INTEGRATED NETWORK PHARMACOLOGY AND MOLECULAR DOCKING STUDY OF *DICHROCEPHALA INTEGRIFOLIA* IN INFLAMMATORY DISORDERS

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Abstract :
Background
Medicinal plants are rich sources of bioactive phytochemicals with multi-target therapeutic potential. *Dichrocephala integrifolia* has been traditionally used for the treatment of inflammation, fever, pain, and infectious disorders. However, the molecular mechanisms underlying its pharmacological activities remain poorly understood.
Objective
The present study aimed to investigate the anti-inflammatory mechanisms of *Dichrocephala integrifolia* using a network pharmacology and molecular docking approach.
Methods
Phytochemicals of *Dichrocephala integrifolia* were collected from literature and phytochemical databases. Potential targets of the identified compounds were predicted using SwissTargetPrediction. Inflammation-related genes were retrieved from GeneCards and DisGeNET databases. Common targets were identified using Venny analysis and further analyzed through Cytoscape-based compound-target network construction and STRING-based protein-protein interaction (PPI) analysis. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using Metascape. Molecular docking studies were carried out using AutoDock Vina to validate compound-target interactions.
Results
A total of 18 phytochemicals and 232 compound-related targets were identified. Among these, 21 overlapping targets associated with inflammation were obtained. Network analysis revealed quercetin and luteolin as key bioactive compounds with highest degree values. PPI analysis identified TNF, PTGS2, MMP9, EGFR, PPARG, HIF1A, and MAPK14 as key hub genes involved in inflammatory signaling pathways. KEGG pathway enrichment analysis demonstrated significant involvement of TNF signaling, MAPK signaling, NF-KB signaling, and cytokine-mediated inflammatory pathways. Molecular docking analysis demonstrated favorable binding interactions of quercetin, luteolin, apigenin, and kaempferol with the target protein (PDB ID:2AZ5), with luteolin exhibiting the strongest binding affinity.
Conclusion
The findings suggest that *Dichrocephala integrifolia* exerts anti-inflammatory effects through multi-component, multi-target, and multi-pathway mechanisms. Quercetin, luteolin, apigenin, and kaempferol were identified as key bioactive compounds interacting with inflammation-associated targets. These results provide scientific support for the traditional medicinal use of the plant and establish a theoretical foundation for future experimental validation.

KEYWORDS: *Dichrocephala integrifolia*; Network Pharmacology; Molecular Docking; inflammation; Bioactive Compounds; KEGG Pathway Analysis; MAPK signaling pathway.

INTRODUCTION

Inflammation is a complex biological response initiated by immune cells and inflammatory mediators against harmful stimuli such as pathogens, tissue injury, and toxins[1,14]. Although acute inflammation is considered protective, chronic inflammation contributes to the progression of various diseases including arthritis, diabetes, cardiovascular disorders, cancer, and neurodegenerative diseases. Pro-inflammatory mediators including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL6), nuclear factor-kappa B (NF-KB), and mitogen-activated protein kinase (MAPK) signaling pathways play critical roles in inflammatory responses[15].

Medicinal plants have been extensively used in traditional healthcare systems due to their therapeutic efficacy, affordability, and lower adverse effects compared to synthetic drugs[2,5]. Unlike conventional drugs that usually target a single molecule, herbal medicines contain multiple phytoconstituents capable of interacting with diverse molecular targets, resulting in synergistic pharmacological effects[1,5,14].

Dichrocephala integrifolia belongs to the family Asteraceae and is traditionally used in various regions for the treatment of fever, pain, inflammation, parasitic infections, and wounds. Previous phytochemical investigations have reported the presence of flavonoids, phenolic compounds, sterols, and terpenoids possessing antioxidant and anti-inflammatory activities[19,20,21].

