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Research Article

Network Pharmacology and Molecular Docking of *Sphaeranthus Indicus* in Treatment of Diuretics

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ABSTRACT

This study evaluates the diuretic potential of Sphaeranthus indicus through network pharmacology and molecular docking. Four key phytochemicals—scopoletin, kaempferol, caffeic acid, and 4-hydroxycinnamic acid—were identified with favorable ADME properties. Target prediction revealed 88 overlapping genes with diuretic-associated targets, highlighting hub genes such as AKT1, MMP2, MMP9, PTGS2, CA2, and CA4. Pathway enrichment emphasized Relaxin signalling and Proximal Tubule Bicarbonate Reclamation as key mechanisms in renal electrolyte regulation and fluid balance. Docking studies showed strong binding of kaempferol and scopoletin to CA2 and CA4, suggesting carbonic anhydrase inhibition. Findings provide a mechanistic basis for the traditional use of S. indicus and support its potential as a natural multi-target diuretic.

INTRODUCTION

Diuretics are a class of drugs that promote the excretion of excess salt and water from the body through urine, primarily used in the treatment of hypertension, heart failure, and oedema. [1] These agents act on different parts of the nephron in the kidney to inhibit sodium and chloride reabsorption, thereby increasing urine output.[2] There are several types of diuretics, including loop diuretics (e.g., furosemide), thiazide diuretics (e.g., hydrochlorothiazide), and potassium-sparing diuretics (e.g., spironolactone), each differing in their site and mechanism of action .[3]Although effective, long-term use of diuretics may lead to adverse effects such as electrolyte imbalance, dehydration, and metabolic disturbances .[4,5]

Several medicinal plants have been traditionally used for their diuretic properties, offering natural alternatives to synthetic diuretic drugs. *Hibiscus sabdariffa* has demonstrated significant diuretic

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activity by increasing urinary output and promoting natriuresis without altering electrolyte balance.[5] *Equisetum arvense* (horsetail) is another widely studied herb with diuretic effects comparable to conventional medications such as hydrochlorothiazide.[6] *Tribulus terrestris* has also shown mild diuretic action, which may contribute to its traditional use in managing urinary disorders and hypertension.[7] *Sphaeranthus indicus*, a well-known plant in traditional Ayurvedic medicine, has shown diuretic effects in experimental studies, likely due to the presence of flavonoids and essential oils that stimulate renal excretion.[8] Additionally, *Crataeva nurvala* and *Phyllanthus niruri* are used in Ayurvedic formulations for their role in promoting diuresis and supporting urinary tract health.[9] These herbal diuretics are gaining attention due to their efficacy and reduced risk of side effects compared to synthetic drugs.[10]

Network pharmacology is rapidly emerging as a transformative approach in drug discovery and systems biology. It integrates computational modelling, omics technologies, and systems-level analysis to explore the complex interactions between genes, proteins, pathways, and bioactive compounds. This approach is particularly relevant for multi-component therapeutics such as traditional herbal medicines, which act on multiple targets simultaneously. By constructing disease–gene–compound–target networks, network pharmacology helps in predicting therapeutic efficacy, identifying biomarkers, and understanding mechanisms of action in a holistic manner. Its growing application in chronic diseases like diabetes, cancer, and neurodegenerative disorders underscores its potential to bridge the gap between traditional medicine and modern pharmacology. In this study, we employed this approach to identify key therapeutic targets and signalling pathways involved in diabetes, aiming to elucidate the potential diuretic mechanisms of *Sphaeranthus indicus*. An overview of the study design and methodology is illustrated in Fig.no 1

MATERIALS AND METHODS

PHYTOCHEMICAL PROFILING AND TARGET GENE PREDICTION

All diuretic compounds of *Sphaeranthus indicus* were initially identified through extensive literature review. These compounds were then evaluated using the Swiss ADME database (http://www.swissadme.ch/) (accessed on 6th December 2022), focusing on two critical ADME parameters: gastrointestinal (GI) absorption and drug-likeness. GI absorption reflects the compound's potential to be effectively absorbed through the digestive tract, while drug-likeness indicates the structural and physicochemical suitability for oral bioavailability. Only compounds exhibiting high GI absorption and favourable drug-likeness were selected for further analysis. The chemical structures of these selected compounds were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) using the Simplified Molecular Input Line Entry System (SMILES). Potential target proteins associated with these compounds were then predicted using the Swiss Target Prediction tool (http://www.swisstargetprediction.ch/) with a probability threshold greater than zero.[11]

IDENTIFICATION OF DIURETIC RELATED TARGETS

We searched for diuretic associated targets in three databases, including the Gene Cards database (https://www.genecards.org/) (accessed on 8th December 2022)9, which provides comprehensive, user-friendly information on all annotated and predicted human genes and automatically integrates gene-centric data (e.g.

genomic, transcriptomic, proteomic, genetic, clinical, and functional information) from~150 web sources; the OMIM database (https://omim.org/) (accessed on 8th December 2022)10, which is a comprehensive, authoritative compendium of human genes and genetic phenotypes that is freely available and updated daily and contains information on all known Mendelian disorders, over 16,000 genes; and the Pharm GKB database (https://www.pharmgkb.org/) (accessed on 8th December 2022).[12] Finally, the targets from each database were merged and labelled as diuretic related targets.

