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UNVEILING THE ANTICANCER POTENTIAL OF *Viola Tricolor*: A NETWORK PHARMACOLOGY APPROACH

Simchu. RB[1*], Mrs. Anusree. S[2], Mrs. Rupitha. NS[3], Akshaya P[4], Jyothi BN[5], Liya S Saji[6], Shabin P[7], Dr. Kiran. KJ[8], Dr. Prasobh. GR[9]

[1,4,5,6,7]Fourth semester M Pharm, Department of Pharmacology, Sree Krishna College of Pharmacy and Research Centre Parassala.

[2] Associate Professor, Department of Pharmacology, Sree Krishna College of Pharmacy and Research Centre Parassala.

[3] Assistant Professor, Department of Pharmacology, Sree Krishna College of Pharmacy and Research Centre Parassala.

[8] Professor and Vice Principal, Department of Pharmacology, Sree Krishna College of Pharmacy and Research Centre Parassala.

[9] Principal, Sree Krishna College of Pharmacy and Research Centre Parassala.

ABSTRACT

Cancer is a complex and multifaceted disease characterized by the uncontrolled growth and spread of abnormal cells. It can affect any part of the body and is a leading cause of death worldwide.Breast cancer is a disease in which malignant cells form in the tissues of the breast, often originating in the ducts or lobules. It is one of the most common types of cancer affecting the women worldwide. Lot of conventional therapies are available for the treatment of cancer, but the main problem associated with the treatment is the occurrence of serious adverse effects. Plants play a vital role in the discovery and development of anticancer agents due to their rich diversity of bioactive compounds with therapeutic potential. Many plant-derived secondary metabolites, such as alkaloids, flavonoids, and terpenoids, exhibit strong anticancer properties by targeting multiple cellular pathways involved in cancer development, including apoptosis induction, inhibition of angiogenesis, and suppression of metastasis.Network pharmacology is an emerging and highly relevant approach in modern drug discovery, especially for complex diseases like cancer. Unlike traditional "one drug, one target" strategies, network pharmacology embraces the complexity of biological systems by examining how drugs interact with multiple targets and pathways simultaneously.*Viola tricolor*, commonly known

as wild pansy**,** heartsease, or Johnny jump-up, is a small annual or perennial herbaceous plant from the Violaceae family, native to Europe and parts of Asia.Phytochemical studies have identified a rich profile of bioactive compounds in *Viola tricolor*, including flavonoids (violanthin, rutin), phenolic acids (Caffeic and p-Coumaric acids), cyclotides, saponins, and carotenoids, which contribute to its wide spectrum of pharmacological activities. Recent in vitro studies have demonstrated that extracts of *Viola tricolor* exhibit cytotoxic effects against various cancer cell lines**,** including human cervical (HeLa), breast (MCF-7), and lung carcinoma cells, indicating potential anticancer activity.Due to its multi-component composition and traditional medicinal relevance, *Viola tricolor* is increasingly being studied through network pharmacology and molecular docking approachesto better understand its potential as a natural source of anticancer agents.The study utilized a network pharmacology approach to explore the anticancer potential of *Viola tricolor*, uncovering its multi-target and multi-pathway therapeutic actions. The analysis revealed that bioactive compounds in *Viola tricolor* interact with key cancer-related proteins such as TP53, PIK3CA, AKT1, JAK2, and ERBB2, which are central nodes in critical signaling pathways governing cell proliferation, apoptosis, and metastasis.

KEY WORDS

Anti-cancer, Apoptosis, Breast cancer, Cytotoxicity, Docking, Metastasis, Viola tricolor.

INTRODUCTION

Cancer is a complex and multifaceted disease characterized by the uncontrolled growth and spread of abnormal cells. It can affect any part of the body and is a leading cause of death worldwide. Cancer develops through a series of genetic mutations that disrupt normal cellular function, often resulting from a combination of genetic, environmental and lifestyle factors. The disease can manifest in various forms such as carcinomas, sarcomas, leukemias and lymphomas. Early detection and treatment have significantly improved survival rates for many types of cancer and ongoing research continues to advance our understanding and management of disease.[1]

Breast cancer is a disease in which malignant cells form in the tissues of the breast, often originating in the ducts or lobules. It is one of the most common types of cancer affecting the women worldwide. Early detection through screening and mammograms has significantly improved treatment outcomes, with common treatments including surgery, radiation therapy, chemotherapy and targeted therapy. Factors such as genetics, hormonal influences and lifestyle can contribute to the risk of developing breast cancer. Breast cancer is a significant health concern in India, with the country reporting 192,020 new cases in 2022, accounting for 26.6% of all cancer cases. It is the most prevalent cancer in India, with a higher incidence among urban premenopausal women in their mid-forties. The median age at first diagnosis is between 40-45 years, which is concerning because breast cancer diagnosed at an early age tends to be more aggressive. [2]

Plants play a vital role in the discovery and development of anticancer agents due to their rich diversity of bioactive compounds with therapeutic potential. Many plant-derived secondary metabolites, such as alkaloids, flavonoids, and terpenoids, exhibit strong anticancer properties by targeting multiple cellular pathways involved in cancer development, including apoptosis induction, inhibition of angiogenesis, and suppression of metastasis. Notably, several widely used chemotherapeutic drugs, such as paclitaxel from the Pacific yew tree and vincristine from the Madagascar periwinkle, are derived from plants, highlighting their effectiveness and clinical relevance. In addition, plant-based compounds often demonstrate lower toxicity and fewer side effects compared to synthetic drugs, improving patient outcomes and tolerability. Traditional medicine systems have long utilized plants for treating various ailments, offering valuable leads for modern anticancer research. Overall, the use of plant sources in anticancer activity is not only scientifically significant but also offers a sustainable and promising path for future cancer therapies. [3]

Network pharmacology is an emerging and highly relevant approach in modern drug discovery, especially for complex diseases like cancer. Unlike traditional "one drug, one target" strategies, network pharmacology embraces the complexity of biological systems by examining how drugs interact with multiple targets and pathways simultaneously. This holistic perspective is particularly valuable in cancer treatment, where multiple genes, proteins, and signaling networks are involved in disease progression and drug resistance. By integrating data from genomics, proteomics, and systems biology, network pharmacology helps identify key nodes and pathways that can be modulated for therapeutic benefit. It is especially useful for studying the mechanisms of natural compounds and traditional herbal medicines, which often exert their effects through multi-target interactions. As a result, network pharmacology enhances our understanding of drug actions, improves target prediction, and facilitates the development of more effective and safer therapies. [4]

***Viola tricolor*:**

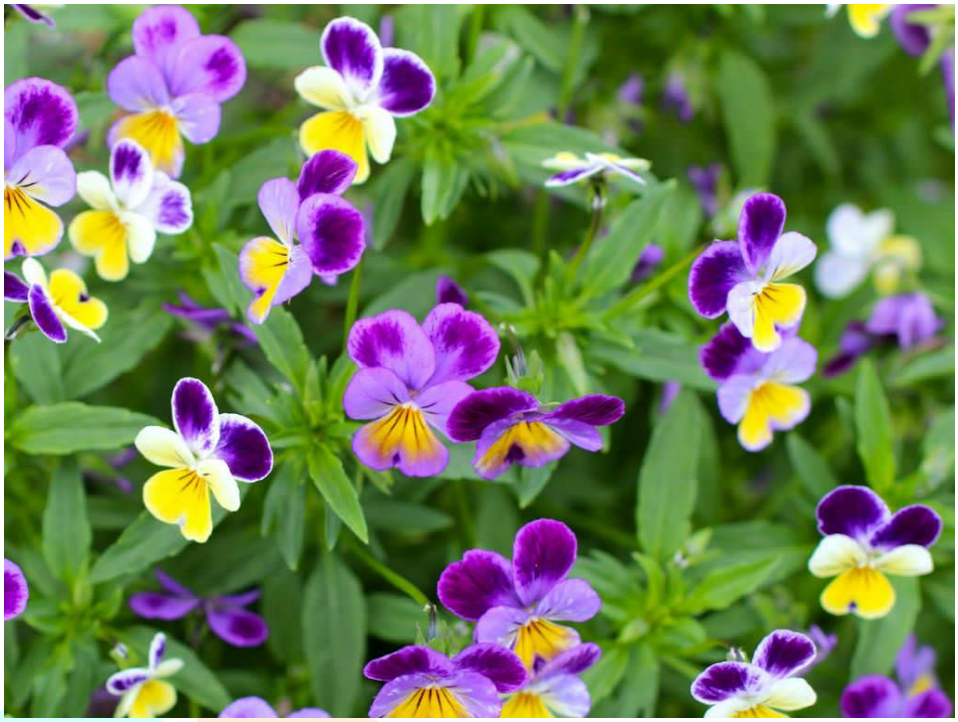


Fig No 1 *Viola tricolor*

Viola tricolor, commonly known as **wild pansy, heartsease**, or **Johnny jump-up**, is a small annual or perennial herbaceous plant from the **Violaceae** family, native to Europe and parts of Asia. It is characterized by its distinctive tricolored flowers—typically violet, yellow, and white—which have made it both an ornamental and medicinal plant of interest. Traditionally, *Viola tricolor* has been used in **European folk medicine** to treat conditions such as skin diseases, bronchitis, and urinary inflammation due to its **anti-inflammatory, diuretic, and expectorant** properties. [5]