Despite its traditional medicinal importance, the precise molecular mechanisms responsible for its therapeutic effects remain unclear. Therefore, a systems pharmacology approach is required to understand the interactions between phytochemicals, molecular targets, and biological pathways[1,5].

Network pharmacology is an emerging interdisciplinary approach integrating pharmacology, bioinformatics, systems biology, and computational biology to elucidate the multi-target therapeutic mechanisms of herbal medicines[1,2,5,7]. This approach provides comprehensive insights into compound-target-pathway interactions and has become increasingly valuable in natural product research.

The present study aimed to investigate the bioactive compounds and anti-inflammatory mechanisms of *Dichrocephala integrifolia* using network pharmacology and molecular docking approaches.

TAXONOMICAL CLASSIFICATION[26]

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Asterales
Family: Asteraceae
Genus: *Dichrocephala*
Species: *Dichrocephala integrifolia*

MATERIALS AND METHODS

1. COLLECTION OF PHYTOCHEMICALS

The phytochemical constituents of *Dichrocephala integrifolia* were collected through extensive literature surveys and phytochemical databases including PubChem and scientific articles. Canonical SMILES and molecular structure of the compounds were obtained from PubChem for further analysis.

Table 1. Major Phytochemicals identified from *Dichrocephala integrifolia*[17].

Compound	PubChem CID	Molecular Formula	Phytochemical Class
Quercetin	5280343	$C_{15}H_{10}O_7$	Flavonoid
Luteolin	5280445	$C_{15}H_{10}O_6$	Flavonoid
Kaempferol	5280863	$C_{15}H_{10}O_6$	Flavonoid
Apigenin	5280443	$C_{15}H_{10}O_5$	Flavonoid
β-Sitosterol	222284	$C_{29}H_{50}O$	Sterol
Stigmasterol	5280794	$C_{29}H_{48}O$	Sterol
Chlorogenic acid	1794427	$C_{16}H_{18}O_9$	Phenolic acid
Caffeic acid	689043	$C_9H_8O_4$	Phenolic acid
Gallic acid	370	$C_7H_6O_5$	Phenolic acid
Ferulic acid	445858	$C_{10}H_{10}O_4$	Phenolic acid

2. ADME SCREENING

The pharmacokinetics properties of the identified phytochemicals were evaluated using SwissADME. Parameters such as oral bioavailability, gastrointestinal absorption, and Lipinski's rule of five were assessed[17].

Table 2. ADME properties of major phytochemicals

Compound	GI Absorption	Lipinski Violations	Drug-likeness
Quercetin	High	0	Good
Luteolin	High	0	Good
Kaempferol	High	0	Good
Apigenin	High	0	Good

3. PREDICTION OF POTENTIAL TARGETS

Potential protein targets of the identified compounds were predicted using SwissTargetPrediction with species limitation set to *Homo sapiens*[13]

4. COLLECTION OF INFLAMMATION-RELATED TARGETS

Inflammation-associated genes were collected from GeneCards and DisGeNET databases using the keyword "inflammation". Duplicate genes were removed to obtain a final list of inflammation-related targets[11,12].

5. IDENTIFICATION OF COMMON TARGETS

The predicted compound-related targets and inflammation-related genes were compared using Venny 2.1 software to identify overlapping targets[18].

6. CONSTRUCTION OF COMPOUND-TARGET NETWORK

The compound-target interaction network was constructed using Cytoscape version 3.9.1. Network topological parameters including degree centrality and betweenness centrality were analyzed to identify key compounds and targets[10].

7. PROTEIN-PROTEIN INTERACTION (PPI) ANALYSIS

The overlapping targets were imported into the STRING database with a confidence score ≥ 0.7 to generate a protein-protein interaction network[9]. The obtained network was visualized using Cytoscape software[10].

8. GO AND KEGG PATHWAY ENRICHMENT ANALYSIS

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using the Metascape platform[19] with a significance threshold of $p < 0.05$.