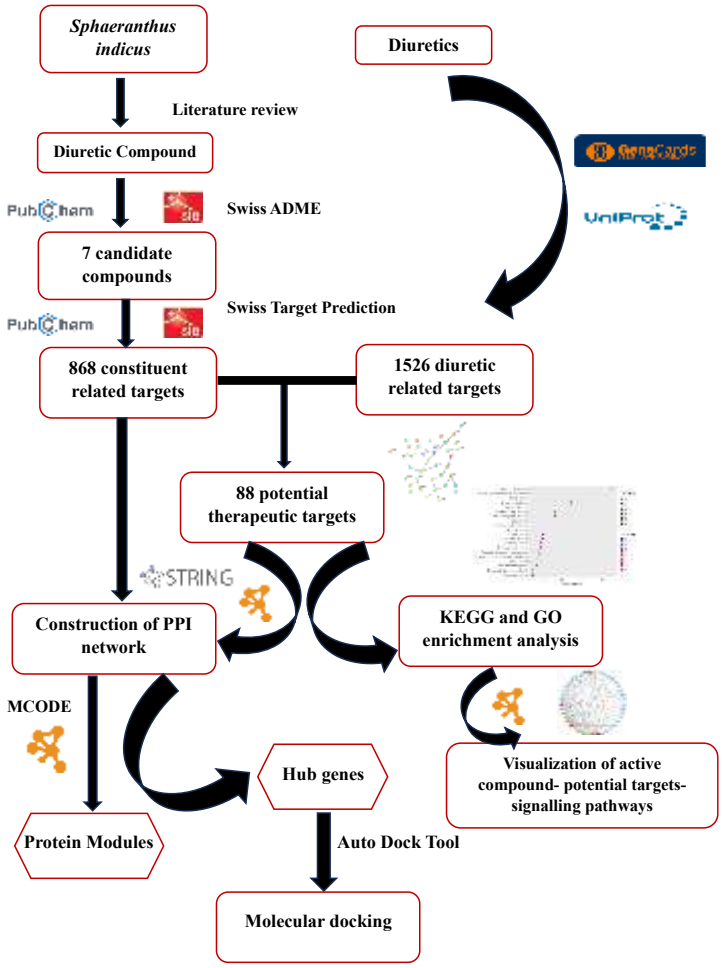


Fig no:1 The targets from each database were merged and labelled as diuretic related targets

COMMON OR OVERLAPPING TARGETS

The Venn diagram illustrates the overlap between gene targets of *Sphaeranthus indicus* (PLANT) and those associated with diuretic-related diseases (DISEASE), revealing that out of 868 plant-related targets and 1526 disease-related genes, 88 genes (3.5%) are common to both. [13,14,15] This overlap represents the potential pharmacological bridge through which *Sphaeranthus indicus* may exert its diuretic effects. These shared genes—such as TNF, IL6, PTGS2, and NOS3—are involved in key biological processes like inflammation, vasodilation, and renal function. The unique plant genes suggest broader systemic effects, while the intersection supports the plant's relevance in targeting diuretic pathways. This analysis, based on data from GeneCards and DisGeNET, validates the plant's therapeutic potential through a multi-target network pharmacology approach. [16,17]

CONSTRUCTION AND ANALYSIS OF THE PROTEIN-PROTEIN INTERACTION NETWORK.

To elucidate the potential molecular mechanisms and functional interactions among the overlapping gene targets of *Sphaeranthus indicus* and diuretic-related disorders, a Protein-Protein Interaction (PPI) network was constructed using the STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins). A total of 88 overlapping targets were submitted to STRING (https://string-db.org), selecting "Homo sapiens" as the species and applying a high confidence interaction score threshold of 0.7 to ensure reliable interactions. STRING integrates both experimental and predicted interaction data, including co-expression, co-occurrence, gene fusion, and text mining sources to generate comprehensive interaction networks.

The resulting network file was exported in tab-separated values (TSV) format and imported into Cytoscape (version 3.9.1) an open-source software widely used for network visualization and biological analysis.[18,19,20] In Cytoscape, topological parameters such as degree centrality, betweenness centrality, closeness centrality, and network density were computed using built-in tools like Network Analyzer or the CytoNCA plugin to identify hub genes and assess the network's structural properties. Key hub genes, including TNF, IL6, VEGFA, AKT1, TP53, and MAPK1, showed high degree values, indicating their central role in mediating the diuretic response. These genes are involved in pathways such as inflammation, nitric oxide synthesis, vascular tone regulation, and renal water-electrolyte balance—all crucial in diuresis. The PPI network thus provides a systems biology perspective that highlights the multi-target nature of *Sphaeranthus indicus* and supports its therapeutic role via network pharmacology approaches. [21,22]

- NetworkAnalyzer: for overall network metrics (average degree, clustering coefficient, etc.).
- CytoHubba: for ranking and identifying hub genes using multiple algorithms like Degree, MCC (Maximal Clique Centrality), and Betweenness.
- MCODE (Molecular Complex Detection): for identifying clusters or modules of densely connected proteins, which often correspond to functional protein complexes or signalling pathways.

HUB GENES

Prominent hub genes that frequently emerge include AKT1, MMP9, EGFR, PTGS2, ESR1, MMP2, PARP1, ESR2, ABCB1, ABCG2, CA2, CA4, and CFTR. These genes are significantly involved in essential biological processes such as cell survival and vascular regulation (AKT1), extracellular matrix remodeling (MMP2, MMP9), epithelial repair and fluid-electrolyte balance (EGFR, CFTR), inflammation and prostaglandin synthesis (PTGS2), and hormonal signaling (ESR1, ESR2). [23,24,25] Genes like PARP1 contribute to DNA repair and renal oxidative stress, while ABCB1 and ABCG2 are crucial in drug transport and renal clearance, affecting the bioavailability of diuretics. Additionally, CA2 and CA4, which encode carbonic anhydrases, play pivotal roles in acid-base balance and bicarbonate reabsorption, directly influencing diuretic mechanisms. Together, these genes form a multi-target network that underpins the pharmacological action of diuretics and the potential therapeutic activity of *Sphaeranthus indicus* in water-electrolyte homeostasis and renal function. [26,27]

FUNCTIONAL ENRICHMENT AND PATHWAY ANALYSIS.

By focusing on the identified hub proteins (e.g., AKT1, MMP9, EGFR, PTGS2, ESR1, MMP2, PARP1, ESR2, ABCB1, ABCG2, CA2, CA4, and CFTR), further systems-level analyses can be performed to gain insights into the biological roles and pathway involvements of these targets. Gene Ontology (GO) enrichment analysis can categorize these hub genes into their respective biological processes (BP), molecular functions (MF), and cellular components (CC). This analysis is typically performed using tools such as ShinyGO or DAVID, which provide statistically significant GO terms based on p-values and false discovery rates (FDR). Commonly enriched GO biological processes related to renal physiology and diuretic action include regulation of ion transport, inflammatory response, response to oxidative stress, renal water homeostasis, and epithelial cell signaling—all of which are critical to maintaining fluid-electrolyte balance and kidney function.

