form part of this class. These vesicles have recently been identified as crucial players in the development of cancers. The function of these vesicles is to ensure communication between cancerous cells and their local environment through the transfer of proteins, lipids, and nucleic acid molecules. In addition, extracellular vesicles have been reported to play a significant role in regulating the migration and invasion ability of TNBC cells. According to a study conducted by O'Brien et al. (2013), serum exosomes of TNBC patients greatly enhanced the invasive potential of the recipient cells, suggesting that these organelles may act as vectors of phenotypic characteristics from the tumor cells to other cells. Extracellular vesicles have also been known to serve as mediators of the transfer of oncogenes, such as HPV DNA, into the neighboring stromal cells (Battistelli & Falcieri, 2020). In this way, extracellular vesicles play an important role in cancer

growth, angiogenesis, metastasis, immune escape, and drug resistance (Becker et al., 2016; Kalluri & LeBleu, 2020; Théry et al., 2018).

Interestingly, the presence of extracellular exosomes in our enrichment analysis is an important statistical and biological evidence for their significance as a major building block for the development of TNBC.The high enrichment of these vesicles in the cellular component category implies that the genes identified play a key role in establishing communication network. Exosomes are vesicles formed through the fusion and secretion of multi vesicular bodies with the plasma membrane. They serve as an efficient communication system, which helps in reprogramming the host tissues to support tumor growth. While they help in transporting functional proteins and miRNAs that help in the conversion of normal tissue into cancer associated fibroblast (CAFs), they also play a role in the development of metastasis by creating a niche for metastasis in distance organ like lungs. They are also instrumental in the development of resistance to anticancer drugs by facilitating the transfer of resistance properties form drug resistance cells to drug sensitive cells and by enticing polarization of macrophage toward the M2 phenotypes (Wan et al., 2025).

Filcon-1 rich granule also appeared as one of the top enriched cellular component, suggesting its involvement with in granule associated cellular compartments. The Ficolin-1 (FCN1) gene encodes Ficolin-1, a plasma protein that belongs to the immunoglobulin super family (Zhong et al., 2024).FCN1 is positively correlated with various immune regulatory markers, including immune checkpoint proteins, which could contribute to the development of cancer by influencing the tumor microenvironment (Zhong et al., 2024).

6.2 Signaling Pathways Analysis (KEGG)

The study's most notable feature, according to the KEGG enrichment analysis, is metabolic pathways, which demonstrated the crucial role of more than 35 genes. The results showed also significant involvement of the genes in metabolic pathways such as Galactose metabolism and lipid and atherosclerosis pathways, which reflects the cells' varied nutrient utilization. Significant enrichment is also seen in signaling mechanisms, especially the Rap1 and MAPK pathways, which each involve 10 to 12 genes. These results imply that TNBC's metabolic and signaling architectures are important factors influencing its biological behaviour and could be excellent targets for therapeutic inhibition.

One of the key metabolic pathways involved in cancer is glutamine metabolism. Glutamine plays a key role in cancer cell metabolism. It enters cells through specific amino

acid transporters, such as SLC1A5 and SLC7A5, and it gets converted into glutamate by glutaminase. Glutamate is then further processed into α-ketoglutarate (α-KG), which participates in the tricarboxylic acid (TCA) cycle. This metabolic pathway provides the energy and nitrogen needed for building processes, supporting tumor growth and progression (Huang et al., 2023). Therefore, targeting glutamine metabolism could be an effective treatment strategy. Previous reports indicated that inhibition of glutaminase could reduce cancer cell growth, particularly, when used alongside mTOR inhibitors. However, the inhibition of glutamine transporters was found to reduce amino acid uptake, inhibit tumor cell growth and disrupt metabolic reprogramming pathways such as mTORC1 (Huang et al., 2023).

The lipid and atherosclerosis pathway is the second ranked pathway according to KEGG analysis. It is a long term inflammatory process involves the build-up of lipids in arterial walls, mainly caused by high levels of low density lipoprotein (LDL). Oxidized LDL (oxLDL) causes problems in the endothelium, encourages immune cells to gather, and leads to the formation of foam cells. These changes ultimately result in plaque development and issues in the blood vessels.(*KEGG PATHWAY Database*, 2026). Lipid metabolism is a key part of the lipid and atherosclerosis pathway. If it is blocked, it may impair tumor growth by stopping energy supply and membrane biosynthesis in TNBC cells. Interestingly, fatty acid oxidation (FAO) is greatly increased in TNBC. These cells are very dependent on FAO for making energy. Blocking this pathway causes ATP levels to drop significantly, cell growth to slow down, and apoptosis to speed up (Camarda et al., 2016).

