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Original Article

Mapping the Anti-Anaemic Signaling Landscape of *Rajanyadi Churna* Using Network Pharmacology-Driven Molecular Docking

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ABSTRACT

Background: Anaemia is a multifactorial hematological disorder influenced by impaired erythropoiesis, chronic inflammation, oxidative stress, and disturbed iron metabolism. *Rajanyadi Churna* is a well-known classical Ayurvedic polyherbal formulation, traditionally prescribed in *Pandu Roga* (anaemia), comprising diverse phytochemicals with possible hematinic, antioxidant, and immunomodulatory activities. **Objectives:** This study systematically explores anti-anaemic mechanisms of *Rajanyadi Churna* using an integrated network pharmacology and molecular docking approach. **Materials and Methods:** Phytoconstituents of *Rajanyadi churna* were collected from literature and public database. Targets of all the compounds was retrieved form the Swiss target predication and highly modulated targets were docked by using Schrodinger software. **Results:** A total of 79 phytoconstituents and 881 anaemia-related genes were screened, out of which 119 shared targets were obtained. PPI analysis highlighted highly connected hub genes including AKT1, IL6, TP53, STAT3, EGFR, MTOR, HIF1A, and TNF. Molecular Complex Detection (MCODE) clustering identified five significant functional modules associated with erythropoiesis, cytokine regulation, apoptosis, oxidative stress response, and iron homeostasis. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis highlighted HIF-1, PI3K-Akt, JAK-STAT, MAPK, glutathione metabolism, and NF-κB signaling as key therapeutic pathways modulated by the formulation. Network construction of compound-target-pathway positioned Apiole and EGFR as central nodes, reflecting a high level of polypharmacological interaction. Results of molecular docking depicted strong binding affinities of several phytoconstituents toward EGFR, wherein Rhoifolin (-12.809) and Taxifolin-3-glucoside (-10.846) outperformed standard inhibitors in specific interactions. **Conclusion:** Overall, the findings highlight that *Rajanyadi Churna* exerts anti-anaemic effects through a synergistic multi-target mechanism.

Keywords: Anaemia, Molecular docking, Network Pharmacology, *Rajanyadi Churna*.

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INTRODUCTION

Anaemia remains one of the most challenging and widespread public health concerns, as this condition is estimated to affect 1.8 billion individuals worldwide and to significantly contribute to morbidity, reduced cognitive performance, impaired immunity, and diminished quality of life (Dhonde *et al.*, 2023; Safiri *et al.*, 2021). Defined primarily by a reduction in the concentration of haemoglobin, haematocrit, or Red Blood Cell (RBC) mass, anaemia develops from diverse etiologies such as nutritional deficiencies, chronic infectious diseases, inflammation-driven erythropoietic suppression, genetic abnormalities, and impaired iron metabolism (Chaparro and Suchdev, 2019; Alli *et al.*, 2017). Iron Deficiency Anaemia (IDA) represents the most common subtype; however, Anaemia of Chronic Disease (ACD), hemolytic anaemia, and anaemia precipitated by oxidative stress and inflammation continue to surge, especially in low- and middle-income countries (Obeagu *et al.*, 2025; Sankar and Villa 2021). Conventional therapeutic regimens include oral iron salts, parenteral iron preparations, erythropoiesis-stimulating agents, and micronutrient supplementation, which, though effective in many instances, often exhibit drawbacks related to gastrointestinal intolerance (Iolascon *et al.*, 2024), poor bioavailability (Kolars *et al.*, 2025), oxidative mucosal injury, risk of iron overload, or high cost (Muñoz *et al.*, 2018). These issues have spurred growing interest in traditional multi-component herbal formulations that possess wide pharmacological spectra and can manage the multifaceted nature of anaemia.

Rajanyadi Churna is a classical Ayurvedic polyherbal powder formulation that is traditionally indicated for *Pandu Roga*, a disease entity analogous to anaemia described in the Ayurvedic

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texts. The formulation finds its place in authoritative sources like *Bhaishajya Ratnavali* (*Pandu Roga Prakaran*) and *Ayurveda Sara Sangraha* and *Astanga Hridaya* (Uttara sthana). *Rajani* or *Curcuma longa* is the principle ingredient that imparts not only the name but also a significant contribution to its therapeutic activity. The general ingredients for *Rajanyadi Churna* are *Curcuma longa* (Rajani), *Cedrus deodara* (*Deva daru*), *Pinus roxburghii* (*Sarala*), *Scindapsus officinalis* (*Shreyasi*), *Solanum indicum* (*Brihati*), *Solanum xanthocarpum* (Kantakari), *Uraria picta* (Prishnaparni), *Anethum sowa* (Shatahva), though minor variations exist in regional practice. These herbs are well-recognized in Ayurvedic pharmacopeia for their *rasayana, deepana-pachana, rakta-vardhaka*, and *shothahara* activities (Jantan *et al*., 2021).

Phytochemical studies on these botanicals have yielded a wide array of bioactive principles comprising curcuminoids, Apiole, gallic acid, diosgenin, piperine, flavonoids, tannins, and terpenoids (Chaudhary *et al*., 2015; Bhardwaj *et al*., 2022; Lorinczova *et al*., 2022). Most of these molecules have demonstrated pharmacological activities related to anaemia: curcumin exhibits antioxidant and anti-inflammatory action; berberine controls hepcidin expression, increases iron absorption, and decreases inflammation-related cytokines; tannins and phenolic acids are involved in the protection against oxidative stress; and piperine increases the bioavailability of co-administrated phytochemicals (Zhai *et al*., 2025). More importantly, the synergistic interaction of these multiple phytoconstituents may explain the wide hematinic and cytoprotective action ascribed to *Rajanyadi Churna* in classical Ayurvedic texts. Despite significant traditional usage, the mechanistic basis for the anti-anaemic activity of *Rajanyadi Churna* has been poorly explained using modern scientific perspectives. Being polyherbal in nature, it is difficult to attribute therapeutic outcomes of Ayurvedic preparations to any single constituent. Here also, therapeutic action frequently arises from multi-target, multi-pathway interactions of various phytoconstituents. Conventional reductionist pharmacology is incapable of mapping such complex interactions; therefore, systems-level approaches like network pharmacology are increasingly important for explaining Ayurvedic formulations.