9. MOLECULAR DOCKING ANALYSIS

Molecular docking studies were performed using PyRx software employing the AutoDock Vina algorithm to validate interactions between major phytochemicals and hub target proteins[16,17]. Three-dimensional structures of TNF, PTGS2, and MAPK14 proteins were downloaded from protein data Bank (PDB). The chemical structure of selected phytochemicals were prepared and optimized prior to docking analysis. Docking interactions and binding affinities were visualized using PyMOL[18].

RESULTS

1. PHYTOCHEMICAL SCREENING

A total of 18 bioactive compounds were identified from *Dichrocephala integrifolia*, including flavonoids, sterols, and phenolic compounds. Among them, quercetin, luteolin, and kaempferol demonstrated favorable pharmacokinetics properties.

2. TARGET PREDICTION AND COMMON TARGET ANALYSIS

A total of 232 compound-related targets and 178 inflammatory-related genes were identified from multiple databases. Venn diagram analysis identified 21 overlapping targets, which were considered potential therapeutic targets and selected for subsequent network construction and pathway enrichment analyses.

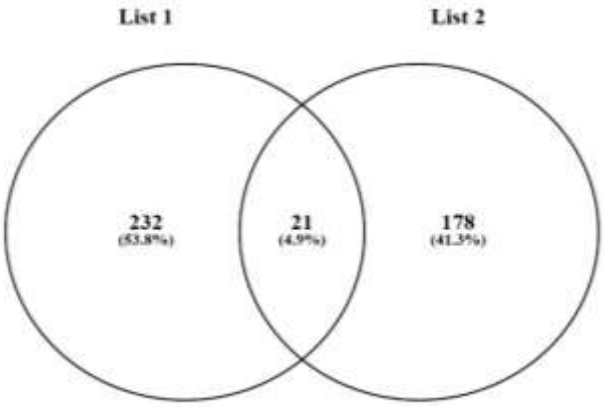

Figure 1. Venn diagram showing the overlap between phytochemical targets and inflammation-related genes. Targets predicted from bioactive compounds of *Dichrocephala integrifolia* were compared with inflammation-associated genes obtained from disease databases. The overlapping genes represent potential therapeutic targets responsible for the anti-inflammatory activity.

3. COMPOND-TARGET NETWORK ANALYSIS

The compound-target interaction network consisted of 21 nodes and 109 edges. Quercetin and luteolin exhibited the highest degree values, including strong multi-target interaction potential.

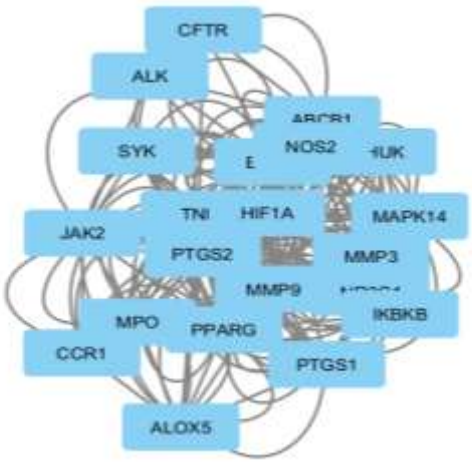

Figure 2. Compound–target interaction network constructed using Cytoscape software. Nodes represent phytochemicals and protein targets, while edges represent interactions.

4. PROTEIN-PROTEIN IDENTIFICATION (PPI) NETWORK ANALYSIS

The overlapping targets were imported into the STRING database to construct a protein–protein interaction (PPI) network. The network consisted of 21 nodes and 109 edges, with an average node degree of 10.4 and an average clustering coefficient of 0.778. The PPI enrichment p-value was found to be < 1.0e−16, indicating that the proteins were biologically connected significantly more than expected for a random set of proteins.

Topological analysis identified PTGS2, TNF, EGFR, MMP9, HIF1A, and MAPK14 as major hub genes based on degree centrality values.