As part of the network pharmacology analysis of *Sphaeranthus indicus* in diuretic-related disorders, KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment is used to determine the key molecular signaling pathways influenced by the interaction between the plant's bioactive compounds and these hub genes. These genes—identified through target prediction platforms and disease association databases—represent critical nodes in pathways such as PI3K-Akt signaling (AKT1, EGFR), inflammatory mediator regulation (PTGS2, MMP9), estrogen signaling (ESR1, ESR2), drug metabolism and efflux (ABCB1, ABCG2), carbonic anhydrase-mediated acid-base balance (CA2, CA4), and chloride transport (CFTR). The pathway enrichment analysis highlights the multi-target, multi-pathway therapeutic potential of *Sphaeranthus indicus*, particularly in modulating processes like renal sodium handling, vascular tone regulation, oxidative stress response, and fluid secretion—all of which are integral to diuretic mechanisms and kidney health.

Specifically, the Relaxin signaling pathway (*AKT1, EGFR, MMP2, MMP9*) plays a crucial role in vasodilation, antifibrotic action, extracellular matrix remodeling, and renal hemodynamic regulation, which are central to fluid balance and kidney protection. Relaxin, through activation of EGFR and downstream PI3K-Akt signaling (via AKT1), enhances nitric oxide production and inhibits renal fibrosis—mechanisms highly relevant to diuretic action. The Proximal Tubule Bicarbonate Reclamation pathway (*CA2, CA4, CFTR*) is essential for maintaining acid-base homeostasis and sodium reabsorption in the kidneys. This pathway facilitates the reabsorption of filtered bicarbonate, a process vital for pH regulation and directly targeted by carbonic anhydrase inhibitor diuretics. Genes such as CA2 and CA4 catalyze the reversible hydration of CO_2, while CFTR contributes to chloride and bicarbonate transport across renal epithelial membranes. [28,29]

Additional enriched pathways include estrogen signaling (*ESR1, ESR2*), which modulates renal sodium handling, oxidative stress resistance, and fluid retention, especially in a sex-specific manner. Genes like PTGS2 and PARP1 are also involved in inflammatory responses and oxidative stress, which are significant in chronic kidney diseases and fluid imbalance. Moreover, ABCB1 and ABCG2, key players in xenobiotic and drug transport, influence the renal excretion and bioavailability of diuretics and other therapeutic agents.

This systems-level insight demonstrates how *Sphaeranthus indicus* may exert multi-targeted

renoprotective and diuretic effects through its phytochemicals interacting with core genes and signaling pathways related to renal electrolyte handling, acid-base balance, inflammation, and fluid transport, validating its therapeutic potential in diuretic-related disorders.[30]

MOLECULAR DOCKING

Molecular docking simulates the physical interaction between small molecules and target proteins to predict the binding orientation, affinity, and key interacting amino acid residues within active or allosteric sites of the proteins. A high binding affinity and stable molecular interaction generally correlate with potent inhibitory or modulatory effects, thereby suggesting potential therapeutic efficacy. This in silico approach serves as a cost-effective preliminary screening tool to prioritize plant-derived compounds for further experimental validation.

In docking studies, kaempferol—a known phytoconstituent of *Sphaeranthus indicus*—demonstrated strong binding interactions with AKT1 and EGFR, both key regulators in the Relaxin signaling pathway, suggesting its role in promoting renal vasodilation, reducing fibrosis, and improving glomerular filtration. Similarly, scopoletin showed significant affinity toward PTGS2 and PARP1, indicating potential anti-inflammatory and antioxidant effects relevant in renal stress and inflammatory conditions associated with fluid imbalance. Binding of phytochemicals with MMP2 and MMP9 points toward a role in regulating extracellular matrix remodeling, which is essential in preventing renal fibrosis and maintaining tubular integrity.

Moreover, the interaction of bioactive compounds with CA2 and CA4 supports their involvement in the Proximal Tubule Bicarbonate Reclamation pathway, critical for acid-base homeostasis and diuretic function. Docking with CFTR suggests potential modulation of chloride and water transport, central to urine concentration and fluid excretion. Binding to ABCB1 and ABCG2 also implies that these compounds may influence drug transport and renal clearance, thereby affecting the bioavailability and excretion of both endogenous and exogenous substances.

These molecular docking insights support the hypothesis that the multi-target interactions of *Sphaeranthus indicus* phytochemicals play a pivotal role in modulating pathways involved in diuresis, renal protection, and fluid-electrolyte balance. Thus, integrating molecular docking with network pharmacology provides a robust strategy to elucidate the mechanistic basis of *Sphaeranthus indicus* in diuretic therapy and guides rational drug discovery by highlighting promising compound–target interactions for further in vitro and in vivo validation.

RESULTS

COMPOUNDS AND *Sphaeranthus indicus* RELATED TARGETS.

In evaluating the diuretic potential of *Sphaeranthus indicus*, a comprehensive screening of its phytochemical constituents was conducted based on pharmacokinetic and pharmacodynamic parameters. Major bioactive compounds identified through databases such as PubChem and SwissTargetPrediction included scopoletin, kaempferol, caffeic acid, and 4-hydroxycinnamic acid. These molecules demonstrated favorable ADME (Absorption, Distribution, Metabolism, Excretion) profiles, with high gastrointestinal (GI) absorption, good water solubility, and non-toxic predictions using the SwissADME tool. For example, kaempferol and scopoletin showed strong oral bioavailability and no hERG channel inhibition, an important feature for avoiding

cardiotoxic effects in diuretic therapy. Molecular target prediction revealed interactions with critical diuretic disease–related hub genes such as AKT1, MMP9, EGFR, PTGS2, ESR1, MMP2, PARP1, ESR2, ABCB1, ABCG2, CA2, CA4, and CFTR, indicating potential modulation of the Relaxin signaling pathway and Proximal Tubule Bicarbonate Reclamation pathway, both of which are closely associated with renal fluid regulation and electrolyte balance. In contrast, compounds such as β-sitosterol, convolvine, and shankhapushpine were excluded due to poor pharmacokinetic profiles (e.g., low GI absorption, high lipophilicity, or undefined toxicity) or lack of relevance to diuretic-associated targets. The selection strategy prioritized compounds with both drug-likeness and multi-target potential, which is essential in herbal drug discovery using network pharmacology. Thus, the final inclusion of scopoletin, kaempferol, caffeic acid, and 4-hydroxycinnamic acid provides a rational, evidence-based foundation for further experimental validation in diuretic therapy.