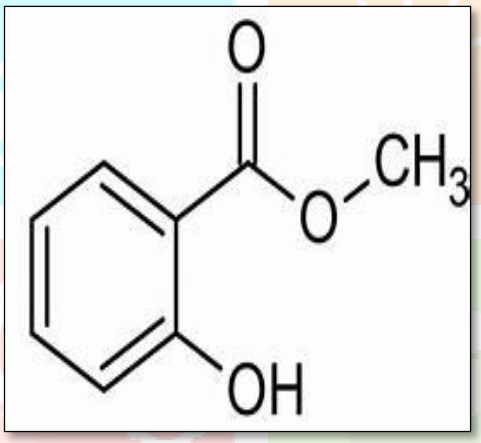
Phytochemical studies have identified a rich profile of **bioactive compounds** in *Viola tricolor*, including **flavonoids (violanthin, rutin), phenolic acids (Caffeic and p-Coumaric acids), cyclotides, saponins, and carotenoids**, which contribute to its wide spectrum of pharmacological activities. Recent in vitro studies have demonstrated that extracts of *Viola tricolor* exhibit **cytotoxic effects against various cancer cell lines,** including human cervical (HeLa), breast (MCF-7), and lung carcinoma cells, indicating potential anticancer activity. [6] These effects are thought to result from **induction of apoptosis, oxidative stress modulation, and inhibition of cell proliferation,** although the precise molecular mechanisms are still under investigation.

Due to its **multi-component composition and traditional medicinal relevance**, *Viola tricolor* is increasingly being studied through **network pharmacology and molecular docking approaches** to better understand its potential as a **natural source of anticancer agents**. These findings support further preclinical and clinical research into its therapeutic applications, particularly in the context of plant-based anticancer drug development.

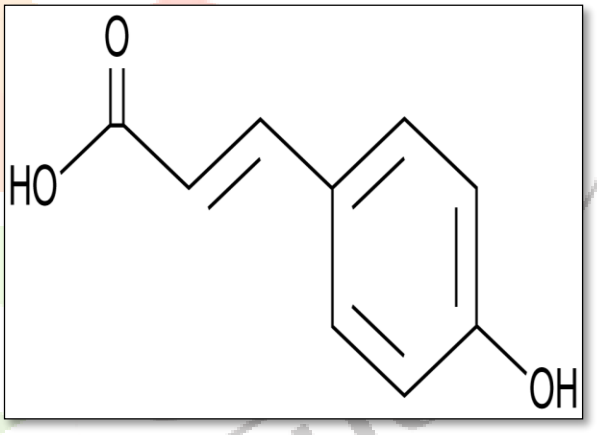
PLANT PROFILE:

- **Botanical name:** *Viola tricolor.*
- **Common name: wild pansy, heartsease**, **Johnny jump-up.**
- **Family:** Violaceae.
- **Kingdom:** Plantae.
- **Division:** Tracheophyta.
- **Class:** Magnoliopsida.
- **Order:** Malpiphiales.
- **Genus:** *Viola*
- **Species:** *V.tricolor*

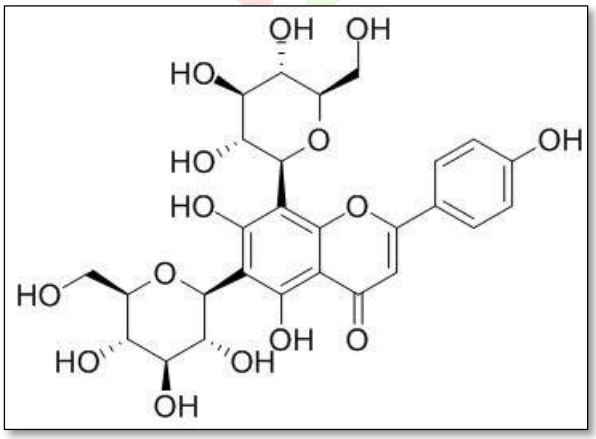
PHYTOCHEMICAL COMPOSITION OF *Viola tricolor*:



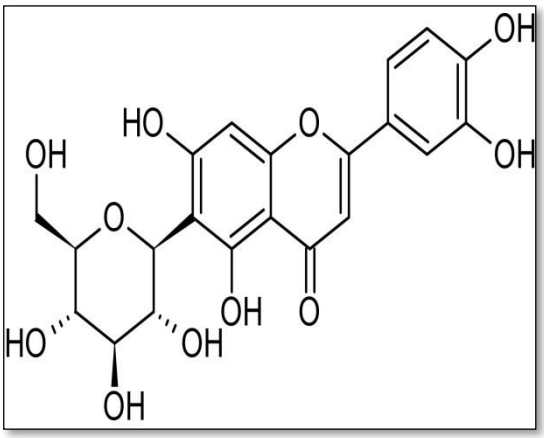
Methyl salicylate



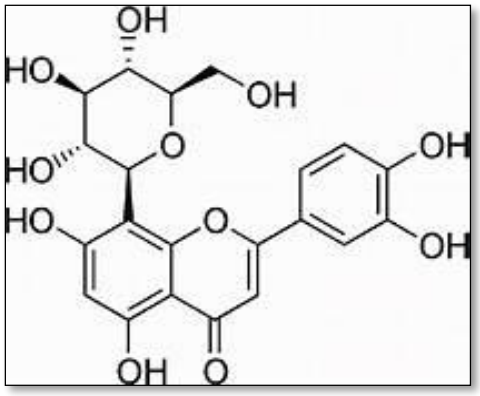
Para Coumaric acid



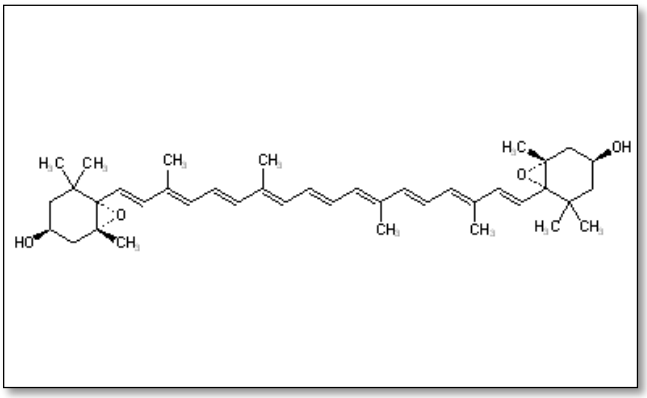
Vicenin 2



Isoorientin



Orientin



Violaxanthin

TRADITIONAL USES:

The various phytochemicals present in plants have been reported to possess great potential in the treatment of multiple diseases. The aerial parts are used as expectorant and diuretic, for skin conditions, bronchitis, cystitis, and rheumatism. The whole of the plant can be used in medicine, such as lowering the temperature, detoxification, dispersing blood stasis, and relieving coughs. [7]

It is a well known herb in traditional medicine, prescribed for the treatment of illnesses such as coughs and inflammatory skin diseases. It is used to treat rheumatic pains, asthma, and respiratory problems. It has been used by folk healers to treat and control a variety of human diseases, including infectious diseases, diabetes, lung diseases, cough, fatigue, and several other conditions. In traditional medicine, fragrant violets played a significant therapeutic role. Sweet violet decoctions were employed as choleretics, blood pressure reducers, and remedies for fever, heart palpitations, and fainting. The plant was commonly prescribed for respiratory issues such as coughs, sore throats, pleurisy, and lung inflammation, as well as disorders of the digestive and urinary systems. The aroma of fresh violet flowers was believed to alleviate headaches, promote calmness, combat insomnia, and counteract various toxins. [8]

Violet oil was historically used to treat dry skin, wounds, and hair loss. Additionally, all parts of the plant—including the stems, leaves, flowers, fruits, and seeds—were used in the management of skin conditions, cystitis, bronchitis, inflammation, and served as a diuretic. A concentrated decoction made from grass and violet roots is traditionally used to induce vomiting. Milder doses serve as a remedy for jaundice and epilepsy. Violet syrup is commonly employed to relieve asthma and airway obstruction. For children suffering from chickenpox, 5–7 drops of fresh plant sap are recommended. An infusion of the herb is applied externally to treat eye conditions, while both internal and external applications are used to address pneumonia, scrofula, and cutaneous tuberculosis. [9]

NETWORK PHARMACOLOGY

Network pharmacology is an interdisciplinary approach that integrates systems biology, bioinformatics, and pharmacology to understand the interactions between drugs, targets, and disease pathways in a holistic, network-based manner. Unlike traditional pharmacology, which often focuses on a single drug and a single target, network pharmacology explores how drugs affect multiple targets and biological pathways simultaneously. [10]

Steps in network pharmacology:

- Identify active compounds.
- Predict compound targets.
- Collect disease associated genes.
- Identify common targets.
- Build protein-protein interaction network.
- Visualize and analyze network.
- Enrichment analysis.
- Construct compound target pathway network.
- Molecular docking.