Network pharmacology therefore integrates phytochemical profiling, target prediction, protein-protein interaction networks, signaling pathway enrichment, and disease-gene association to construct a holistic mechanistic view of multi-component therapeutics (Obeagu, 2025). This approach aligns well with the basic Ayurvedic principle wherein diseases arise from system-wide imbalances rather than from isolated molecular defects (Ye *et al*., 2020). In the context of anaemia, for instance, several biological processes such as iron absorption and transport, erythropoietin signaling, heme biosynthesis, inflammation modulation, redox balance, mitochondrial function, and hypoxia adaptation are simultaneously implicated (Gudasi *et al*., 2024). Network pharmacology thus allows the systematic mapping of how the diverse phytoconstituents in *Rajanyadi Churna* impinge on these pathways. Complementing these network pharmacology findings, molecular docking offers atomistic insight into the binding potential and interaction strength of phytochemicals against key anaemia-associated proteins. Docking-based interaction analysis helps identify lead compounds with strong binding affinity, potential modulatory effects, and relevance to anaemia-associated pathways (Gudasi *et al*., 2023). The present study, through an integrated approach, aspires to deconstruct the complex mechanistic matrix through which *Rajanyadi Churna* exerts its therapeutic potential.

The aim of this work is to provide a comprehensive mechanistic profile of *Rajanyadi Churna* in anaemia by connecting classical Ayurvedic knowledge with modern computational approaches. Using network pharmacology and molecular docking, the paper aims to identify major bioactive metabolites, core target proteins, important signaling pathways, and potential synergy. Such an approach gives scientific credence to traditional uses of *Rajanyadi Churna* and forms the modern, evidence-based rationale for its therapeutic applicability in anaemia. This work therefore has helped bridge traditional medicine with modern pharmacology for eventual validation through future *in vitro, in vivo*, and clinical studies.

MATERIALS AND METHODS

Preparation of *Churna*

The preparation of *Rajanyadi Churna* was carried out following standard Ayurvedic pharmaceutics procedures. High-quality, authentic raw herbs were sourced from a certified supplier to ensure purity and pharmacognostic integrity. All ingredients were thoroughly cleaned and washed to remove dust and foreign particles, then shade-dried completely to prevent moisture retention. Each dried herb was powdered separately using a mechanical grinder and passed through a fine mesh sieve (typically #80) to obtain a uniform particle size. The sieved powders of all eight ingredients were then combined in equal proportions and mixed thoroughly to achieve a homogeneous blend. The final *churna* was transferred to a clean, dry, airtight container and stored in a cool, moisture-free environment to preserve its stability and therapeutic potency.

Computational studies

Identification of Metabolites and Their Targets

The phytoconstituents of *Rajanyadi Churna* were systematically compiled from academic publications, classical Ayurvedic literature, and phytochemical databases such as IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics) and Dr. Duke's Phytochemical Database. PubChem CIDs for each reported compound were obtained from the PubChem database, and their corresponding SMILES (Simplified Molecular Input

Line Entry System) structures were curated for computational analysis. Potential protein targets of these phytoconstituents were predicted using the SuperPred tool based on SMILES input, and the resulting target proteins were standardized using the UniProt database, restricting entries to *Homo sapiens* for biological relevance (Pote *et al.*, 2025).

Intersection Analysis of Phytoconstituent and Anaemia-Related Targets

Anaemia-associated genes were retrieved from the GeneCards database, and the overlapping targets shared between the predicted compound targets and disease-related genes were identified using the Venny 2.0 online tool. This intersection provided the set of common therapeutic targets potentially modulated by the bioactive constituents of *Rajanyadi Churna*.

Protein-protein interaction and Network construction

Protein-Protein Interaction (PPI) analysis was conducted using the STRING database by incorporating the common targets shared between *Rajanyadi Churna* phytoconstituents and anaemia-related genes, applying a confidence threshold of 0.400 and excluding isolated nodes. The resulting PPI network was imported into Cytoscape 3.10.2 for detailed topological and centrality analysis, where nodes represented target proteins and edges denoted functional interactions. Cluster identification was performed using the MCODE plugin to detect highly interconnected subnetworks. Additionally, a compound-target network was constructed in Cytoscape 3.10.2 to visualize interactions between the phytocompounds and their predicted targets. Network Analyzer was used to compute key parameters, including degree, betweenness, eccentricity, neighborhood connectivity, and in-/out-degree distributions. Nodes in this integrated network represented active compounds, target genes, and enriched pathways, while edges reflected their interactions, enabling evaluation of node importance based on degree values. This comprehensive network analysis provided insights into the multi-target and multi-pathway pharmacological behaviour of *Rajanyadi Churna* (Gudasi *et al.*, 2025).

Gene Ontology and KEGG Pathway Enrichment Analyses for Anaemia Using Clue GO and Clue Pedia Plugins.

Gene Ontology (GO) and KEGG pathway enrichment analyses were performed using ClueGO (https://apps.cytoscape.org/apps/cluego) and CluePedia (https://apps.cytoscape.org/apps/cluepedia) within the Cytoscape platform. ClueGO facilitated the visualization and functional interpretation of gene-related biological processes and pathways. It applies statistical methods, such as the hypergeometric test, to evaluate the significance of enrichment, helping to pinpoint key biological processes and pathways associated with the dataset. Additionally, CluePedia was integrated with ClueGO to provide deeper insights into the functional networks. This tool enhanced the analysis by uncovering potential novel biomarkers and illustrating connections between genes and pathways, offering a more comprehensive understanding of the biological significance of the results (Gharge *et al.*, 2025; Kambalyal *et al.*, 2024; Desaipatti *et al.*, 2025).

Molecular Docking

Protein preparation

The molecular docking study was conducted using Schrödinger's Small Molecule Drug Discovery Suite 2023-1, utilizing the crystal structures of the EGFR tyrosine kinase domain obtained from the Protein Data Bank (PDB ID:1M17). The selected phytoconstituents were sketched in Maestro and processed through LigPrep to generate relevant tautomers and stereoisomers, correct chiral centers, and perform energy minimization with the OPLS4 force field, while protonation states were adjusted at pH 7.0±0.5 using Epik. Protein preparation was executed using the Protein Preparation Wizard, which involved adding missing hydrogens, assigning proper bond orders, correcting mislabeled atoms, forming disulfide bonds, and removing crystallographic water molecules beyond 5 Å from heteroatoms. Protonation of ionizable residues was performed at pH 7.0 using Epik, followed by optimization of the hydrogen-bonding network. Finally, restrained minimization of the protein structure was carried out with the OPLS4 force field until the heavy-atom RMSD converged to 0.3 Å, ensuring a fully optimized receptor model for docking (Patil, 2025).