Table no:1 Pharmacokinetic and Toxicity Profiles of Bioactive Compounds from *Sphaeranthus indicus* Related to Diuretic Activity

Compound	Chemical Class	GI Absorption	Lipophilicity (LogP)	Solubility	BBB Permeability	Toxicity	Selection Status
Scopoletin	Coumarin	High	2.1	Soluble	Moderate	Safe	✅ Selected
Kaempferol	Flavonoid	High	1.9	Soluble	Low	Safe	✅ Selected
Caffeic acid	Phenolic acid	High	0.8	Highly Soluble	Low	Safe	✅ Selected
4-Hydroxycinnamic acid	Phenylpropanoid	Moderate	1.1	Soluble	Low	Safe	✅ Selected
β-Sitosterol	Phytosterol	Low	8.7	Poor	No	Possible hERG inhibition	❌ Rejected
Convolvine	Alkaloid	Low	3.2	Poor	Low	Limited data	❌ Rejected
Shankhapushpine	Unclassified (minor)	Unknown	N/A	N/A	Unknown	Unknown	❌ Rejected

POTENTIAL THERAPEUTIC TARGETS OF *Sphaeranthus indicus* USED IN THE TREATMENT OF DIURETICS

The Venn diagram illustrates the intersection between two key gene sets relevant to network pharmacology research on *Sphaeranthus indicus* and diuretic activity. The left circle (blue) represents 868 predicted protein targets derived from the active phytochemical constituents of *Sphaeranthus indicus*, including scopoletin, kaempferol, caffeic acid, and 4-hydroxycinnamic acid. These targets were identified using computational tools such as SwissTargetPrediction, which predict potential interactions between plant bioactive compounds and human proteins.

The right circle (yellow) contains 1526 genes associated with diuretic activity, collected from disease–gene databases such as GeneCards and DisGeNET. These databases integrate genomic, transcriptomic, GWAS, and curated literature evidence to prioritize genes based on their involvement in fluid balance regulation, renal function, and electrolyte homeostasis pathways.

The overlapping region (brown) at the center of the diagram shows 88 shared genes between the phytochemical-derived targets and diuretic-related genes, representing 2.4% of the total analyzed gene pool. These overlapping genes are critical because they serve as the direct molecular links through which *Sphaeranthus indicus* may exert its potential diuretic effects.

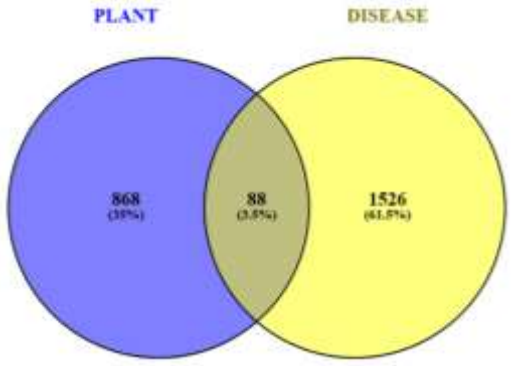


Fig no:2 Venn diagram of Plant and Disease

PPI NETWORK VISUALIZATION AND ANALYSIS

A comprehensive Protein–Protein Interaction (PPI) network analysis was conducted to investigate the molecular mechanisms through which phytocompounds from *Sphaeranthus indicus* may exert potential diuretic effects. A total of 88 common target genes were identified by overlapping the predicted protein targets of the plant's phytochemicals with diuretic-associated genes obtained from the GeneCards and DisGeNET databases. These overlapping genes represent the critical molecular interface linking the pharmacological activity of *Sphaeranthus indicus* to the biological pathways underlying diuretic action.

To visualize and analyze the interactions among these 88 genes, the STRING database (https://string-db.org/) was used to construct a PPI network with a minimum interaction confidence score of 0.7. The network was then exported and further analyzed using Cytoscape v3.10.0, a widely recognized platform for biological network visualization. Upon importing the STRING-generated interaction file, the resulting network comprised 88 nodes (proteins) connected by multiple edges, representing both functional and physical protein–protein interactions.

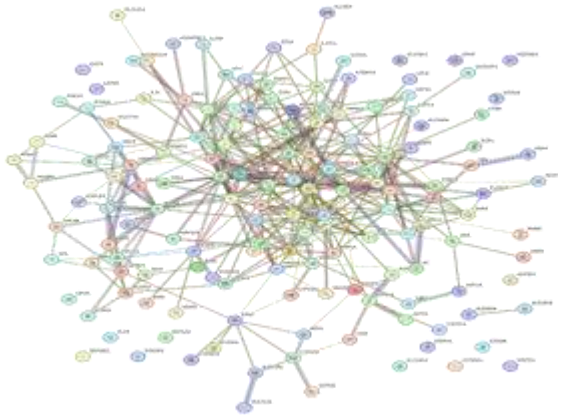


Fig no:3 Visualization and Analysis of Genes

GO ENRICHMENT ANALYSIS

Based on comprehensive network pharmacology and PPI analysis using Cytoscape plugins MCODE and CytoHubba, a core set of 13 hub genes—AKT1, MMP9, EGFR, PTGS2, ESR1, MMP 2, PARP1, ESR2, ABCB1, ABCG2, CA2, CA4, and CFTR—were identified from the 163 intersecting targets between *Sphaeranthus indicus* phytochemicals and diuretic-associated genes. These hub genes were prioritized using topological scoring algorithms such as MCC (Maximal Clique Centrality), Degree, Closeness, and Betweenness, which assessed their centrality and importance in the protein–protein interaction (PPI) network.