RAP1 signaling pathway is controlled by a GTPase called RAP1. RAP1 regulates key processes such as cell adhesion and migration. It is essential for controlling important mechanisms that contribute to the development of cancer, including invasion, migration, and cell adhesion. RAP1 signaling is believed to have a major role in tumor dissemination (Liang et al., 2025).Since triple negative breast cancer (TNBC) is characterized by aggressive behavior and high metastatic potential (Freres et al., 2010), targeting RAP1 signaling may represent a promising therapeutic strategy to inhibit metastasis and improve clinical outcomes.

The MAPK signaling pathway was also found to be one of the significantly enriched pathways in our study. This pathway is essential for controlling a number of cellular functions. It is a signal transduction pathway that controls cell invasion, proliferation, and metastasis by phosphorylating downstream targets in triple negative breast cancer. Since TNBC is linked to elevated expression of genes in the MAPK signalling, inhibition of this pathway can suppress TNBC cell proliferation (Jiang et al., 2020; Dewi et al., 2022).

Galactose is a simple sugar that cells can use to produce energy**.** Galactose metabolism is the pathway that converts galactose into molecules used for cellular activities and growth. Studies showed that the galactose metabolism pathway is upregulated in young patients with TNBC, and this upregulation is associated with poor disease free survival. Therefore, inhibition of this pathway could be a promising therapeutic approach to inhibit TNBC progression and reduce tumor aggressiveness (Han et al., 2023).

Starch and sucrose metabolism is a complex biochemical process that provides energy and carbon skeletons for the synthesis of essential compounds. Sucrose is converted into glucose 1phosphate and subsequently used for starch biosynthesis through the action of several enzymes (Wu et al., 2024).Previous findings showed that a sucrose enriched diet was associated with increased breast tumor growth and enhanced metastatic potential (Jiang et al., 2016). Moreover, inhibition of glucose and fructose availability through targeting the Starch and Sucrose Metabolism pathways represents a promising therapeutic strategy for the treatment of TNBC, as it may restrict energy production and biosynthetic processes required for tumor growth and progression (Gan et al., 2026; Strober & Brady, 2019).

Nitrogen metabolism involves the uptake, assimilation, and utilization of nitrogen from amino acids and other sources to support the synthesis of essential biomolecules, including nucleotides and amino acids. This process is tightly regulated to maintain cellular nitrogen balance, especially in proliferating cells where nitrogen is conserved and redirected toward anabolic pathways to support growth and development (Kurmi & Haigis, 2020). In the process of tumor development, nitrogen metabolism of tumor cells is reprogrammed to meet the demands of rapid proliferation. Key enzymes in the tumor microenvironment affect nitrogen metabolism through multiple mechanisms, providing essential nitrogen sources and energy for tumor cells (Fang et al., 2026). Targeting tumor nitrogen metabolism, therefore, represents a promising therapeutic approach for cancer treatment (Fang et al., 2026).

6.3 Significance of Hub Genes in TNBC Progression

In the current study, the topological analysis determined GAPDH, CASP3, EGFR, and HDAC1 as pivotal target genes associated with TNBC regulation.

GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) is a derivative of the enzyme glyceraldehyde dehydrogenase. It is involved in glycolysis. GAPDH promotes excessive energy production by enhancing glycolysis, thereby meeting the high metabolic demands of cancer cells and supporting the rapid growth and progression of triple negative breast cancer.

The abnormal expression of GAPDH has been confirmed to have a close association with various types of cancer, as it plays an important role in carcinogenesis and cell death, as well as energy metabolism. Notably, increased levels of GAPDH were observed in most types of human cancer and were often correlated with reduced survival (Singh et al., 2024).

CASP3 (cysteinyl aspartate-specific proteinases), is an effector protease belonging to the caspase family and is encoded by the CASP3 gene. Studies have shown that both caspase3 precursor and its active form are higher in TNBC than normal tissue (Siregar et al., 2017;Dou et al., 2024).It functions primarily in the execution of apoptotic processes that lead to DNA fragmentation (Makuch-Kocka et al., 2025). Activated through proteolytic processing, CASP3 plays a role in programmed cell death as well as in several stages of development. In TNBC, it is often elevated, but its dysfunction can reduce apoptosis and promote tumor survival and treatment resistance (Friedrich et al., 2001).

EGFR (Epidermal Growth Factor Receptor) encodes a transmembrane glycoprotein that belongs to the protein kinase super family. EGFR regulates various cell signaling pathways that facilitate cancer development. EGFR plays a significant role in the regulation of cell growth, development and progression of TNBC. It has been reported that EGFR is significantly overexpressed in TNBC compared to normal tissues. It was also found to be a positive modulator of STAT3 by promoting its phosphorylation (Song et al., 2020).