Grid generation

EGFR exhibits a bilobed kinase architecture comprising the N-lobe (β-strands and αC-helix) and the larger C-lobe (predominantly α-helical). These lobes form a cleft that constitutes the ATP/erlotinib binding pocket. To define this active site region, a receptor grid was generated using the SiteMap module within Schrödinger's Glide suite, allowing accurate characterization of the ligand-accessible binding environment (Maledavar *et al.*, 2025).

Ligand-Protein docking

Docking simulations for all prepared ligands were executed using the Glide XP (Extra Precision) algorithm. During docking, van der Waals radii of ligand nonpolar atoms were scaled by 0.80, with a partial charge cutoff of 0.15, enabling improved ligand flexibility and interaction sampling. Up to ten binding poses were generated per ligand, and the highest-ranking poses-based on Glide scores were selected for downstream interaction analysis. Each docked complex was visually inspected to assess critical interactions, including hydrogen bonds, hydrophobic contacts, and π-π stacking, within the defined EGFR binding pocket (Sepay *et al.*, 2022).

Statistical Analysis

Functional enrichment analysis was performed using the ClueGO plugin in Cytoscape, where enrichment significance was evaluated using the hypergeometric test. P-values were adjusted for multiple comparisons using the Bonferroni step-down method, and terms with an adjusted p-value < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Physico chemical charcterstics of churna

The quality evaluation of *Rajanyadi Choornam* (Batch No. 24C1602) demonstrated full compliance with Ayurvedic Pharmacopoeia of India (API) standards. The formulation appeared as a yellow, fine powder with a characteristic herbal odour and a salty-bitter taste. The pH of the 10% solution was 5.66, falling within the prescribed range of 5.30-6.30. Physicochemical parameters were within acceptable limits, with loss on drying recorded at 5.11% (NMT 6%), total ash at 5.78% (NMT 7%), acid-insoluble ash at 2.48% (NMT 3%), water-soluble extractive at 13.53% (NLT 8%), and alcohol-soluble extractive at 1.13% (NLT 0.50%). Microbial load was well within permissible limits, showing total aerobic count of 25 × 10^2 cfu/g (NMT 10^5) and yeast-mould count of 27 cfu/g (NMT 10^3). Pathogenic microorganisms including *E. coli, Salmonella* spp, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were absent. Overall, the sample met all physicochemical and microbiological quality specifications, confirming its suitability for safe therapeutic use.

Network Pharmacology

Mining of phytoconstituents and targets

A comprehensive review of literature and database resources revealed 79 chemical components of *Rajanyadi Churna*. The chemical composition of all 27 components was confirmed by using Pub chem software and these compounds found to act on 501 host targets.

Selection of targets

A total of 881 genes were retrieved from the GeneCard databases using "anaemia" as a keyword. These were compared with the target genes of the active ingredients, identifying potential anaemia target genes for each ingredient through Venny 2.0. After comprehensive analysis of *Rajanyadi Churna* potential anaemia targets, 119 candidate targets were selected (Figure 1), suggesting that all components may have a therapeutic effect on anaemia through these targets.

Protein-Protein interaction and cluster analysis

Protein-protein interaction network of (Rajanyadi Churna) - anaemia regulated genes

The 119 common targets were imported into the STRING database, and 6 unconnected nodes were discarded. The PPI network comprised 119 nodes and 1804 edges, with an average node degree of 30.3 and an average local clustering coefficient of 0.673 (Figure 2). The PPI network was then exported to Cytoscape 3.10.2 for further analysis.

Cluster networks and hub genes identification using MCODE plugin for anaemia regulated genes

MCODE analysis was performed to identify highly interconnected protein subnetworks within the *Rajanyadi Churna*-anaemia interaction network, providing deeper insight into its core therapeutic mechanisms (Figure 3). Five significant clusters emerged, with Cluster 1 (Score 35.05) representing the central functional module containing key regulatory proteins such as AKT1, TP53, TNF, IL6, EGFR, STAT3, MTOR, and HIF1A. These nodes are closely associated with red blood cell formation, control of inflammation, cutting oxidative stress, and survival signaling. This may infer that *Rajanyadi Churna* acts by rebalancing the process of blood formation and lessening anemia of cytokines driven. This cluster consists of a group of mediators for inflammation-like IL1B, MPO, SERPINE1, and metabolic regulators such as IDH1 and PKM (Table 1). The third to the fifth clusters relate to lysosomal functions and coagulation pathways, leading to efficient iron management and stabilized coagulation. Overall, the MCODE analysis shows the wide, multi-targeting influence of *Rajanyadi Churna* across pathways in mitigating anemia.

Gene Ontology (GO) enrichment analysis

The integrated Gene Ontology (GO) analysis reveals that the therapeutic response in anaemia arises from a systems-level

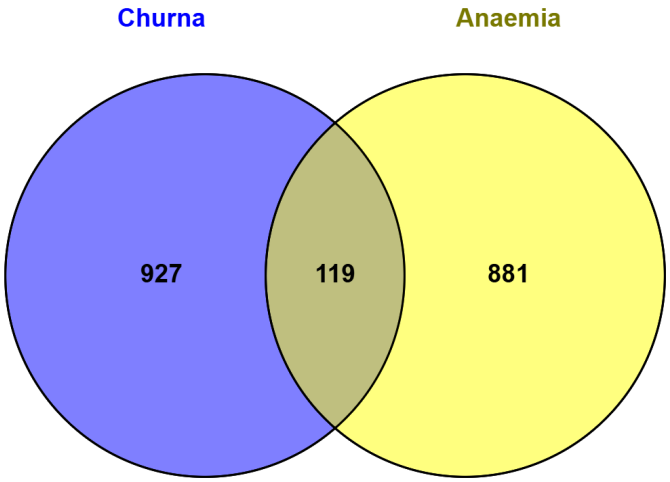

Figure 1: Intersection analysis of phytoconstituents and Anaemia related to targets like mapping by Venny 2.0.