Cytoscape is the software employed for performing network pharmacology. For the identification structures Swiss target prediction is used. The common genes were identified by using the software Venny 2.0. The network analysis done by using the software String. The molecular docking was performed using the software MZ dock.

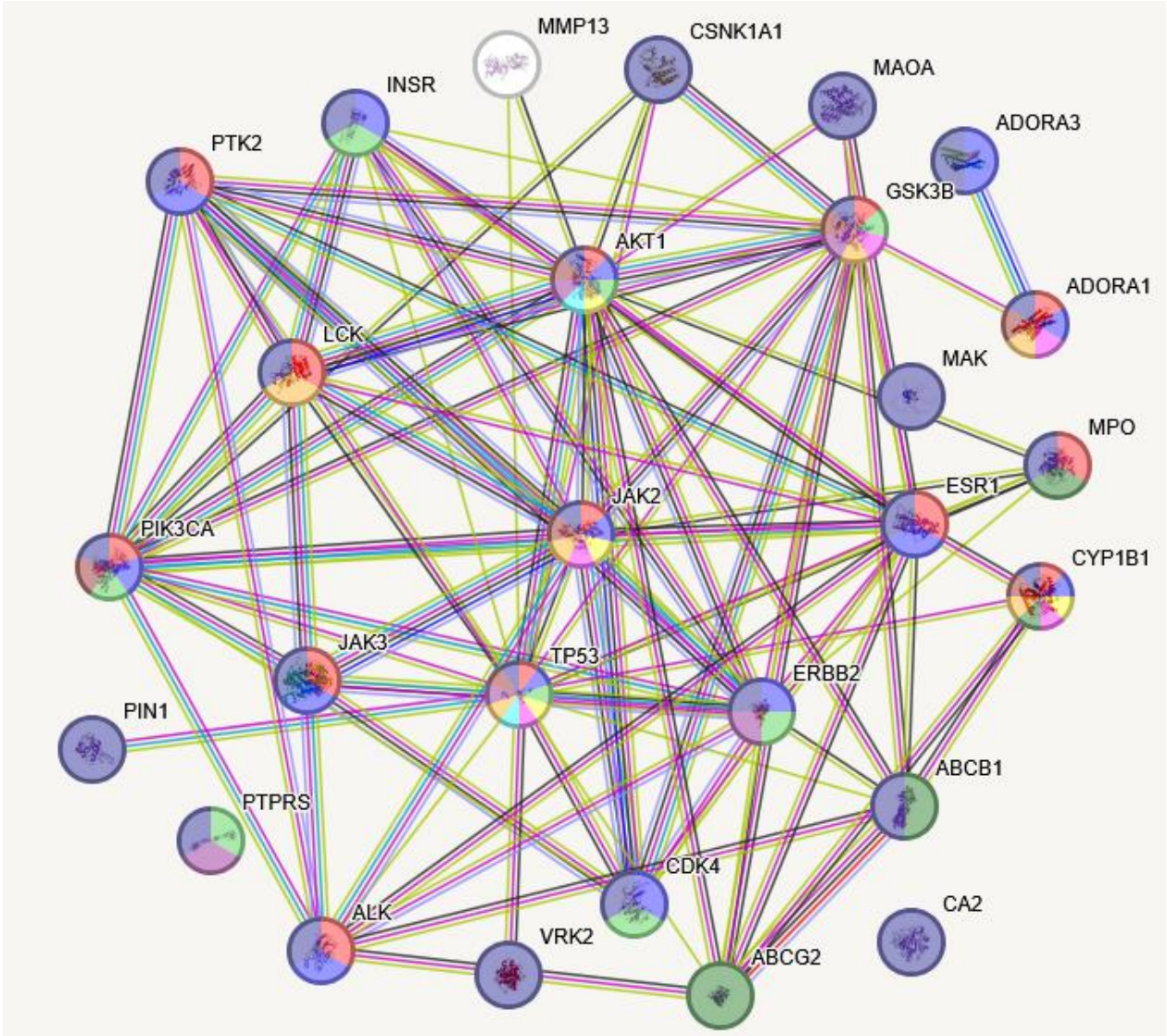


Fig No 2 String analysis

Network status:

- Number of nodes: 27
- Number of edges: 98
- Average node degree: 7.26
- Average local clustering coefficient: 0.691
- Expected number of edges: 39
- PPI enrichment p-value: 1.33e- 15

Network analysis:

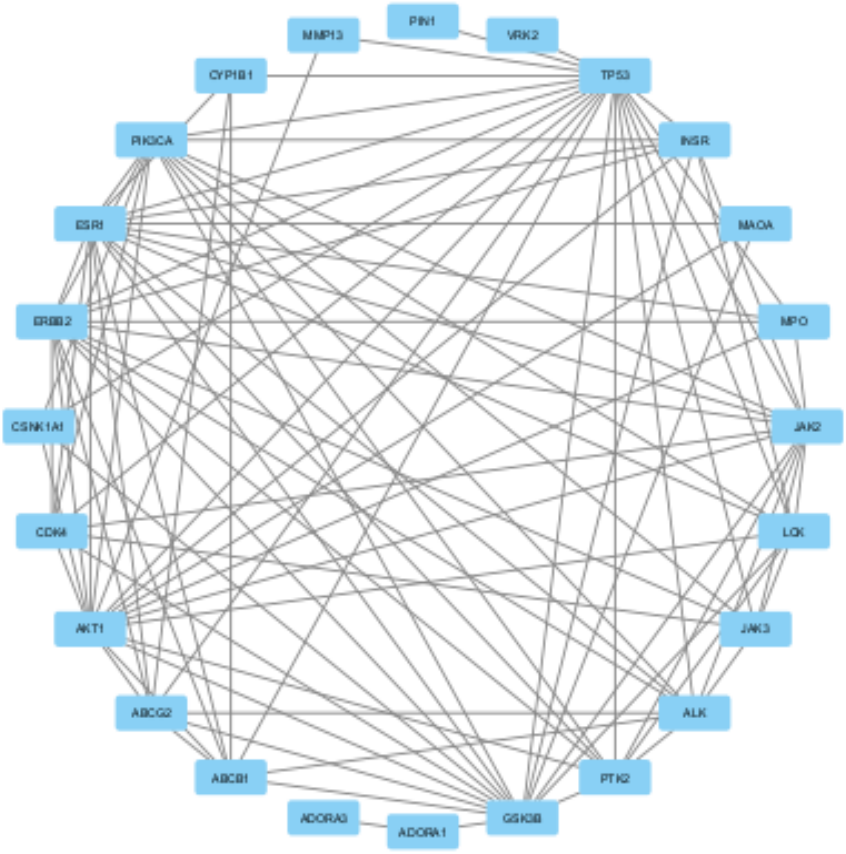


Fig No 3 Network analysis

The figure illustrates the **protein-protein interaction (PPI) network** of key target proteins associated with cancer-related pathways. Each node represents a protein (e.g., TP53, ESR1, AKT1), and the edges denote known or predicted interactions obtained from databases such as STRING. The circular layout reveals the complexity of molecular interactions involved in cancer progression. Highly connected **hub proteins** such as TP53, ESR1, and AKT1 play critical roles in regulating processes like apoptosis, cell proliferation, and oncogenic signaling. This network visualization supports the concept of **network pharmacology**, which emphasizes the importance of multi-target therapeutic strategies over the traditional one-drug-one-target approach. Such analysis helps in identifying potential biomarkers and designing more effective and personalized anticancer treatments.