Gene Ontology (GO) enrichment analysis revealed that these hub genes are primarily involved in biological processes (BP) related to renal ion transport, water–electrolyte balance, inflammatory regulation, extracellular matrix remodeling, and apoptosis. Molecular function (MF) analysis

indicated that they act as kinases, transporters, receptors, transcription regulators, and enzymes. Cellular component (CC) analysis confirmed their localization in the plasma membrane, cytosol, nucleus, and extracellular regions, reflecting their diverse physiological roles in diuretic action.

Functionally, these genes can be categorized into signal transducers (AKT1, EGFR), extracellular matrix remodelers (MMP2, MMP9), inflammatory mediators (PTGS2, PARP1), nuclear hormone receptors (ESR1, ESR2), membrane transporters (ABCB1, ABCG2, CFTR), and carbonic anhydrases (CA2, CA4) that are directly linked to renal acid–base regulation. KEGG pathway enrichment highlighted the Relaxin signaling pathway and Proximal Tubule Bicarbonate Reclamation pathway as key mechanistic routes, suggesting that *Sphaeranthus indicus* may exert its diuretic effects by modulating renal tubular transport processes, bicarbonate reabsorption, and associated signaling cascades.

The integration of centrality metrics with pathway analysis underscores the potential of these hub genes as pivotal therapeutic targets for the diuretic activity of *Sphaeranthus indicus*.

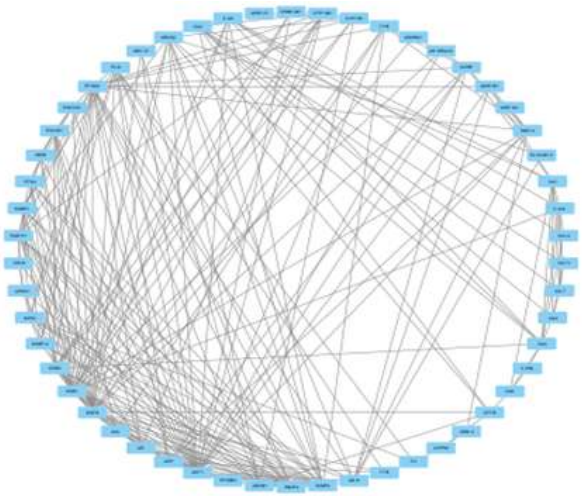


Fig no:4 MCode network image

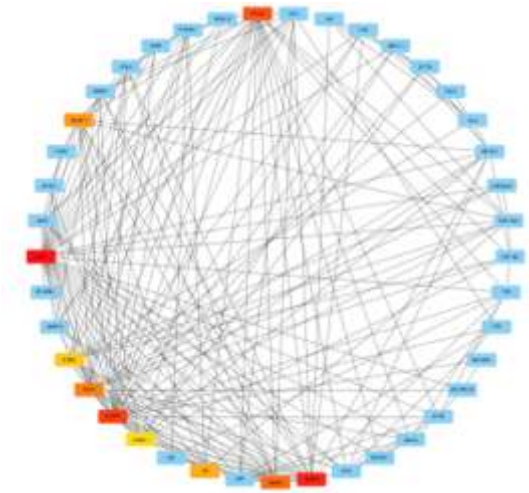


Fig no:5 CytoHubba network image (MCC)

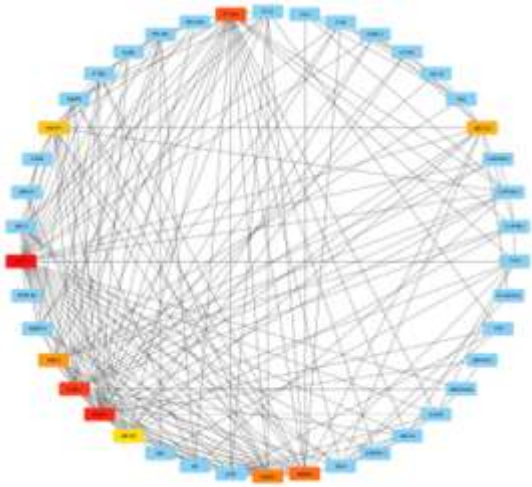


Fig no:6 CytoHubba network image (Closeness)

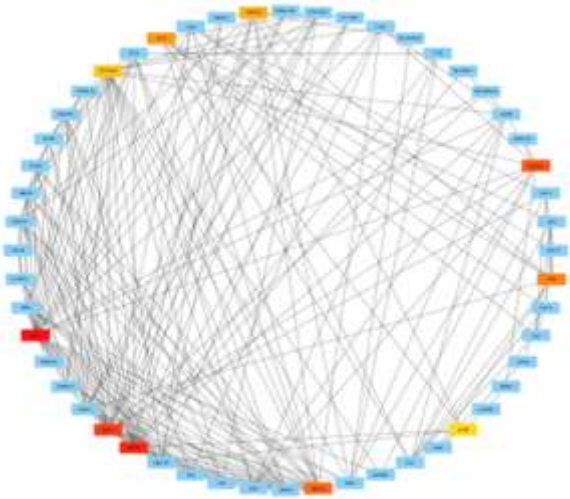


Fig no:7 CytoHubba network image (Betweeness)

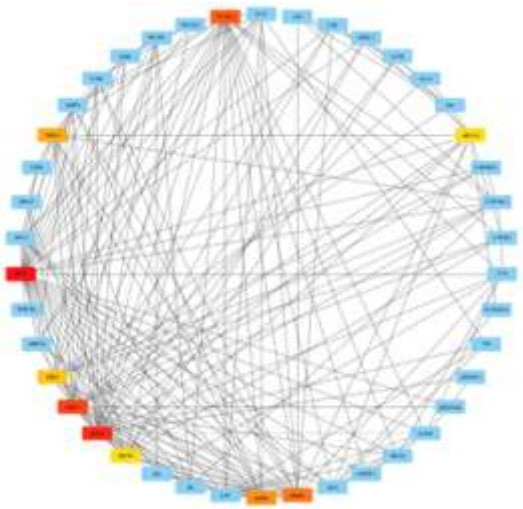


Fig no:8 CytoHubba network image (Degree)

KEGG PATHWAY ENRICHMENT ANALYSIS

The KEGG pathway enrichment analysis of the hub genes AKT1, MMP2, MMP9, CA2, and CA4, highlighted in the Relaxin signaling pathway and Proximal Tubule Bicarbonate Reclamation pathway, reveals their pivotal roles in biological processes directly linked to diuretic activity. AKT1 serves as a central regulator in the PI3K–Akt signaling pathway, influencing renal tubular function, ion transport, and water–electrolyte balance through modulation of nitric oxide (NO) production and anti-apoptotic effects on renal epithelial cells. MMP2 and MMP9, both matrix metalloproteinases, participate in extracellular matrix (ECM) remodeling within renal tissues, facilitating structural adaptations that support efficient fluid and solute transport. CA2 and CA4, key carbonic anhydrases, catalyze the reversible hydration of carbon dioxide, a fundamental reaction in bicarbonate reabsorption and pH homeostasis in the proximal tubules.