Table 3. Top Hub Genes Identified from PPI Analysis

GENE	DEGREE
TNF	38
PTGS2	36
MMP9	36
EGFR	32
PPARG	32
HIF1A	30
MAPK14	22
NR3C1	20
JAK2	20
NOS2	20

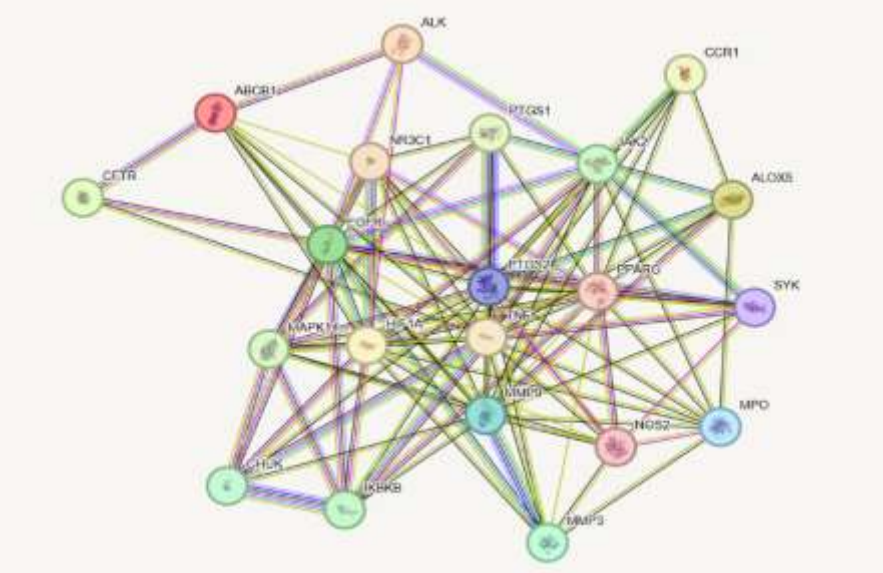

Figure 3. Protein–protein interaction (PPI) network of overlapping targets obtained from the STRING database. The network highlights interactions among inflammation-related proteins. Hub genes such as **TNF**, and **MAPK14** were identified based on topological parameters such as degree centrality.

5. GO ENRICHMENT ANALYSIS

Gene Ontology enrichment analysis indicated that the identified targets were mainly associated with:

- Inflammation response
- Cytokine-mediated signaling pathways
- Regulation of immune response
- Response to oxidative stress
- Cellular response to lipopolysaccharide

6. KEGG PATHWAY ENRICHMENT ANALYSIS

KEGG pathway analysis demonstrated significant enrichment in:

- TNF signaling pathway
- MAPK signaling pathway
- NF-$_K$B signaling pathway
- Cytokine-cytokine receptor interaction