Analysis of Maximal Clique Centrality:

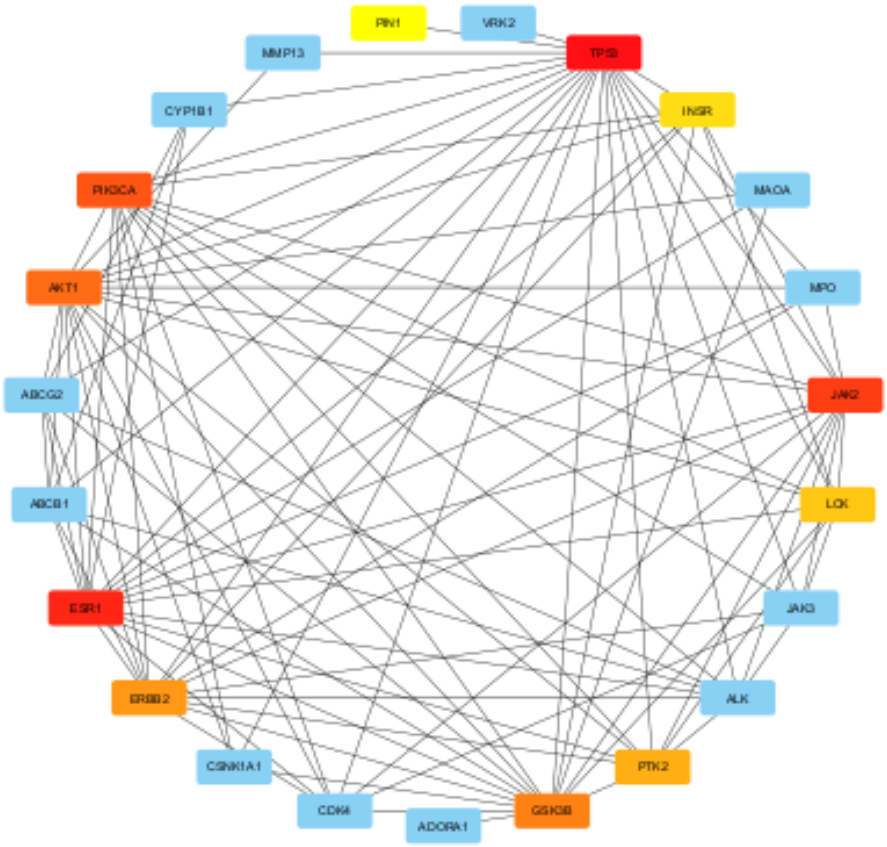


Fig No 4 MCC analysis

The image represents the **protein-protein interaction (PPI) network** of cancer-related targets, analyzed using **Maximal Clique Centrality (MCC)**—a topological algorithm used to identify highly influential nodes within complex biological networks. In this network, nodes represent proteins, while edges denote known or predicted functional interactions. The color gradient indicates MCC scores, with **red and orange nodes** representing proteins with the highest centrality values. Notably, **TP53, ESR1,** and **JAK2** are highlighted as key **hub proteins**, possessing high MCC scores and suggesting their central roles in regulating critical oncogenic processes such as apoptosis, cell proliferation, hormone response, and signal transduction. These proteins often participate in multiple protein cliques, emphasizing their influence across various cancer-related pathways. Identifying such high-centrality nodes is crucial in network pharmacology, as they serve as potential **multi-target drug candidates or biomarkers** for cancer treatment. The use of MCC enhances the ability to prioritize targets that may offer **greater therapeutic impact** through network-based interventions.

Analysis of degree between targets:

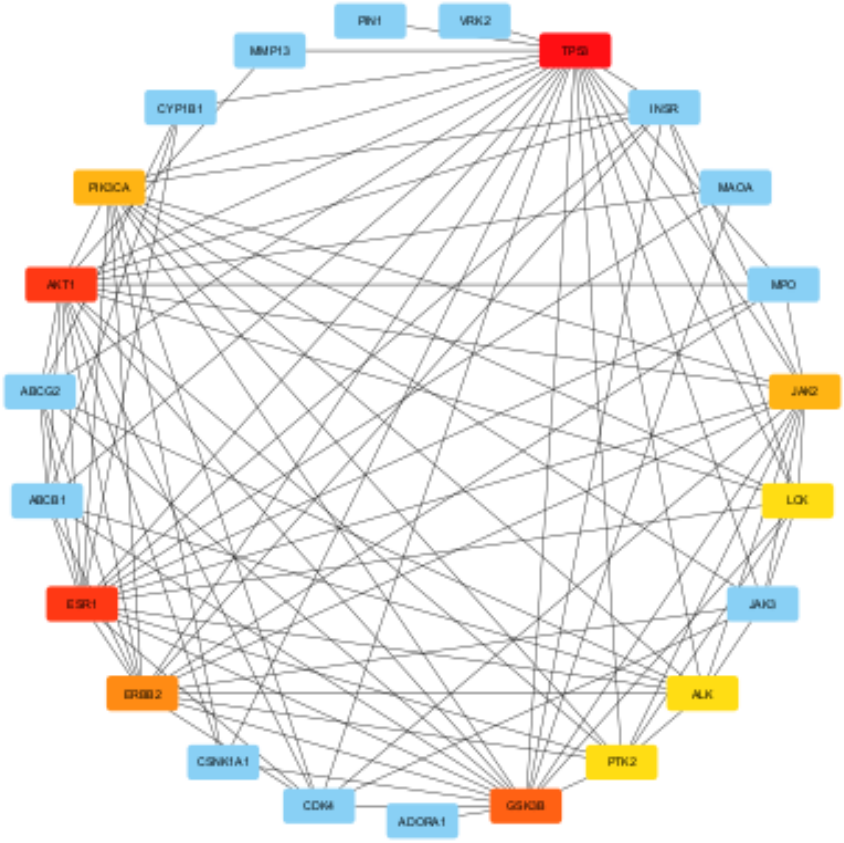


Fig No 5 Degree analysis

The image depicts the **protein-protein interaction (PPI) network** of key targets associated with cancer, analyzed using **degree centrality** to identify the most interconnected and influential proteins within the network. Each node represents a protein, and edges denote their direct functional or physical interactions. Proteins are color-coded based on their degree values, with **red and orange nodes** indicating **high-degree targets**—those interacting with numerous other proteins. **TP53**, a well-known tumor suppressor gene, is shown as the top-degree node, underscoring its central role in multiple cancer-related pathways, including apoptosis, cell cycle control, and DNA repair. Other high-degree proteins such as **ESR1, AKT1,** and **JAK2** also display significant connectivity, suggesting their importance as **key regulators in oncogenic signaling** and potential **multi-target candidates for drug development**. Degree centrality thus helps prioritize these proteins for therapeutic targeting, as their extensive network involvement implies that modulating their activity could disrupt multiple pathological processes in cancer.

Analysis of closeness between targets:

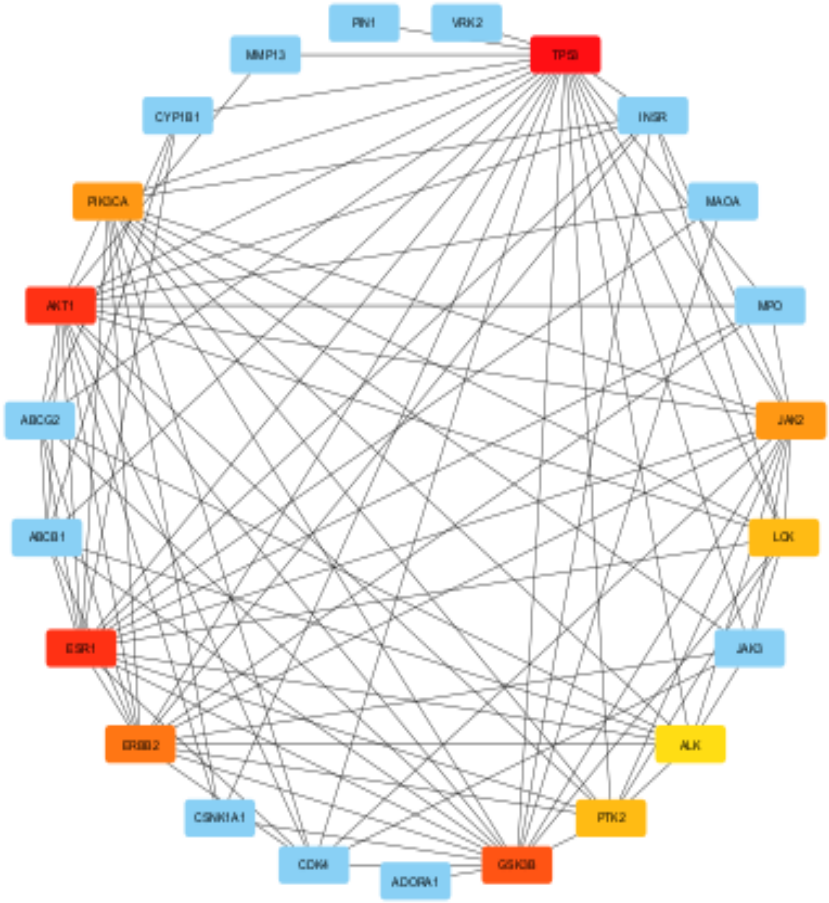


Fig No 6 Closeness analysis

The image illustrates a **protein-protein interaction (PPI) network** of cancer-associated targets, analyzed using **closeness centrality** to evaluate the relative distance of each protein to all others in the network. In this visualization, each node represents a protein, and edges indicate functional or physical interactions. Nodes are color-coded by closeness values, with **red and orange nodes** representing proteins with the **highest closeness centrality**. Notably, **TP53**, **ESR1**, and **AKT1** emerge as central nodes, indicating that these proteins have the **shortest average path to all other nodes** and thus occupy highly influential positions in the network. High closeness centrality suggests that these proteins can rapidly transmit signals or influence a wide range of biological processes, making them critical regulators in cancer pathways such as apoptosis, proliferation, and signal transduction. From a network pharmacology perspective, targeting proteins with high closeness centrality may lead to **faster and broader therapeutic effects**, as these nodes serve as key conduits in the molecular interaction network among the targets underlying the key factor among the progress and survival of the disease cancer among the targets.