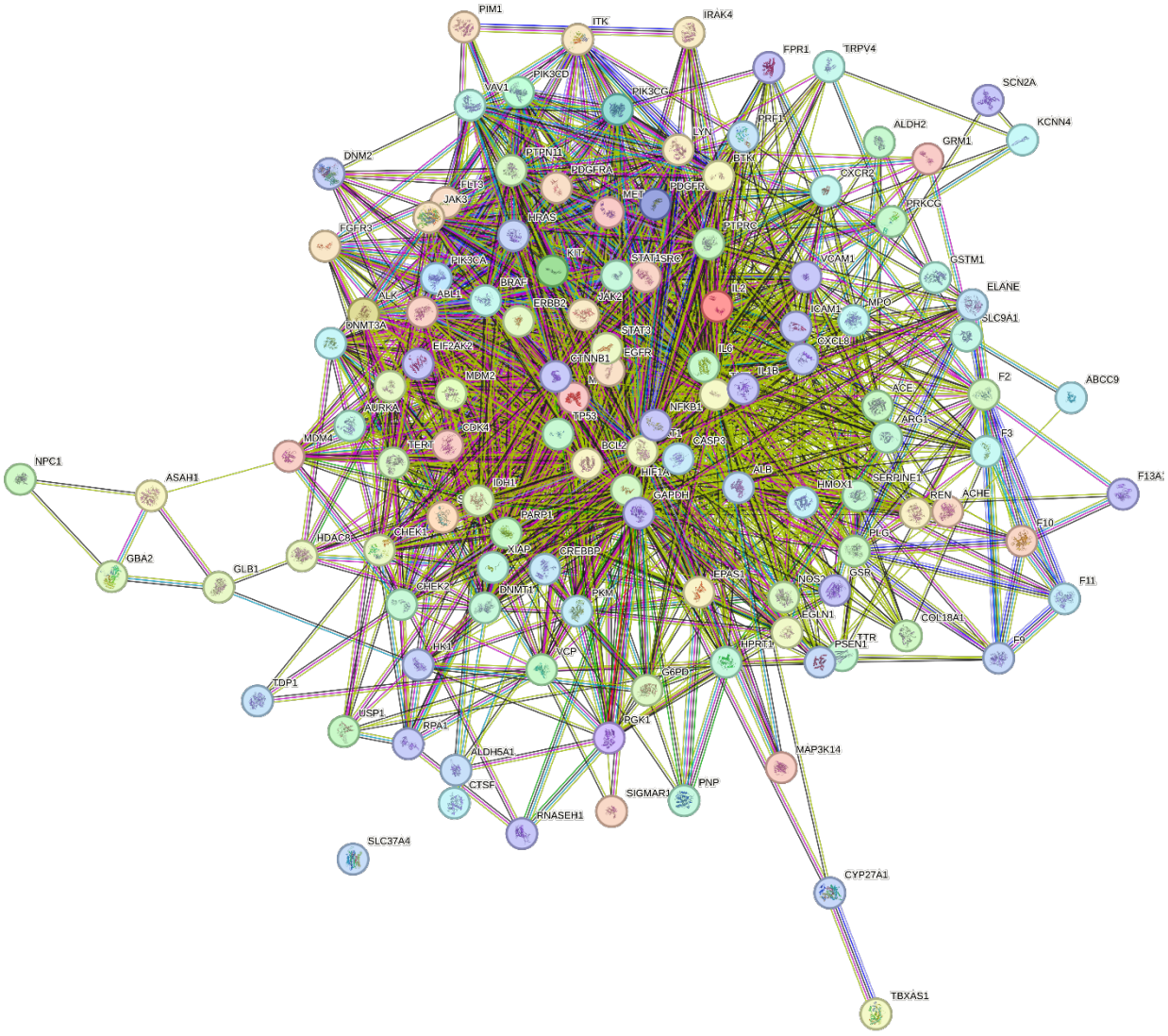

Figure 2: The Protein protein interaction of common targets between phytoconstituents and anaemia targets.

Table 1: MCODE cluster analysis PPI network.

Cluster	Score	Nodes	Edges	Node IDs
1	35.05	41	701	AKT1, GAPDH, TP53, TNF, IL6, EGFR, STAT3, BCL2, HIF1A, ALB, NFKB1, CASP3, SRC, CTNNB1, ERBB2, MTOR, JAK2, IL2, STAT1, KIT, MDM2, PTPRC, HRAS, CXCL8, PIK3CA, EZH2, ABL1, ICAM1, PARP1, PTPN11, PDGFRB, BRAF, TERT, CDK4, PDGFRA, MET, FLT3, HMOX1, EPAS1, DNMT1, XIAP
2	6.083	25	73	IL1B, IDH1, VCAM1, MPO, SERPINE1, LYN, CREBBP, PKM, CHEK1, PLG, JAK3, PIK3CD, PIK3CG, BTK, ACE, DNMT3A, ALK, CXCR2, ARG1, SHH, REN, AURKA, NOS2, CHEK2, PRF1
3	3.333	4	5	GLB1, NPC1, ASAH1, GBA2
4	3	3	3	F2, F3, ELANE
5	3	3	3	F9, F10, F11

interplay of biological processes, molecular functions, and cellular localizations that collectively restore erythropoiesis, iron balance, and hematopoietic stability (Figure 4). Enrichment of biological processes such as response to stress, oxidative injury, chemicals, and oxygen-containing compounds highlights the role of cytoprotective genes like HMOX1, EPAS1, EGLN1, TP53, STAT3, IL6, and AKT1 in mitigating oxidative damage, regulating hypoxia pathways, and enhancing erythropoietin-driven red blood cell formation. Simultaneously, the regulation of cell death and apoptosis through key mediators including BCL2, CASP3, JAK2, PIK3CA, and MTOR ensures improved survival and maturation of erythroid precursors, counteracting the excessive apoptosis commonly observed in anaemia. Complementing this, immune-associated processes governed by IL2, TNF, PRF1, MAP3K14, and PTPRC help suppress chronic inflammation, thereby restoring normal hematopoietic activity. The Cellular