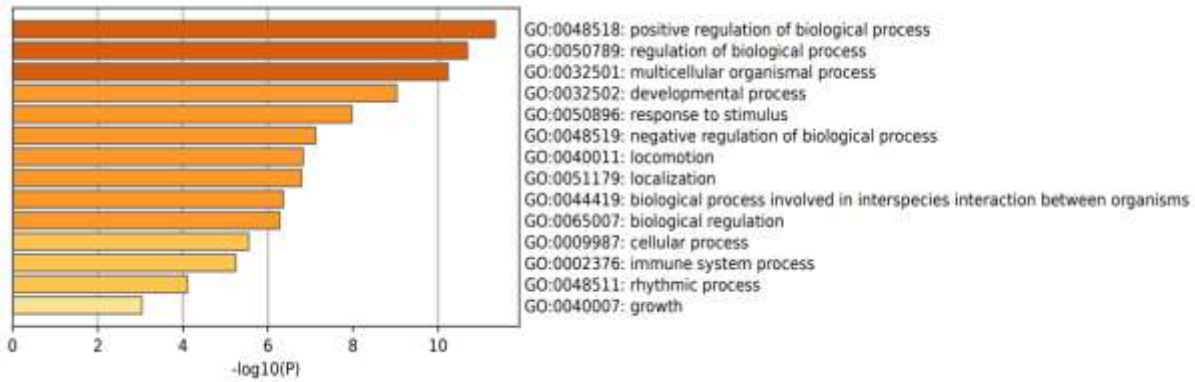

Figure 4. KEGG pathway enrichment analysis of the common targets. The enriched pathways include TNF signaling pathway, MAPK signaling pathway, and other inflammation-related biological pathways, suggesting that *Dichrocephala integrifolia* exerts therapeutic effects through multi-pathway regulation.

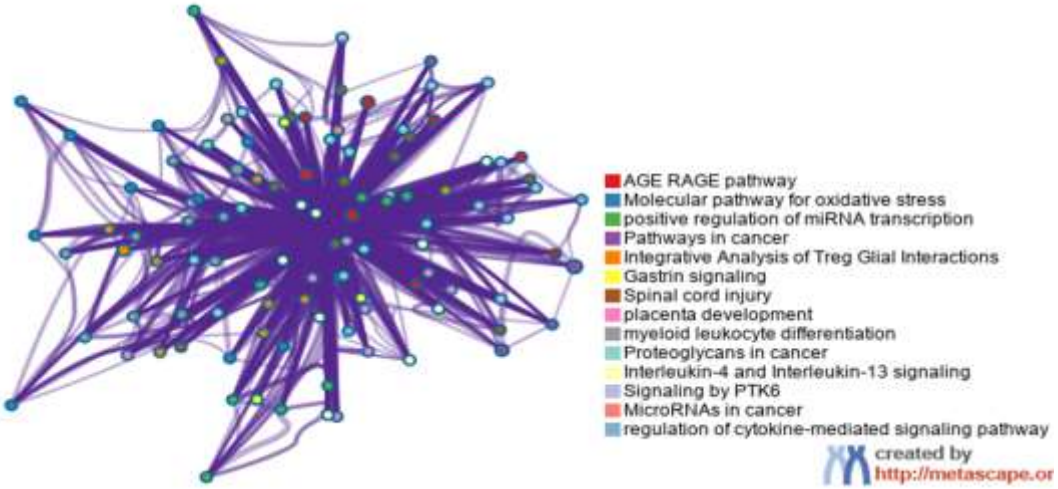

Figure 5. Functional enrichment interaction network of common targets generated using Metascape analysis. Different node colours represent enrichment biological pathways and functional clusters associated with inflammatory responses.

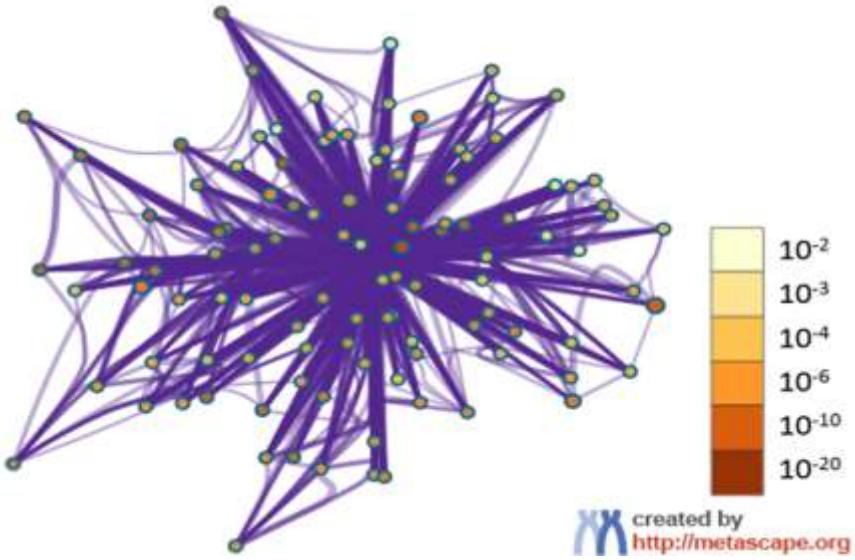

Figure 6. KEGG pathway enrichment interaction network showing significantly enriched pathways associated with the identified targets. Node colors represent pathway enrichment significance based on enrichment p-values