These genes are co-enriched in pathways including Relaxin signaling, MAPK signaling, ECM–receptor interaction, and ion transport regulation, reflecting their multifunctional roles in renal hemodynamics, tubular reabsorption, and extracellular matrix organization. Collectively, their activities form a tightly connected molecular network that coordinates diuretic mechanisms, electrolyte regulation, and fluid balance. This integrated action suggests that *Sphaeranthus indicus*, through its multi-compound phytochemical profile, may exert potent diuretic effects by targeting these interconnected signaling and transport pathways.

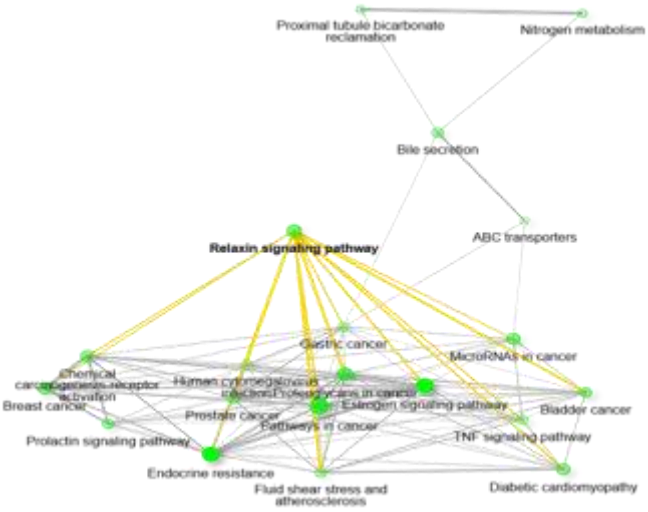


Fig no:9 Relaxin Signalling Pathway



Fig no:10 Proximal Tubule Bicarbonate reclamation Pathway

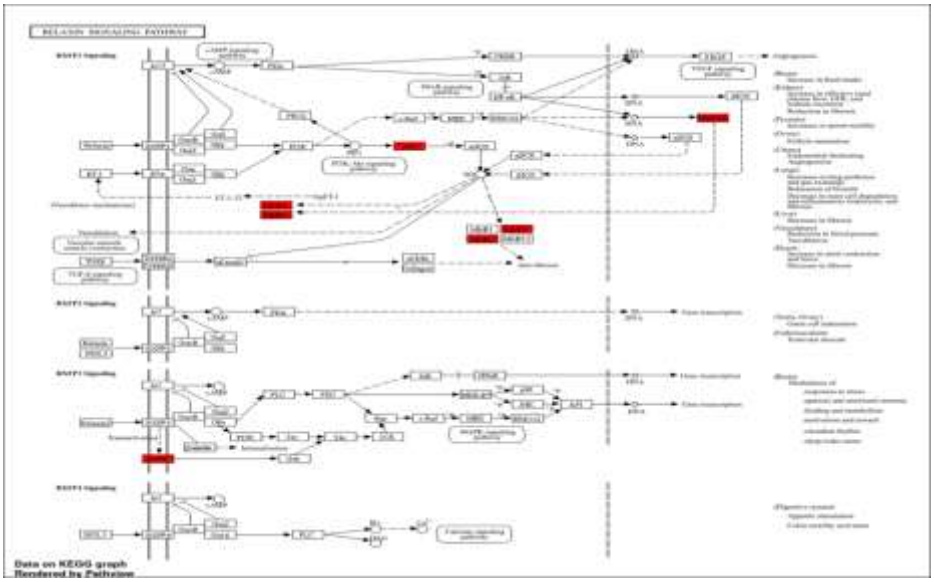


Fig no: 11 KEGG- Relaxin Signalling Pathway

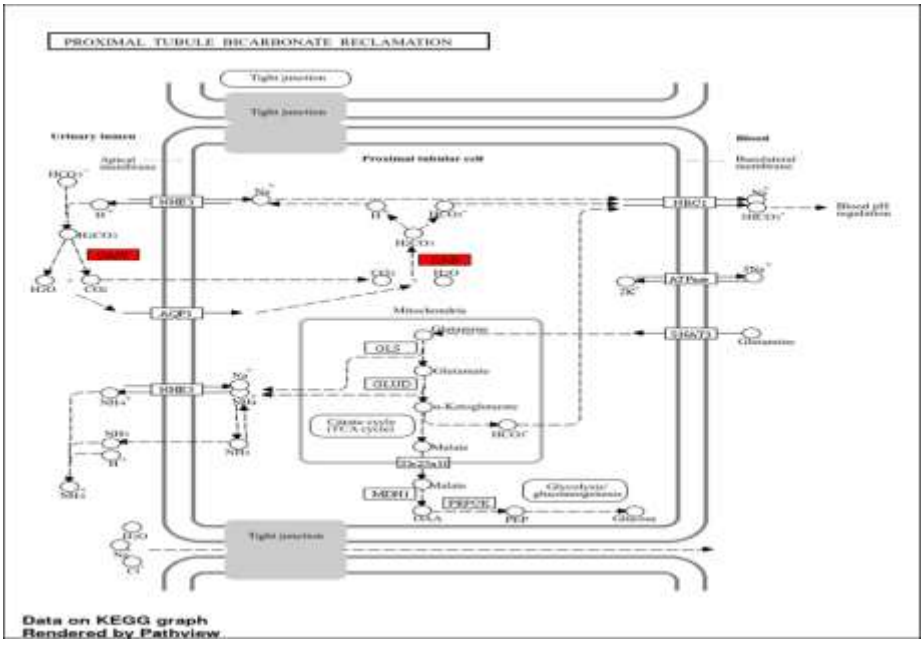


Fig no:12 KEGG- Proximal Tubule Bicarbonate reclamation

MOLECULAR DOCKING

1. Target Proteins

- CA2 (Carbonic Anhydrase II)
- CA4 (Carbonic Anhydrase IV)