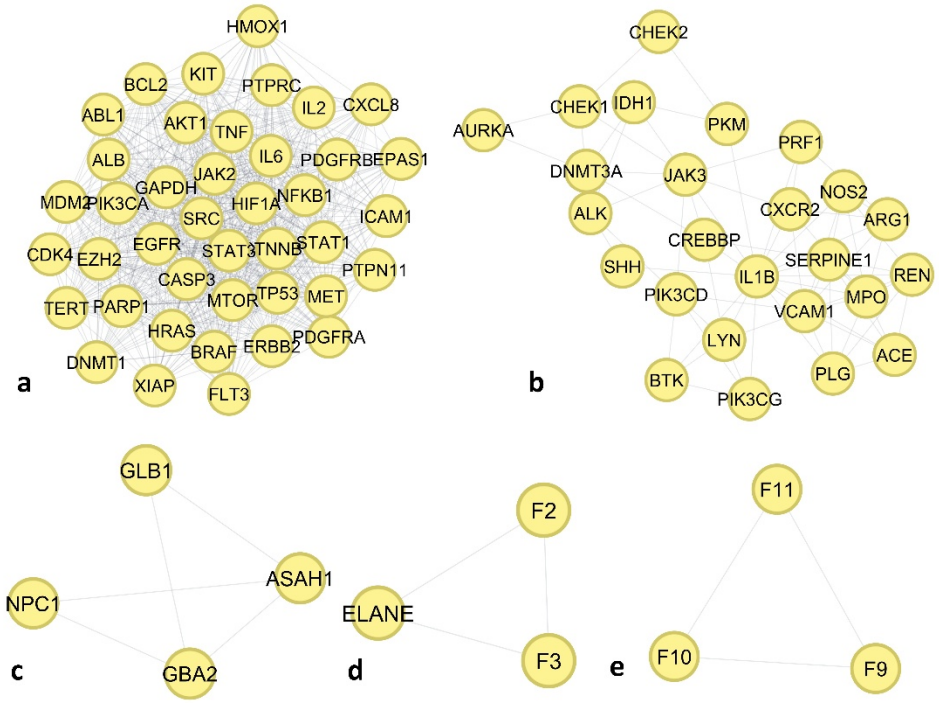

Figure 3: Cluster analysis of *churna* PPI network.

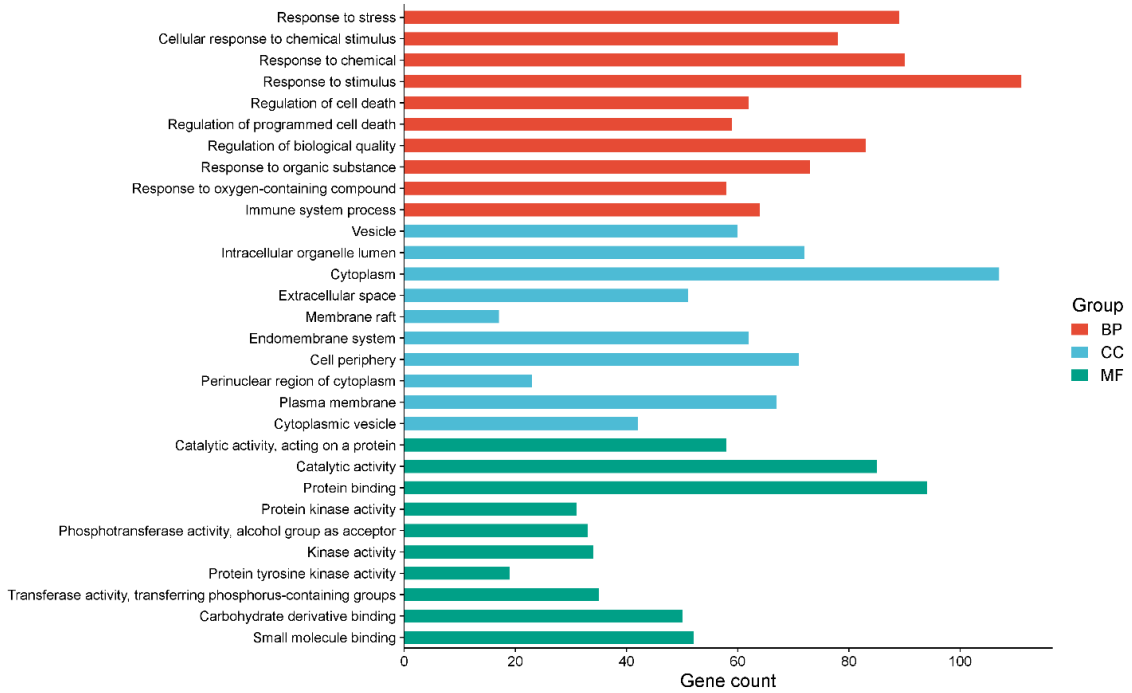

Figure 4: Gene ontology enrichment analysis.