Analysis of betweenness among targets:

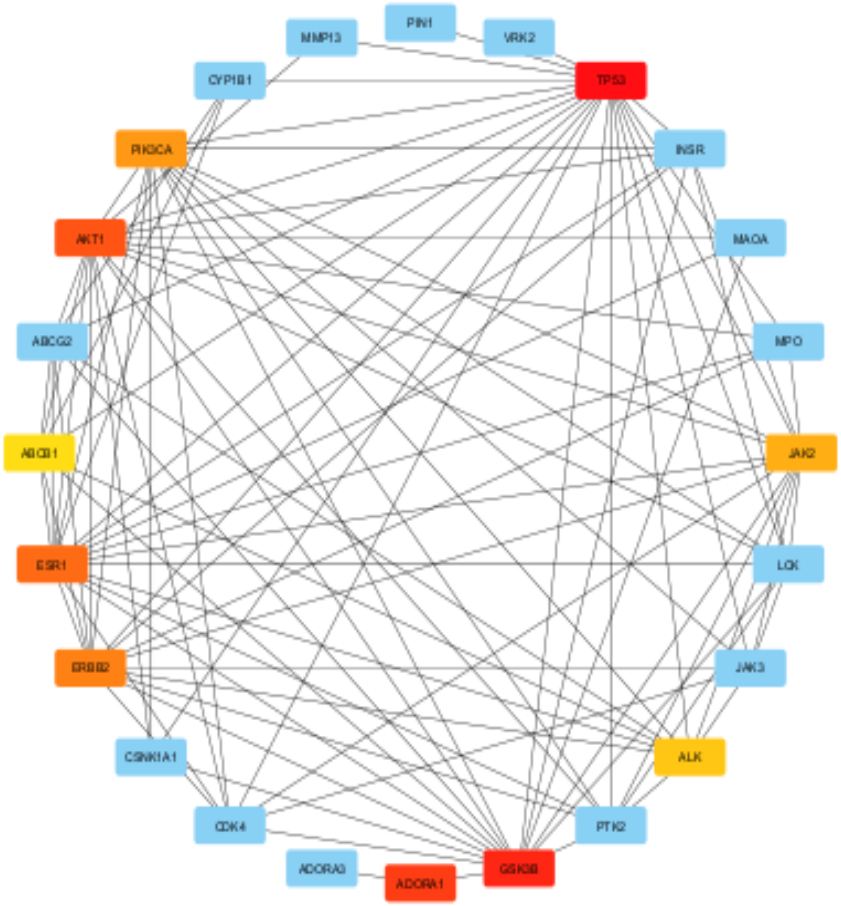


Fig No 7 Betweenness analysis

The image represents the **protein-protein interaction (PPI) network** of cancer-associated targets, analyzed through the lens of **betweenness centrality**, which identifies nodes that serve as critical bridges within the network. Each node corresponds to a protein, and edges indicate functional or physical interactions. Proteins are color-coded according to their betweenness scores, with **red and orange nodes** indicating those with the highest values. Among these, **TP53**, **AKT1**, **GSK3B**, and **ADORA1** emerge as **key bottleneck proteins**—occupying positions on many of the shortest paths connecting other proteins. Their high betweenness suggests they play essential roles in **information flow and signal transduction** across the network. In the context of cancer, such proteins often act as regulatory gatekeepers, controlling multiple pathways involved in tumor progression, survival, and metastasis. From a **network pharmacology** perspective, targeting high-betweenness nodes can disrupt key communication routes within the cancer network, making them promising candidates for **multi-targeted therapeutic interventions** aimed at weakening the overall disease network structure.

KEGG Pathway:

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a comprehensive bioinformatics resource that provides information on the biological functions of genes and proteins within the context of cellular and organism processes. The **KEGG Pathway** database is a key component that maps genes and molecular interactions onto curated **biological pathways**, including those involved in **metabolism**, **genetic information processing**, **environmental information processing**, **cellular processes**,

organism systems, **human diseases**, and **drug development**. In network pharmacology, **KEGG pathways** are widely used to understand how drug targets interact within complex biological systems.

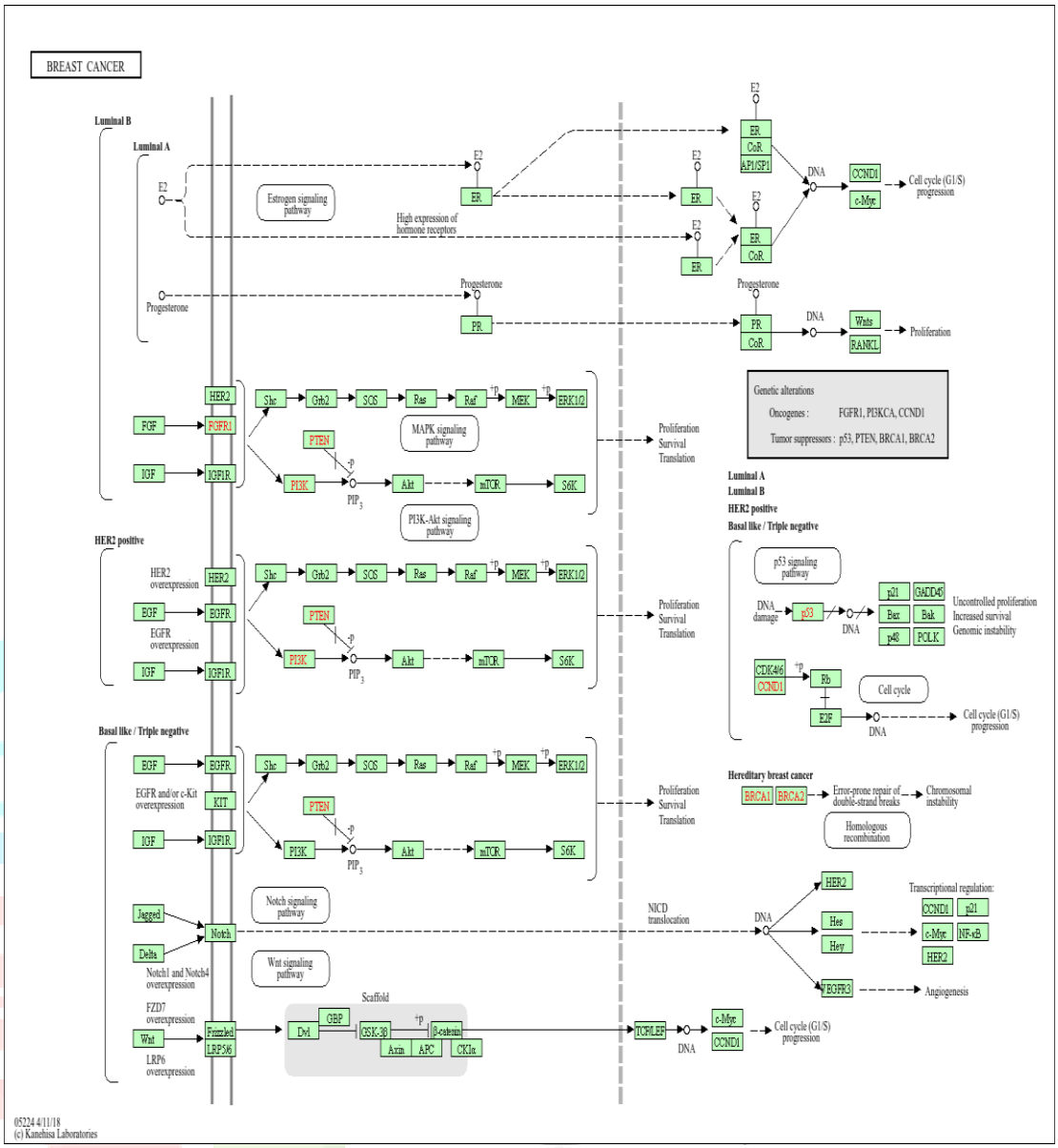


Fig No 8 KEGG pathway

The image represents the **KEGG Breast Cancer Pathway**, highlighting the complex molecular mechanisms and signaling pathways involved in the initiation and progression of breast cancer. It categorizes breast cancer into major subtypes—**Luminal A**, **Luminal B**, **HER2-positive**, and **Basal-like/Triple-negative**—each characterized by distinct molecular markers and pathway activations.