7. MOLECULAR DOCKING ANALYSIS

Molecular docking studies demonstrated strong binding interactions between major phytochemicals and hub proteins.

Table 7. Molecular Docking Results

COMPOUND	TARGET PROTEIN	BINDING ENERGY (kcal/mol)
Luteolin	2AZ5	-8.9
Quercetin	2AZ5	-8.8
Kaempferol	2AZ5	-8.0
Apigenin	2AZ5	-7.3

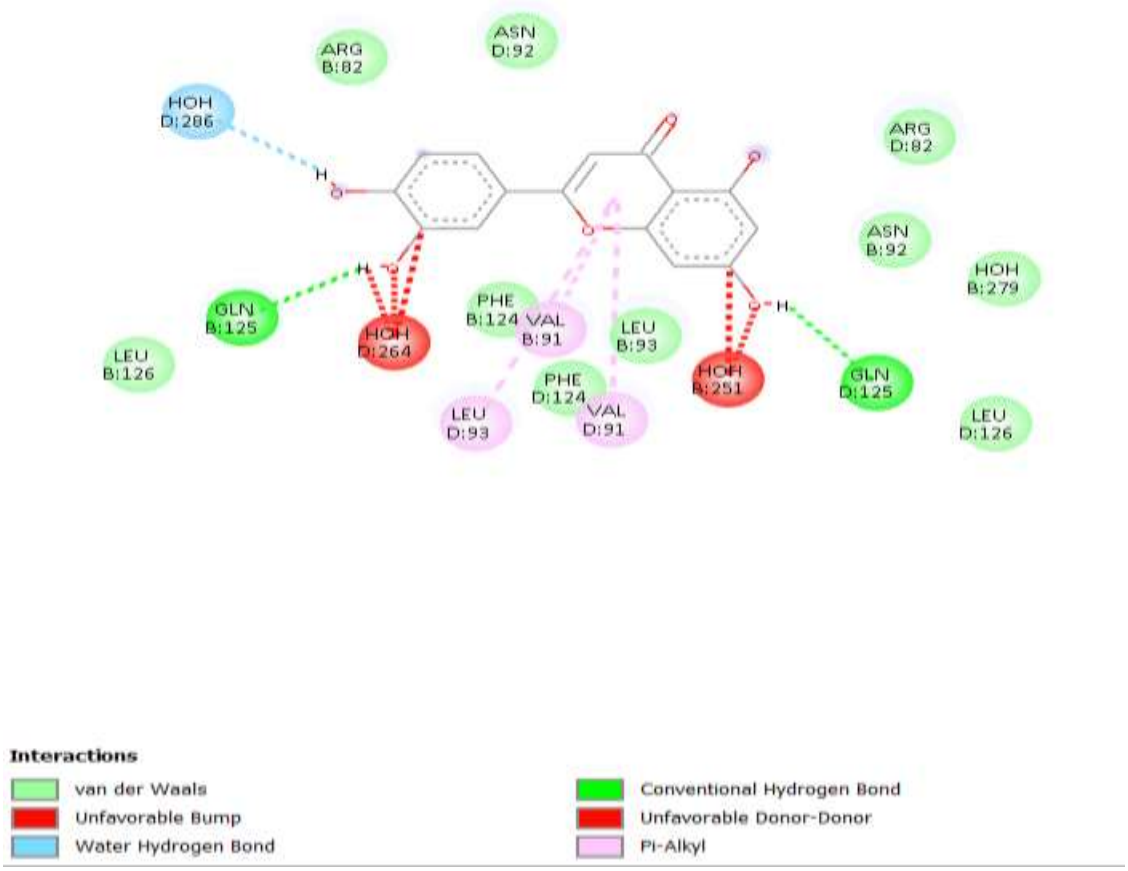

Figure 5. Molecular Docking Interactions

Docking visualization revealed hydrogen bonding and hydrophobic interactions between phytochemicals and target proteins.