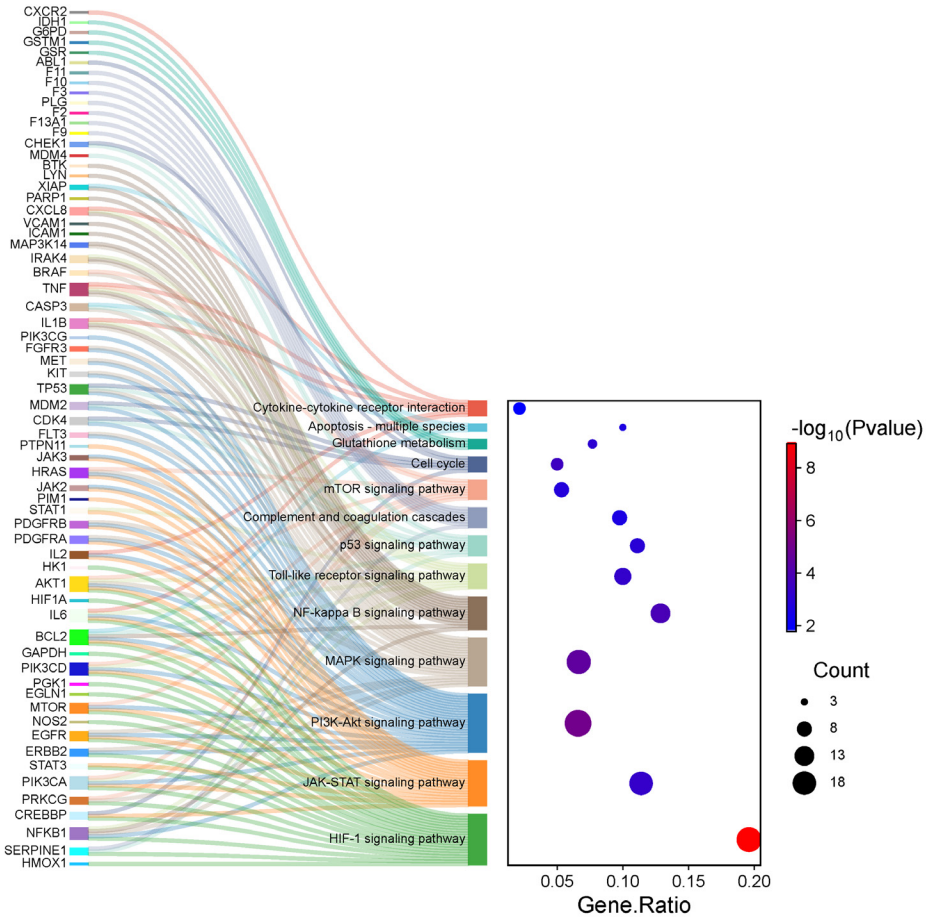

Figure 5: KEGG pathway analysis of selected signaling pathways involved in the treatment of Anaemia.

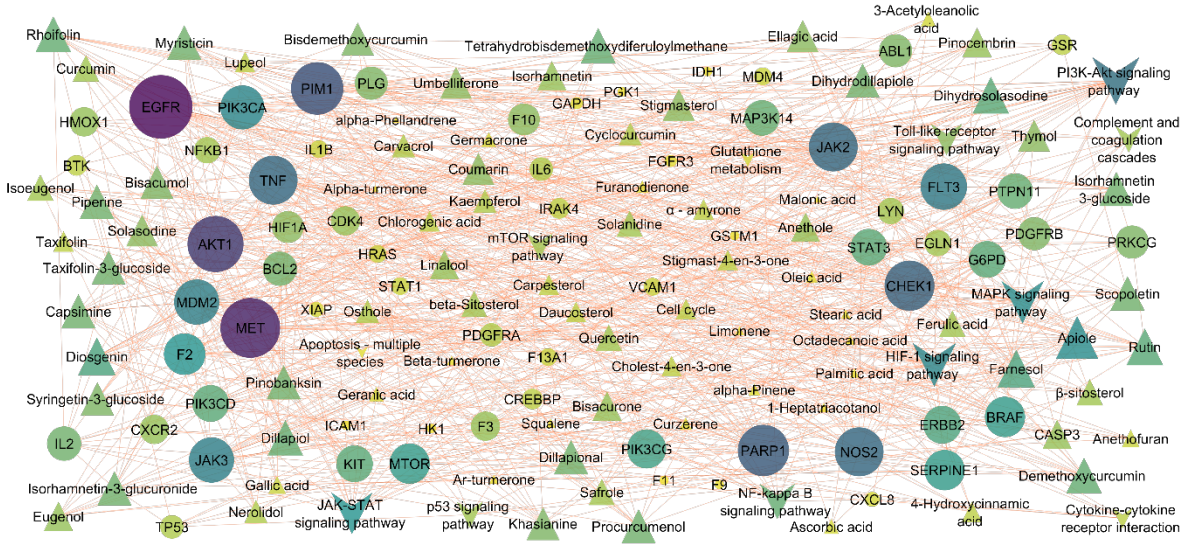

Figure 6: Network Construction between compounds, Targets, and Signaling Pathways.

Component enrichment shows that these regulatory proteins localize across essential compartments cytoplasm, plasma membrane, intracellular vesicles, lysosomes, nucleus, and extracellular space supporting coordinated iron recycling, receptor-mediated erythropoietic signaling, redox homeostasis, and controlled inflammatory responses. Molecular Function enrichment further emphasizes dominant catalytic activities, kinase functions, transferase actions, and protein/small molecule

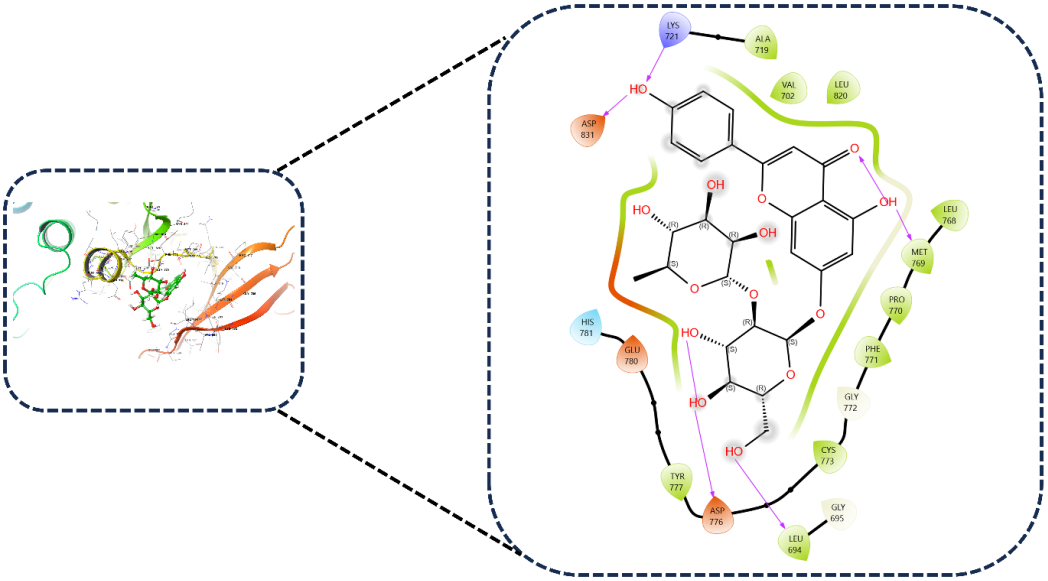

Figure 7: Binding orientation of Rhoifolin in the ligand binding domain of EGFR.