Key signaling cascades such as the **MAPK** and **PI3K-Akt pathways** are prominently featured, showing how over expression or mutation of upstream receptors like **HER2**, **EGFR**, and **IGF1R** activates downstream effectors (e.g., **Ras**, **Raf**, **MEK**, **ERK**, **Akt**), promoting **cell proliferation**, **survival**, and **translation**. In hormone receptor-positive subtypes (Luminal A/B), **estrogen (ER)** and **progesterone (PR)** signaling drive tumor growth via nuclear transcription factors, influencing genes like **Cyclin D1 (CCND1)** and **c-Myc**, which are critical for **G1/S cell cycle progression.**

The pathway also illustrates tumor suppressor gene involvement, such as **TP53**, **PTEN**, **BRCA1**, and **BRCA2**. Mutations in these genes lead to **genomic instability**, **impaired DNA repair**, and **uncontrolled cell division**, particularly evident in basal-like or hereditary breast cancers. The diagram includes the **Notch**, **Wnt**, and **p53 signaling pathways**, emphasizing their roles in stem cell maintenance, transcription regulation, and apoptotic responses.

Barplot:

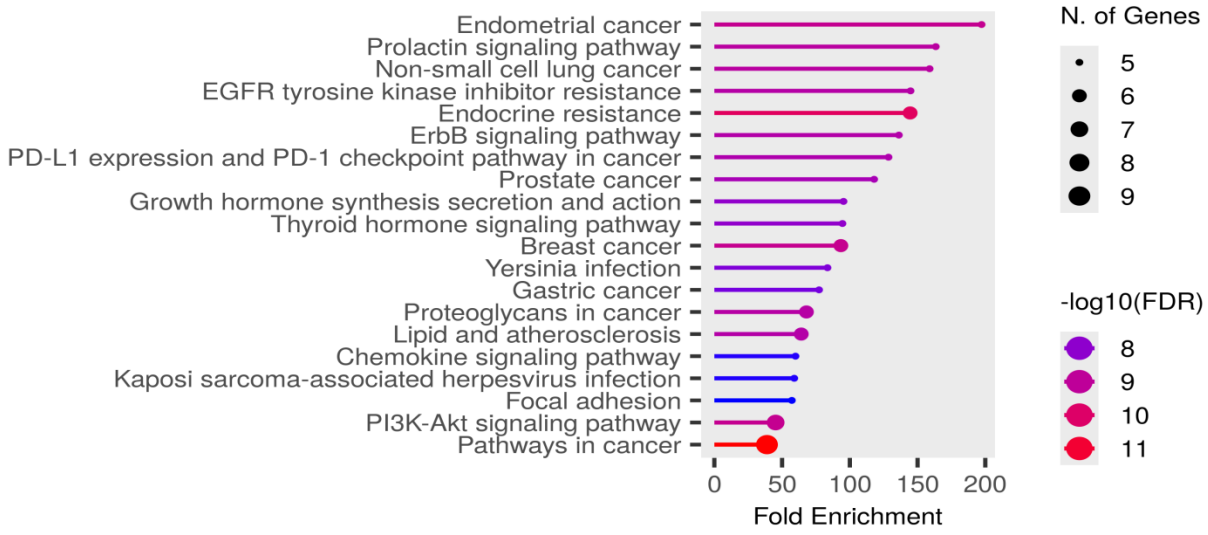


Fig No 9 Barplot

The figure presents a **KEGG pathway enrichment analysis**, visually highlighting the most significantly enriched biological pathways associated with the input gene set. The x-axis represents **fold enrichment**, indicating the degree to which each pathway is overrepresented in the gene list compared to a random distribution. Pathways with higher fold enrichment are more likely to play key roles in the underlying biological condition—in this case, cancer.

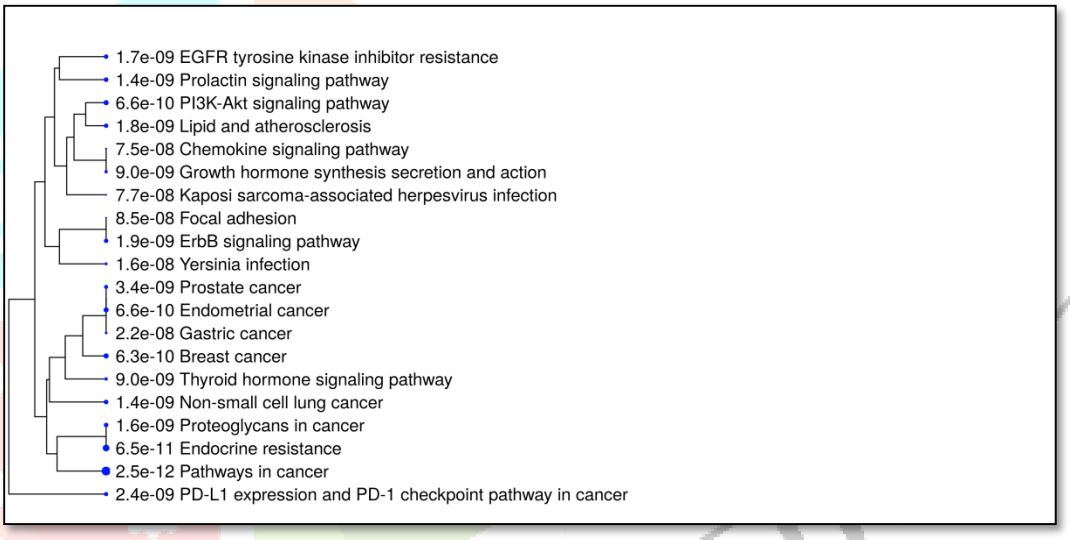
Each horizontal bar corresponds to a KEGG pathway, while the **size of the circles** at the end of each bar represents the **number of genes** involved in that pathway. The **color intensity** of each dot

reflects the **statistical significance**, measured by the –log10 of the false discovery rate **(FDR)**, where red shades indicate the **most significant** pathways.

Prominent among the enriched pathways are several **cancer-related signaling routes**, such as **Pathways in cancer**, **PI3K-Akt signaling**, **ErbB signaling**, **EGFR tyrosine kinase inhibitor resistance**, **Endocrine resistance**, and **Breast cancer**, all of which are strongly associated with tumor progression, proliferation, and treatment resistance. Additionally, pathways like **PD-L1/PD-1 checkpoint signaling**, **Non-small cell lung cancer** and **endometrial cancer** reflect broader **oncogenic mechanisms** shared across multiple tumor types.

This enrichment analysis reinforces the biological relevance of the gene set under investigation and highlights key **molecular targets and signaling networks** that may be explored further for drug discovery, therapeutic intervention, or biomarker identification in cancer research.

Tree plot:



Enrichment plot network:

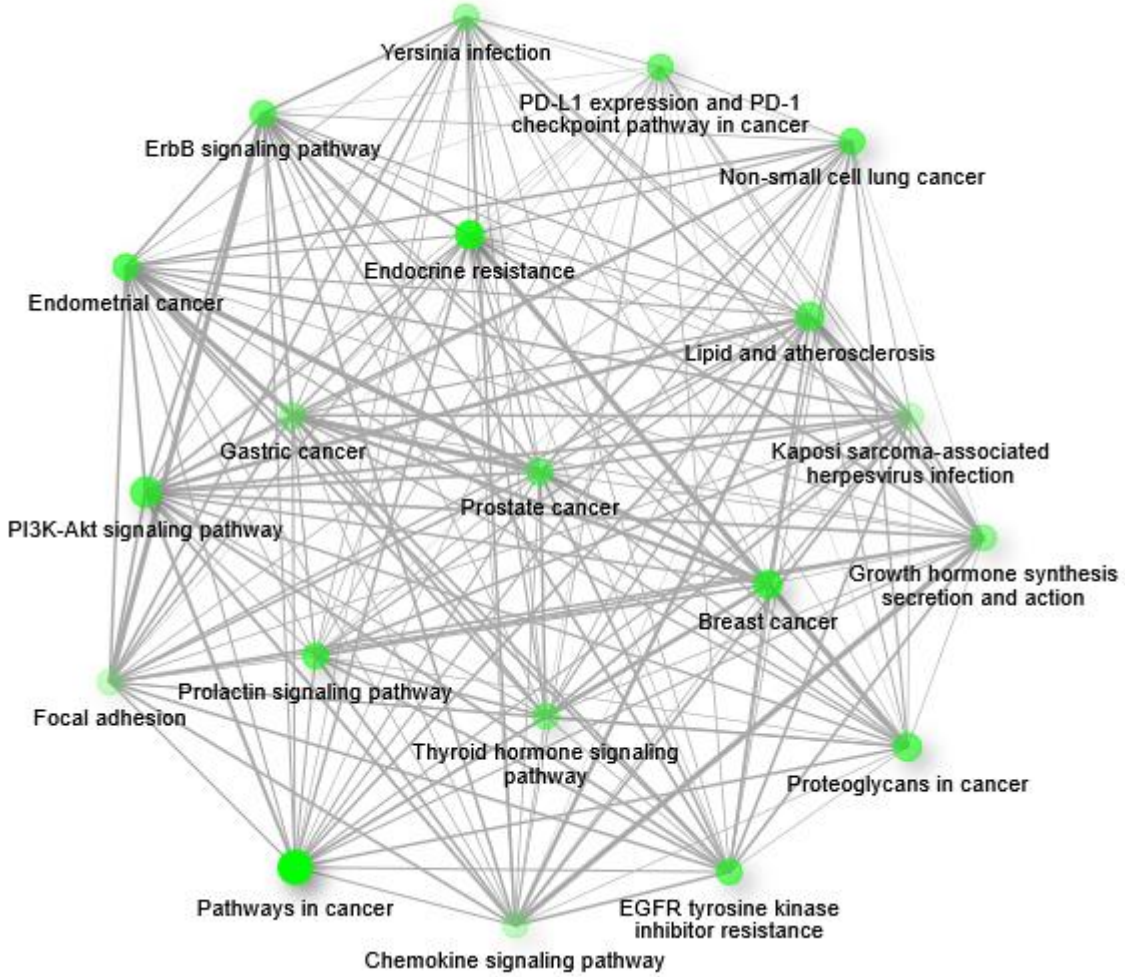


Fig No 10 Enrichment plot network

The image represents the **KEGG pathway enrichment network**, constructed to visualize the interconnectivity among significantly enriched biological pathways related to cancer. Each **node (green circle)** represents a distinct KEGG pathway, while the **edges (gray lines)** indicate shared genes or functional overlaps between those pathways. This network map helps to understand how biological processes are interconnected at the systems level.

Notably, pathways such as **"Pathways in cancer"**, **"Breast cancer"**, and **"ErbB signaling pathway"** are centrally located and **highly connected**, **"PI3K-Akt signaling pathway",** highlighting their pivotal roles in oncogenesis and their frequent involvement in multiple signaling cascades. Their central positions suggest that these pathways may serve as key regulatory hubs in cancer progression.

Additional cancer-related pathways, such as **"Endometrial cancer"**, **"Non-small cell lung cancer"**, **"Prostate cancer"**, and **"Gastric cancer"**, are also tightly integrated within the network, reflecting shared molecular mechanisms across different cancer types. Immune-related and resistance pathways like **"PD-L1/PD-1 checkpoint pathway"**, **"Endocrine resistance"**, and **"EGFR tyrosine kinase inhibitor resistance"** further emphasize the relevance of immune evasion and drug resistance in cancer biology.

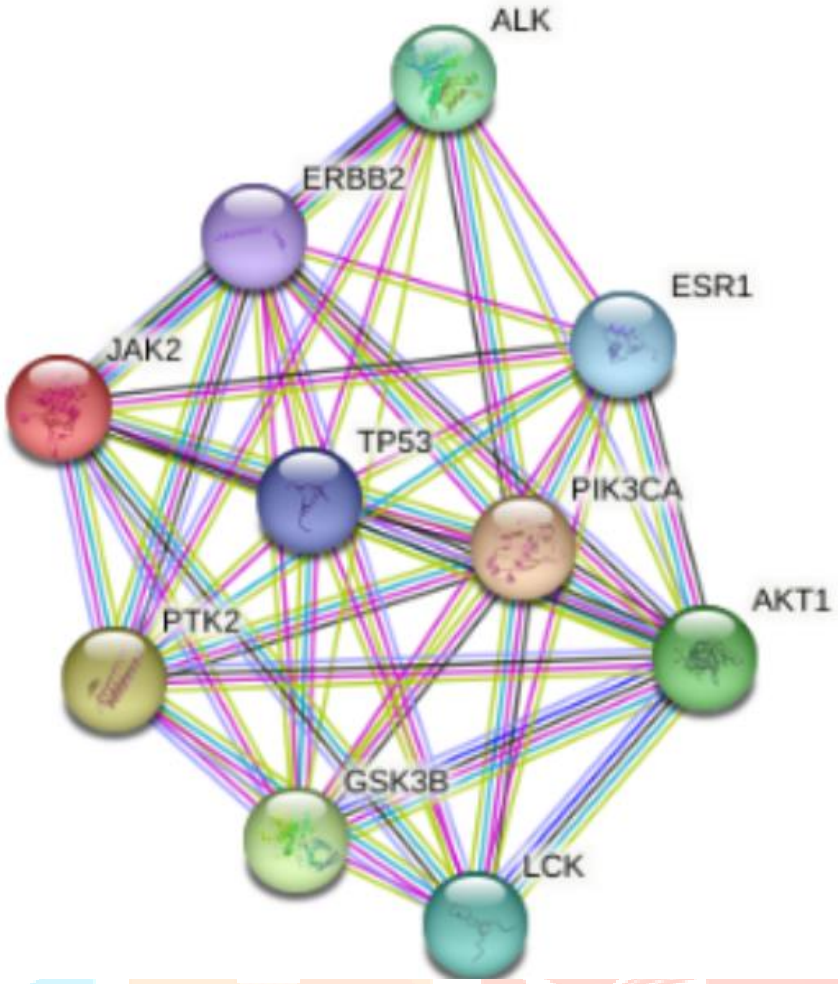


Fig No 11 String network

The image depicts the string interaction among the common genes including ALK, ERBB2, JAK2, ESR1, TP53, PIK3CA, PTK2, GSK3B, LCK and AKT1.

This network shows a complex interaction map of cancer-related proteins, with **TP53** at the center, indicating its critical role as a tumor suppressor and key regulatory hub. The proteins shown—such as **PIK3CA, AKT1, JAK2, ERBB2, ESR1, and ALK**—are well-known oncogenes or signaling proteins implicated in various cancers.

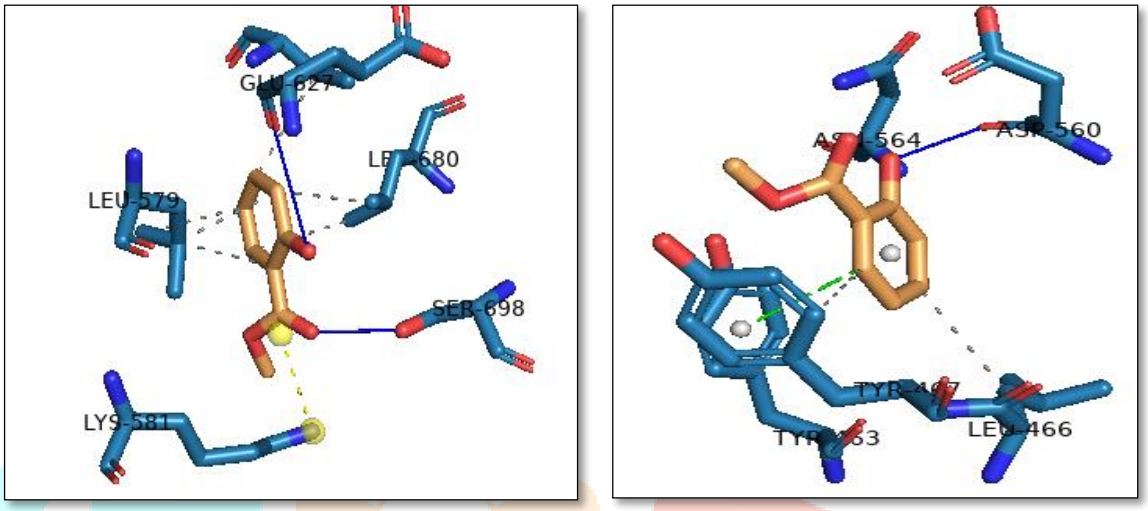
The dense interconnectivity reflects **significant crosstalk between pathways involved in cell growth, survival, apoptosis, and proliferation,** which are often dysregulated in cancer. Targeting these interaction hubs (especially **TP53, PIK3CA, and AKT1**) could be crucial for developing effective cancer therapies.

MOLECULAR DOCKING

Molecular docking is a computational method used in drug discovery and molecular biology to predict the preferred orientation of one molecule to a second molecule when bound to each other to form a stable complex. It is a key tool in structure based drug design. It involves stimulating the interactions between molecules such as proteins and ligands and predicting the most stable binding conformation.