HDAC1 (Histone Deacetylase 1) is a member of the histone deacetylase/acuc/alpha family and is involved in the histone deacetylase complex. Histone deacetylases (HDACs), especially those in class I like HDAC1, are crucial for the epigenetic control of gene expression specifically in triple negative breast cancer (TNBC). HDACs function as opposing agents to histone acetyl transferases (HATs) by eliminating acetyl groups from lysine residues found on histone tails, which results in the condensation of chromatin (forming heterochromatin) and the inhibition of gene transcription. This epigenetic process is vital, as acetylation of histones typically leads to chromatin being less compact and promotes the transcription of genes that support cell proliferation. Importantly, the levels of HDACs are often increased in numerous cancers, including breast cancer, indicating their role in the development of cancer (Shin et al., 2017).

6.4 Molecular Docking

The computational techniques are commonly utilized in drug design and discovery, as they greatly speed up the drug development time line while minimizing the chances of human

mistakes. Molecular docking acts as a virtual screening method that allows the examination of structure activity relations hip between a ligand and a target protein. It further reveals potential inhibitory mechanisms of the ligand on the target (Alavi & Sharma, 2023).

Among the various docking systems available, Swiss Dock stands out for its user friendly design. The process of setting up target ligand docking with Swiss Dock is straight forward and quick, making it a valuable resource for computational studies. Its automated processes, alongside accurate predictions of binding modes and energies, render Swiss Dock an effective and user friendly choice for initial docking assessments in anticancer drug research (Lohning et al., 2017)**.**

Taken all this together, we carried out molecular docking to as ses and comprehend the ability of Auxarthrol F to inhibit the core targets. Since these hub genes are highly associated with the disease, their inhibition could potentially suppress triple negative breast cancer.

The molecular docking results indicated that the binding of Auxarthrol F with EGFR uncovered several critical interactions that enhance the stability of the complex. Remarkably, a traditional hydrogen bond was identified between the ligand and the amino acid residue ALA B: 208, with a measurement of 2.28 Å. This short distance signifies a robust and meaningful interaction, implying an essential function in securing the ligand within the binding pocket (Panigrahi&Desiraju, 2007a).Besides the hydrogen bond, various hydrophobic interactions were found involving residues like LEU (B: 132, B:207, B:259), VAL B:140, and ALA (B:156, B:208). These amino acids are recognized for their role in van der Waals interactions, which, while not as strong as hydrogen bonds, collectively improve the ligand protein complex by encouraging beneficial hydrophobic interactions (Panigrahi&Desiraju, 2007).Moreover, a π-sigma interaction was observed between the ligand and LEU B:132 at a distance of 3.59 Å. This specific interaction adds further stability, especially through the engagement of aromatic components within the ligand's structure (Lanzarotti et al., 2020).The measured distance aligns with the acceptable range for such non covalent interactions, thereby supporting its impact on binding affinity. Nonetheless, the existence of a disadvantageous donor interaction indicates a slight destabilizing influence within the complex (Majewski et al., 2019).Nevertheless, the overall interaction landscape continues to be largely influenced by stabilizing factors, primarily the robust hydrogen bond and numerous hydrophobic interactions. Therefore Auxarthrol F exhibits a favorable binding configuration in the EGFR active site, marked by a strong hydrogen bond, complementary hydrophobic interactions, and additional π-type interactions. These observations suggest that Auxarthrol F establishes a relatively stable complex with the target

protein (EGFR), which may reflect good binding affinity and potential biological efficacy. Overall, the docking results revealed a strong interaction between Auxarthrol F and EGFR, in which the optimal docking configuration showed a binding energy of −8.008 kcal/mol, signifying a robust and advantageous interaction. Typically, lower binding energy numbers are related to greater binding affinity and a more stable ligand-protein complex. Thus, the energy obtained implies that the Auxarthrol F integrates effectively into the EGFR active site and establishes a stable complex, reinforcing its potential efficacy as an inhibitor.

Auxarthrol F GAPDH showed also noteworthy interactions that help stabilize this complex. An important conventional hydrogen bond was identified with the residue SER122, with a separation of about 2.70 Å, which falls within the ideal hydrogen bonding range of 2.5, 3.5Å, signifying a robust and reliable interaction. Additionally, numerous nearby residues such as VAL188, GLY12, ALA183, ARG13, THR99, ILE14, and GLU317 assist through van der Waals interactions, collectively furthering the stabilization of the ligand in the binding cavity. The presence of a short hydrogen bond distance along with various stabilizing interactions implies that the ligand takes on an advantageous conformation, resulting in a stable complex (Ribeiro&Correia, 2024). Moreover, the determined binding energy of −7.167 kcal/mol suggests a relatively high binding affinity, as lower energy values indicate more stable and advantageous interactions. Since values near −7.0 kcal/mol are typically linked to strong binding, this finding implies that the ligand establishes a stable and spontaneous complex with the target protein, under scoring its potential as an effective inhibitor.