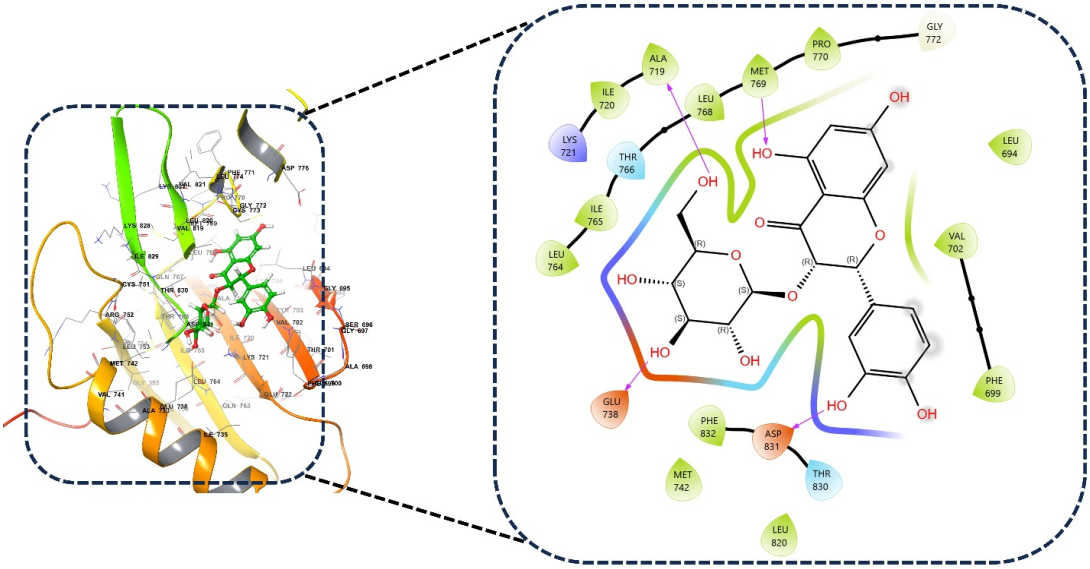

Figure 8: Binding orientation of Taxifolin-3-glucoside in the ligand binding domain of EGFR.

binding, involving enzymes and signaling proteins such as GSR, PKM, DNMT1, PARP1, KIT, FLT3, EGFR, and BTK, which activate core pathways like PI3K-Akt, JAK-STAT, and MAPK to drive erythroid proliferation and differentiation. Enhanced glycolytic and metabolic enzyme activity (HK1, PGK1) provides the energy needed for rapid erythroblast expansion. Together, these GO attributes depict a harmonized therapeutic mechanism in which oxidative stress reduction, hypoxia adaptation, anti-inflammatory action, metabolic regulation, and powerful kinase-driven signaling converge to re-establish healthy red blood cell production and effectively ameliorate anaemia.

KEGG Pathway Enrichment

KEGG pathway enrichment points to several mechanisms by which particular pathways work in the combined treatment of anaemia (Figure 5). One of the major pathways that are implicated in the treatment of anaemia relates to the HIF-1 pathway (hsa04066) where several genes like HMOX1, EGLN1, HIF1A, IL6, AKT1, MTOR, and PGK1 are entangled in regulating the cellular response to hypoxia by inducing mechanisms of red blood cell production. On the other hand, pathways such as JAK-STAT pathway (hsa04630) and PI3K-Akt pathway (hsa04151) are major in the proliferation of hematopoietic stem cells. Various genes like IL2, STAT3, PDGFRA, JAK2, BCL2, and EGFR are intertwined in different mechanisms of differentiating red blood cells in addition to the mechanisms employed by JAK-STAT in

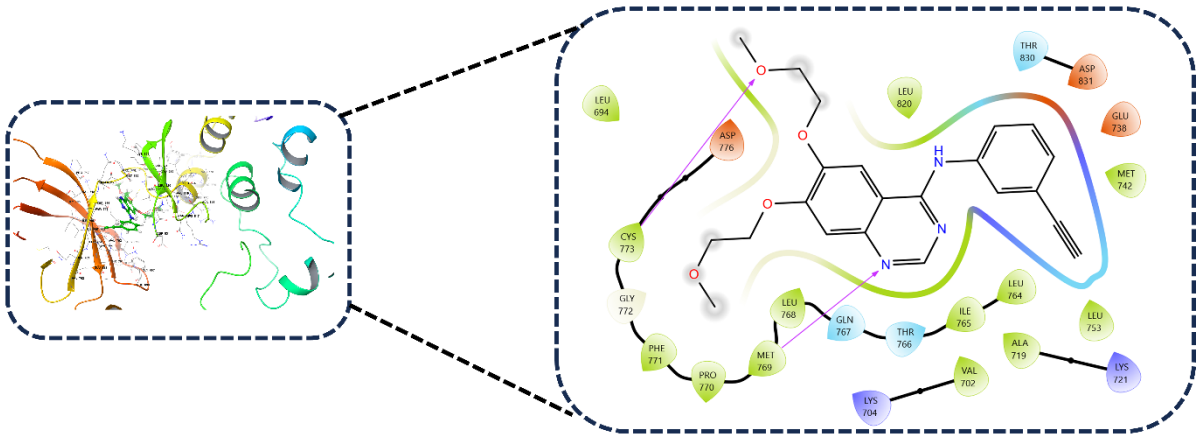

Figure 9: Binding orientation of Co-crystal in the ligand binding domain of EGFR.

shielding progenitor cells from apoptosis. In contrast, pathways such as the MAPK pathway (hsa04010) are vital in stimulating the proliferation of red blood cells. Inflammation-related pathways like NF-κB signaling pathway (hsa04064) and Toll-like receptor signaling pathway (hsa04620) are important in managing the equilibrium of different cytokines like IL1B, TNF, and CXCL8. Apart from that, pathways like p53 signaling pathway (hsa04115) and Apoptosis pathway (hsa04215) are intertwined in regulating the mechanisms of shielding different precursors against apoptosis by different genes like CASP3, TP53, and BCL2. On the other hand, pathways like the mTOR pathway (hsa04150) are important in different mechanisms related to regulating the level of different nutrients. Other pathways such as Complement and coagulation cascades pathway (hsa04610) and Glutathione metabolism pathway (hsa00480) are important in the mechanisms by which different pathways regulate the basic mechanisms related to the stabilization of the blood vessels and decreasing oxidative stress, in addition, in managing different pathways related to the treatment of cancer.