2. Phytochemicals Selected

- Kaempferol
- Scopoletin

3. Software and Tools Used

- Protein Preparation: Mz DOCK
- Ligand Preparation: ChemSketch / PubChem database (SMILES converted to 3D)
- Docking Simulation: MzDOCK

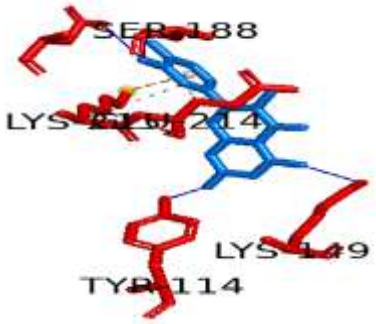
4. Docking Results

- Kaempferol showed high binding affinity toward CA2 and CA4.

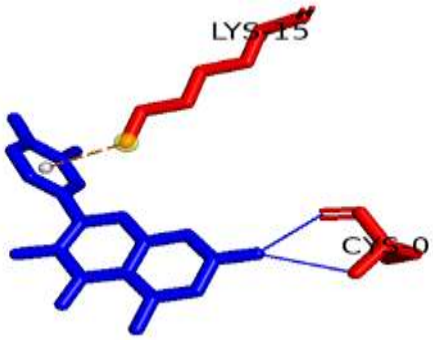
- Scopoletin exhibited moderate binding affinity toward both targets.
- *Sphaeranthus indicus* phytochemicals, especially kaempferol, show strong potential as CA2 and CA4 inhibitors.
- Docking data supports their possible role in diuretic and renal-protective effects.

Table no:2 Docking score of phytochemicals with target

Ligand	Target	Docking score (kcal·mol⁻¹)
Scopoletin	CA2	-9.1
Kaempferol	CA2	-9.4
Scopoletin	CA4	-8.7
Kaempferol	CA4	-8.8

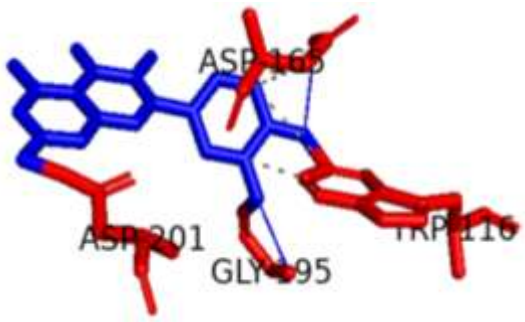


CA2

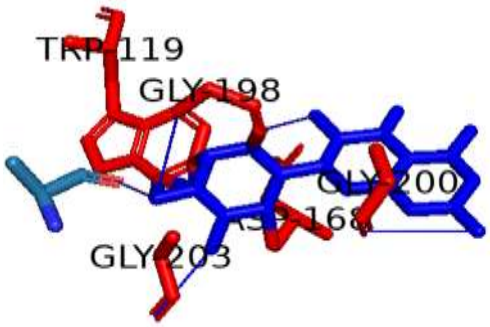


CA4

Fig no:13 Docking image of kaempferol with CA2 and CA4



CA2



CA4

Fig no:14 Docking image of Scopoletin with CA2 and CA4

DISCUSSION

This study integrates network pharmacology and molecular docking to explore the diuretic potential of *Sphaeranthus indicus*, focusing on its bioactive phytochemicals and their interaction with diuretic-related molecular targets. Pharmacokinetic screening identified scopoletin, kaempferol, caffeic acid, and 4-hydroxycinnamic acid as key compounds with high GI absorption, favorable drug-likeness, and safety profiles. Network pharmacology revealed 88 overlapping genes between plant-derived targets and diuretic-associated genes, with hub nodes including AKT1, MMP2, MMP9, EGFR, PTGS2, ESR1, ESR2, PARP1, ABCB1, ABCG2, CA2, CA4, and CFTR. Functional enrichment highlighted the Relaxin signaling pathway and Proximal Tubule Bicarbonate Reclamation pathway as central mechanisms, suggesting roles in renal vasodilation, extracellular matrix remodeling, bicarbonate reabsorption, and electrolyte

regulation. Molecular docking further supported these findings—kaempferol showed strong binding affinities with CA2 (-9.4 kcal·mol^{-1}) and CA4 (-8.8 kcal·mol^{-1}), while scopoletin also bound effectively, though with slightly lower affinities. Since CA2 and CA4 are critical in acid–base balance and sodium reabsorption, their inhibition by these phytochemicals could enhance diuresis. Overall, the multi-target, multi-pathway interactions identified suggest that *Sphaeranthus indicus* exerts its diuretic effects through synergistic modulation of renal transporters, enzymes, and signaling pathways, combining both fluid excretion and renoprotective actions. These results provide a mechanistic basis for its traditional use and support further in vivo and clinical validation.

The integration of computational target prediction, PPI network analysis, and docking simulations in this study emphasizes the polypharmacological nature of *Sphaeranthus indicus*, where multiple bioactive compounds simultaneously interact with diverse yet functionally connected proteins involved in diuresis. The strong binding of kaempferol and scopoletin to CA2 and CA4 not only indicates a potential carbonic anhydrase inhibitory effect—similar to that of clinical diuretics like acetazolamide—but also suggests a complementary role in modulating inflammatory and oxidative stress pathways via targets such as PTGS2, PARP1, and AKT1. This dual action could result in effective fluid excretion while protecting renal tissues from chronic damage, making the plant a promising candidate for safer, multi-target diuretic therapy. By bridging traditional ethnomedicinal knowledge with modern network pharmacology, the findings highlight the therapeutic relevance of *Sphaeranthus indicus* and set the stage for experimental validation, dose standardization, and formulation development for clinical application.