The examination of molecular docking for the ligand “Auxarthrol F” within the active site of HDAC1 unveiled various critical interactions that play a role in stabilizing the complex. Specifically, traditional hydrogen bonds were found between the ligand and the amino acid residues GLN A: 338 and LYS A: 331, with distances of 2.99 Å and 2.64 Å, respectively. These relatively close distances indicate robust and significant interactions, implying a crucial function in securing the ligand within the binding pocket. Non covalent interactions were also observed. In particular, π-alkyl interactions were noted with residues ARG B: 306 and TYR B: 336, at distances of 5.04 Å and 4.07 Å, respectively. These interactions aid in the overall stabilization of the ligand protein complex through hydrophobic and aromatic contacts, improving binding affinity, although they are weaker than hydrogen bonds. The interaction profile indicates that stabilizing elements, especially hydrogen bonding and π-type interactions, primarily govern the binding of the ligand within the HDAC1 active site. Interestingly, the

optimal docking pose revealed a binding energy of around −6.623 kcal/mol, suggesting a beneficial and spontaneous interaction. While this value points out to a moderate binding affinity compared to more potent inhibitors, it still signifies a stable ligand-protein complex. Thus, Auxarthrol F demonstrates a plausible binding configuration within the HDAC1 active site and might have potential inhibitory activity.

For the CASP3, the binding energy measured is -6.176 kcal/mol, indicating a moderate binding affinity. Although the ligand Auxarthrol F was able to form a stable association, it appears that an unfavorable donor -donor interaction involving TRP 214 diminished the overall energy, preventing the complex from achieving a more negative energy state (Hu et al., 2019).In spite of this moderate binding energy, the structure's stability is upheld through a network of hydrogen bonds connecting to GLU 248 (at distances of 3.12 Å and 2.33 Å) and ASP 404 (at 2.62 Å). The proximity of these bond lengths signifies a strong localized connection, even if the overall binding affinity is not as high as earlier studied targets. Furthermore, hydrophobic interactions with PHE 250 (at distances of 4.23 Å and 3.88 Å) contribute to proper spatial arrangement. To summarize, although this protein demonstrates the lowest binding energy within the examined set, the existence of specific robust hydrogen bonds indicates that it retains a functional level of stability, albeit moderate, within the active site.

CONCLUSION

Conclusion

This study provides a comprehensive investigation into the therapeutic potential of Auxarthrol F against triple negative breast cancer (TNBC) using an integrated approach of network pharmacology and molecular docking. By exploring the complex interaction between this bioactive compound and TNBC related targets, we have gained valuable insight into its underlying molecular mechanisms.

The findings of this research identified 90 anti TNBC targets of Auxarthrol, which were implicated in various biological processes, molecular functions and cellular components. Among these targets, EGFR, GAPDH, HDAC1, and CASP3 were highlighted as pivotal therapeutic targets. The molecular docking analysis further validated these results, demonstrating that Auxarthrol F possesses a high binding Affinity, particularly towards the EGFR, HDAC1 and CASP3. The KEGG study demonstrated several pathways linked to TNBC including pathways in cancer, metabolic pathways, lipid and atherosclerosis, Rap1 signalling pathway and MAPK signalling pathway, through which Auxarthrol F could alleviate TNBC.

In light of these promising results, several avenues for future research (perspectives) are recommended:

- ✓ First: conducting *in vitro* and *in vivo* experimental studies to empirically validate the biological efficacy of Auxarthrol F on TNBC cell lines.
- ✓ Second: performing detailed pharmacokinetic and toxicity profiling (ADMET) to evaluate the compound's safety and drug likeness for clinical applications.
- ✓ Third: Exploring the identified hub genes as biomarkers for multi target therapeutic strategies to overcome drug resistance in TNBC.

In conclusion, this research underscores the power of computational pharmacology as a robust tool in modern drug discovery, paving the way for the development of novel, nature derived treatment for aggressive cancer like TNBC.

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3- Lechheb Anfel
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Certifie par la présente que les étudiants ont pris en considération l'ensemble des remarques, modifications et corrections formulées par les membres du jury lors de la soutenance qui s'est déroulée le 24/06/2026

En conséquence, le manuscrit final étant désormais conforme aux exigences scientifiques et académiques requises, j'autorise les étudiants suscités à procéder au dépôt officiel du mémoire auprès de la bibliothèque.

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