Network Construction between Bioactives, Shared Key Targets, and Signalling Pathways

In an effort to assess unambiguously the molecular mechanism of action of *Rajanyadi Churna* against anaemic disorders, a compound-target-pathway interaction map was designed using Cytoscape 3.10.3 (Figure 6). The compound-target-pathway map with 157 nodes and 775 edges succinctly explains the complex interconnectivity between bioactively functioning phytoconstituents, target genes, and participating signaling pathways. In this graphical representation, triangle, ellipse, and V-shaped nodes respectively indicate functional compounds, target genes, and significantly enriched pathways. The graph not only clearly explains the multi-target, multi-pathway pharmacological action of *Rajanyadi Churna*, wherein compounds target multiple genes, and multiple genes take part in multiple signal transduction, but it can be seen clearly that PI3K-Akt and HIF 1 signal pathway stand at nodal points of graphs, thereby interact with 23 and 20 genes, respectively, to represent pivotal involvement in reducing anaemic disorders using *Rajanyadi Churna*. In 79 identified phytoconstituents, connectivity with 19 major proteins, namely EGFR, MET, AKT 1, PIM 1, PARP 1, and CHEK 1, and pivotal involvement in thwarting anaemic disorders through PI3K-Akt and HIF 1 signal pathway, is sole property of Apiole. Moreover, EGFR acts as major target protein, wherein 29 compounds interact with EGFR to generate major anaemic signals through six prominent signal pathways.

Molecular Docking

To determine the docking poses and interaction characteristics of the bioactive compounds present in *Rajanyadi Churna*, molecular docking analysis was conducted. The results revealed that all scrutinized metabolites had a positive non-bonding interaction inside the EGFR active pocket. Among them, Rhoifolin had the highest binding affinity, indicated by the highest docking score of -12.809 kcal/mol and most favorable glide energy of -64.212 kcal/mol. Rhoifolin established strong hydrogen bonding interactions with essential amino residues LYS721, ASP831, ASP776, LEU694, and MET769, shown in Figure 7. In order, the closest compound was found to be Taxifolin-3-glucoside, with a docking score of -10.846 and glide energy of -50.554 kcal/mol, establishing hydrogen bonding ties with amino residues ASP831, ALA719, GLU738, and MET769, shown in Figure 8. Validation of docking approaches was carried out by redocking a ligand of EGFR (PDB ID: 1M17), which had a docking score of -12.809 and established characteristic ligand interactions with MET769 and CYS773, shown in Figure 9. In addition, eight plant components had a superior and comparative affinity with a commercial EGFR inhibitor, named osimertinib, explained in detail in Table 2. These results suggest that some bioactives of *Rajanyadi Churna* had great potential for interacting with EGFR and show potential characteristics of enhancing hematopoietic and anti-anaemic pathways.

Table 2: Binding affinity and interaction results of bioactives in the ligand binding domain of EGFR (4ZAU) protein.

Compound	Docking Score	Glide Energy (kcal/mol)	Ligand atom interaction (H-Bond)/ π-cation
Rhoifolin	-12.809	-64.212	-OH of ligand to ASP 776 -OH of ligand to LEU 694 -OH of ligand to MET 769 -OH of ligand to ASP 831 -OH of ligand to LYS 721 -O of ligand to MET 769
Taxifolin-3-glucoside	-10.846	-50.554	-OH of ligand to ASP 831 -OH of ligand to MET 769 -OH of ligand to GLU 738 -OH of ligand to ALU 719
Rutin	-10.597	-70.11	-OH of ligand to ASP 776 -OH of ligand to ASN 818 -OH of ligand to MET 769 -OH of ligand to ASP 831 -OH of ligand to GLU 738
Quercetin	-10.06	-44.859	-OH of ligand to ASP 831 -OH of ligand to MET 769 -O of ligand to MET 769
Syringetin-3-glucoside	-10.041	-62.385	-OH of ligand to ARG 817 -OH of ligand to ASN 818 -OH of ligand to THR 830 -OH of ligand to ASP 831 -OH of ligand to LYS 721 -OH of ligand to ALA 719
Taxifolin	-9.752	-45.207	-OH of ligand to GLU 738 -OH of ligand to MET 769 -OH of ligand to THR 766 -O of ligand to MET 769
Isorhamnetin 3-glucoside	-9.371	-55.395	-OH of ligand to ARG 817 -OH of ligand to ASN 818 -OH of ligand to ASP 831 -OH of ligand to GLU 738 -OH of ligand to MET 769 -O of ligand to THR 830
Co-crystal	-9.331	-65.367	-N of ligand to MET 779 -O of ligand to CYS 773

CONCLUSION

The current research work offers a broad mechanistic insight into the activity of *Rajanyadi Churna* in the treatment of anaemia using systemic computational tools and approaches. Network pharmacology identified 119 core targets related to erythropoiesis, inflammation, oxidative stress, and cell survival, proving the multi-compound, multi-target character of the formulation. Dominant pathways like HIF-1, PI3/Akt, JAK/STAT, NF-κB, and MAPK indicate the role of the formulation in the simultaneous augmentation of RBC production and the inhibition of inflammatory suppression and oxidative damage, which are primary pro-anaemic mediators in the conditions' development. Molecular docking also confirmed the binding ability of the active phyto-compounds, and Rhoifolin, Taxifolin-3-glucoside, and Rutin proved the strongest binders to EGFR and other hematopoietic targets. The results overall indicate the significant scientific validity of the traditional usages of the *Rajanyadi Churna* formulation as a potential anaemia-relieving agent, suggesting the initiation of future research work on the formulation in consequence.

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ABBREVIATIONS

ACD: Anaemia of Chronic Disease; **API:** Ayurvedic Pharmacopoeia of India; **EGFR:** Epidermal Growth Factor Receptor; **IDA:** Iron Deficiency Anaemia; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **PPI:** Protein-Protein Interaction.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Dr. Savita Sunadholi conceptualized the study and performed network pharmacology analysis. Drafted the manuscript.

Dr. Azizahmed I Arbar supervised the study and guided the methodology. Reviewed and approved the manuscript.

Dr Isha Kandwal assisted in data analysis and interpretation.

All authors approved the final manuscript.

SUMMARY

This study elucidates the anti-anaemic mechanisms of *Rajanyadi Churna* using network pharmacology and molecular docking. The formulation modulates key targets and pathways involved in erythropoiesis, inflammation, oxidative stress, and iron homeostasis, supporting its traditional use through a synergistic, multi-target therapeutic framework.

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