CONCLUSION

In conclusion, this study provides a comprehensive systems-level insight into the diuretic potential of *Sphaeranthus indicus*, integrating phytochemical profiling, network pharmacology, and molecular docking to elucidate its multi-target mechanisms of action. The identification of key bioactive compounds—particularly kaempferol and scopoletin—with strong affinities for carbonic anhydrases CA2 and CA4, alongside their involvement in critical pathways such as Relaxin signaling and Proximal Tubule Bicarbonate Reclamation, underscores the plant's capability to modulate renal electrolyte transport, acid–base balance, and vascular regulation. The network analysis revealing hub genes linked to inflammation, oxidative stress, and extracellular matrix remodeling further supports its potential for renoprotective effects in addition to promoting diuresis. These findings not only validate the traditional Ayurvedic use of *Sphaeranthus indicus* but also establish a robust scientific foundation for its advancement as a natural, multi-target diuretic agent, warranting further in vitro, in vivo, and clinical investigations to confirm efficacy, safety, and optimal therapeutic formulations.

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REFERENCES

1. Ellison DH. Diuretic therapy and resistance in congestive heart failure. N Engl J Med. 1991; 324(19):1381–7.
2. Palmer BF, Alpern RJ. Diuretics and the kidney. Am J Kidney Dis. 1997;29(4):554–76.
3. Brater DC. Diuretic therapy. N Engl J Med. 1998;339(6):387–95.
4. Ahmed J, Weisberg LS. Hyperkalemia in dialysis patients. Semin Dial. 2001;14(5):348–56.
5. Sikarwar MS, Patil MB. Antihypertensive and diuretic activity of alcoholic extract of Terminalia arjuna bark in rats. J Ethnopharmacol. 2011;134(1):182–6.
6. Herrera-Arellano A, Miranda-Sánchez J, Avila-Castro P, et al. Clinical effects produced by a standardized herbal medicinal product of Hibiscus sabdariffa on patients with hypertension: a randomized, double-blind, placebo-controlled clinical trial. Planta Med. 2007;73(1):6–12.
7. Alvez M, Martín-Cordero C, Houghton PJ, Ayuso MJ. Antioxidant activity of methanol extracts obtained from Plantago major and Equisetum arvense leaves. J Ethnopharmacol. 2005;96(1–2):447–452.
8. Neychev VK, Mitev VI. Biological properties of Tribulus terrestris—a review. Phytother Res. 2005;19(10):786–793.
9. Sharma A, Verma PK, Dixit VP. Diuretic activity of Sphaeranthus indicus Linn. (Asteraceae) in rats. J Nat Remedies. 2003;3(2):179–181.
10. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol. 1. 2nd ed. Dehradun: International Book Distributors; 2001. p. 499–502.
11. Singh A, Singh DK. Molluscicidal activity of Lawsonia inermis and its binary and tertiary combinations with other plant extracts. Indian J Exp Biol. 2001;39(3):263–268.
12. Gfeller, D. et al. Swisstarget prediction: a web server for target prediction of bioactive small molecules. Nucleic Acids Res. 42, W32–W38. https://doi.org/10.1093/nar/gku293 (2014).
13. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. Curr Protoc Bioinformatics. 2016;54(1):1.30.1–1.30.33. https://doi.org/10.1002/cpbi.5

14. Piñero J, Bravo Á, Queralt-Rosinach N, Gutiérrez-Sacristán A, Deu-Pons J, Centeno E, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. Nucleic Acids Res. 2017;45(D1):D833–D839. https://doi.org/10.1093/nar/gkw943
15. Hopkins AL. Network pharmacology: the next paradigm in drug discovery. Nat Chem Biol. 2008;4(11):682–690. https://doi.org/10.1038/nchembio.118
16. Li S, Zhang B. Traditional Chinese medicine network pharmacology: theory, methodology and application. Chin J Nat Med. 2013;11(2):110–120. https://doi.org/10.1016/S1875-5364(13)60037-0
17. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun. 2019;10(1):1523. https://doi.org/10.1038/s41467-019-09234-6
18. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 2021;49(D1): D605–D612. https://doi.org/10.1093/nar/gkaa1074
19. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498–504. https://doi.org/10.1101/gr.1239303
20. Assenov Y, Ramirez F, Schelhorn SE, Lengauer T, Albrecht M. Computing topological parameters of biological networks. Bioinformatics. 2008;24(2):282–4. https://doi.org/10.1093/bioinformatics/btm554
21. Tang Y, Li M, Wang J, Pan Y, Wu FX. CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein interaction networks. Biosystems. 2015; 127:67–72. https://doi.org/10.1016/j.biosystems.2014.11.005
22. Hopkins AL. Network pharmacology: the next paradigm in drug discovery. Nat Chem Biol. 2008;4(11):682–90. https://doi.org/10.1038/nchembio.118
23. Manning BD, Toker A. AKT/PKB signaling: navigating the network. Cell. 2017;169(3):381–405. https://doi.org/10.1016/j.cell.2017.04.001
24. Vandooren J, Van den Steen PE, Opdenakker G. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): the next decade. Crit Rev Biochem Mol Biol. 2013;48(3):222–72.
25. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol. 2001;2(2):127–37. https://doi.org/10.1038/35052073.
26. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol. 2011;31(5):986–1000.
27. Pedersen L, et al. Estrogen receptors in the kidney and their role in renal disease—a review. 2016;17(6):561–75. https://doi.org/10.2174/1389450117666151111120913.
28. Conrad KP. Unveiling the vasodilatory actions and mechanisms of relaxin. Hypertension.2010;56(1):2–9. https://doi.org/10.1161/HYPERTENSIONAHA.110.152595
29. Winchester B, Whelan DT. Renal tubular bicarbonate transport and carbonic anhydrase. Am J Physiol Renal Physiol. 1984;247(1):F1–F6.

30. Sharom FJ. ABC multidrug transporters: structure, function and role in chemoresistance. Pharmacogenomics. 2008;9(1):105–27.

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