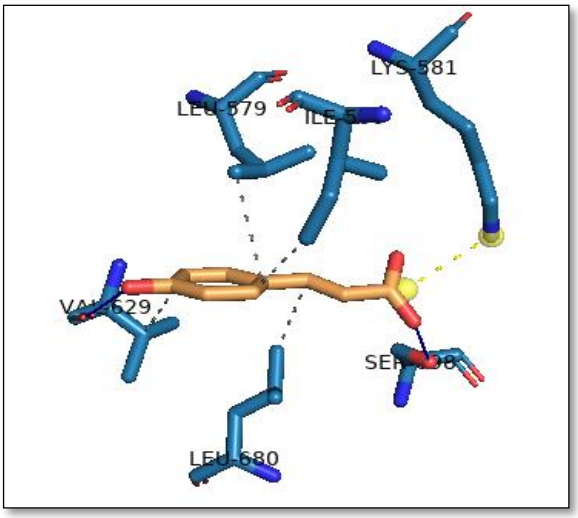
The chemical constituents present in *Viola tricolor* responsible for anticancer property includes Methyl salicylate, Para Coumaric acid, Isoorientin, Orientin and Vicenin 2. The binding affinity of methyl salicylate, Para Coumaric acid and Vicenin 2 with the receptors JAK2 and PIK3CA were analyzed using docking studies. MZ dock was the software used to perform the docking studies.

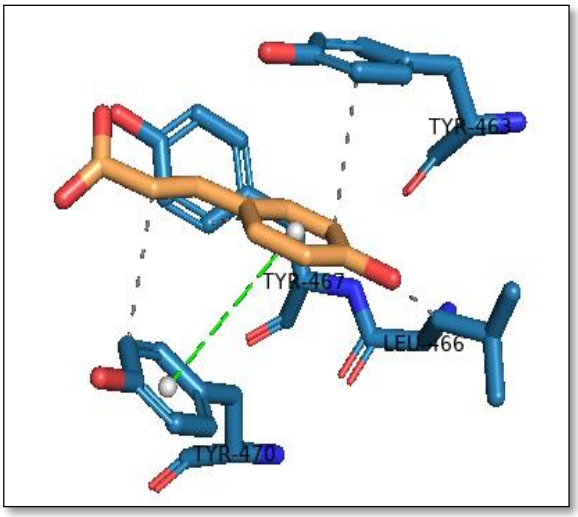
Molecular docking images and docking score of Methyl salicylate with the receptors JAK2 and PIK3CA



Receptors	Number of hydrogen bond interactions	Binding affinity (Kcal/mol)
JAK2	2	-6.2
PIK3CA	2	-6.6

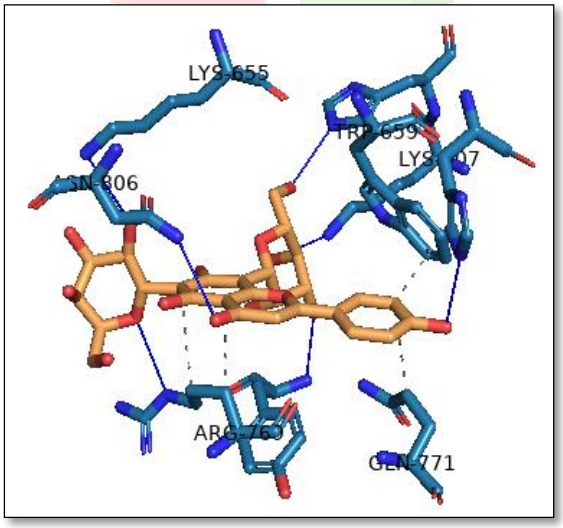
Molecular docking images and docking score of Para Coumaric acid with the receptors JAK2 and PIK3CA

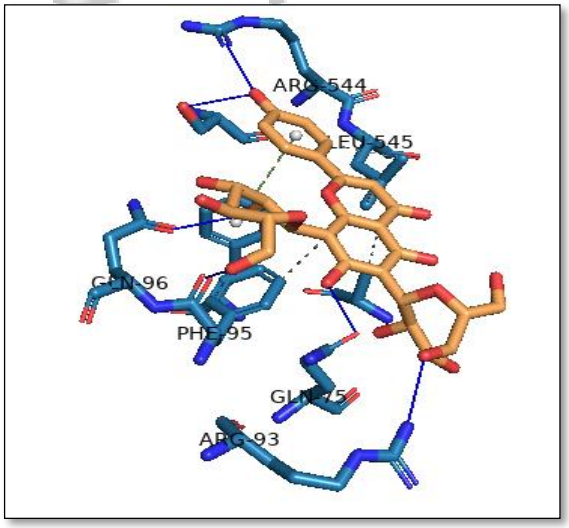




Receptors	Number of hydrogen bond interactions	Binding affinity (Kcal/mol)
JAK2	2	-6.7
PIK3CA	1	-6.3

Molecular docking images and docking score of Vicenin 2 with the receptors JAK2 and PIK3CA





Receptors	Number of hydrogen bond interactions	Binding affinity (Kcal/mol)
JAK2	7	-10.9
PIK3CA	6	-9.4

Among the chemical constituents Vicenin 2 show greater binding affinity with the receptors JAK2 and PIK3CA followed by Para Coumaric acid and Methyl salicylate.

DISCUSSION

The present study highlights the anticancer potential of *Viola tricolor* through a network pharmacology approach, providing valuable insights into its multi-target and multi-pathway mechanisms of action. Unlike conventional chemotherapeutic agents that often act on a single molecular target, network pharmacology embraces the complexity of cancer pathophysiology by exploring the interactions of multiple phytochemicals with interconnected signaling networks. This perspective is particularly relevant for cancer, a disease characterized by genetic heterogeneity, dysregulated pathways, and adaptive resistance mechanisms (Hopkins, 2008; Li & Zhang, 2013).

Phytochemical analysis of *Viola tricolor* has revealed the presence of flavonoids (violanthin, rutin), phenolic acids (caffeic acid, p-coumaric acid), cyclotides, and saponins, which are known to possess diverse pharmacological properties (Kähkönen et al., 1999; Campos et al., 2019). Many of these compounds have been reported in literature to exert cytotoxic, anti-proliferative, and pro-apoptotic effects against cancer cells (Panche et al., 2016; Desai et al., 2008). In silico predictions in this study further support these findings by demonstrating significant interactions of these bioactive molecules with key oncogenic and tumor suppressor proteins such as TP53, PIK3CA, AKT1, JAK2, and ERBB2. These targets play a central role in regulating apoptosis, cell cycle progression, angiogenesis, and metastasis—processes that are hallmarks of cancer (Hanahan & Weinberg, 2011).

The observed ability of *Viola tricolor* compounds to modulate multiple targets suggests its potential to overcome limitations of current single-target therapies, particularly drug resistance. For example, TP53 activation can induce apoptosis in tumor cells, while inhibition of PI3K/AKT signaling may suppress proliferation and survival mechanisms (Liu et al., 2009). Similarly, modulation of JAK2 and ERBB2 pathways could inhibit metastatic progression, which is a major cause of cancer-related mortality (Cai et al., 2019). Thus, the multi-component and synergistic nature of *Viola tricolor* offers a holistic therapeutic approach.

Another significant advantage of plant-derived therapies is their potential for reduced toxicity compared to conventional chemotherapeutics. However, the safety, pharmacokinetics, and bioavailability of *Viola tricolor* compounds need to be rigorously evaluated through preclinical and clinical studies before translation into therapeutic applications (Cragg & Newman, 2005). While in vitro and in silico findings are promising, in vivo validation remains essential to confirm the pharmacological relevance of these interactions in complex biological systems.

Overall, this study provides a scientific basis for the traditional use of *Viola tricolor* in herbal medicine and underscores its potential as a natural reservoir of anticancer agents. The network pharmacology approach not only validated its multi-target actions but also paved the way for future molecular docking, in vivo studies, and structure-activity relationship analyses to identify lead compounds with high therapeutic efficacy.

CONCLUSION

The study utilized a network pharmacology approach to explore the anticancer potential of *Viola tricolor*, uncovering its multi-target and multi-pathway therapeutic actions. The analysis revealed that bioactive compounds in *Viola tricolor* interact with key cancer-related proteins such as **TP53, PIK3CA, AKT1, JAK2, and ERBB2**, which are central nodes in critical signaling pathways governing cell proliferation, apoptosis, and metastasis.

The protein-protein interaction (PPI) network highlights the herb's ability to modulate complex biological systems, supporting its potential as a **promising source of natural anticancer agents**. These findings lay the groundwork for further experimental validation and pave the way for the development of *Viola tricolor*-derived compounds as complementary or alternative therapies in cancer treatment.

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