DISCUSSION

The present study employed a network pharmacology and molecular docking approach to investigate the therapeutic potential of *Dichrocephala integrifolia* against inflammatory disorders. The findings demonstrated that the bioactive phytochemicals present in the plant may exert pharmacological effects through modulation of multiple targets and signaling pathways. This multi-target therapeutic mechanism supports the traditional medicinal applications of the plant and highlights the importance of herbal medicines in complex disease management.

Network pharmacology analysis identified several important target proteins associated with inflammation and immune response pathways. The enrichment analysis suggested the involvement of key biological pathways related to inflammatory regulation, cellular signaling, and immune modulation. These findings indicate that the therapeutic activity of *Dichrocephala integrifolia* may result from synergistic interactions among multiple phytoconstituents acting on various molecular targets simultaneously.

Molecular docking studies were performed to validate the interactions between selected phytochemicals and the target protein 2AZ5. Among the compounds analyzed, Luteolin showed the strongest binding affinity (-8.9 kcal/mol), followed by Quercetin (-8.8

kcal/mol), Apigenin (-7.3 kcal/mol), and Kaempferol (-8.0 kcal/mol). The negative binding energy values indicated stable ligand–protein interactions and favorable binding conformations within the active site of the target protein.

Interaction analysis further revealed the presence of hydrogen bonding, hydrophobic interactions, van der Waals interactions, and pi-alkyl interactions between the ligands and amino acid residues such as GLN125, LEU126, ASN92, ARG82, PHE124, VAL91, and LEU93. These interactions contribute to stabilization of the ligand–protein complex and may enhance the biological activity of the compounds.

The stronger binding affinities observed for flavonoid compounds such as Luteolin and Quercetin may be associated with the presence of multiple hydroxyl groups that facilitate stronger intermolecular interactions within the binding pocket. These findings are consistent with previous reports demonstrating the anti-inflammatory and antioxidant potential of flavonoid-rich medicinal plants.

Despite the promising in-silico findings, the present study has certain limitations. The results are based primarily on computational predictions and therefore require further experimental validation through *in-vitro* and *in-vivo* studies. Additional investigations focusing on pharmacological activity, toxicity assessment, pharmacokinetic properties, and clinical efficacy are necessary to confirm the therapeutic potential of the identified phytochemicals.

Overall, the study provides scientific evidence supporting the medicinal significance of Dichrocephala integrifolia and demonstrates the usefulness of integrated network pharmacology and molecular docking approaches in identifying potential therapeutic compounds and molecular mechanisms involved in inflammatory disorders.

LIMITATIONS OF THE STUDY

Although the present study provides important insights into the anti-inflammatory mechanisms of *Dichrocephala integrifolia*, certain limitations should be considered:

1. The study is entirely based on computational and in-silico analysis.
2. Experimental validation through in-vitro and in-vivo studies was not performed.
3. Target prediction depends on publicly available databases and algorithms.
4. Molecular docking results require biological confirmation.

Future studies involving experimental pharmacological investigations are necessary to validate the predicted therapeutical mechanisms.

CONCLUSION

This study employed an integrated network pharmacology and molecular docking approach to investigate the anti-inflammatory mechanisms of *Dichrocephala integrifolia*. Network pharmacology analysis identified multiple bioactive phytochemicals and their associated targets involved in key inflammatory pathways, highlighting the multi-component, multi-target, and multi-pathway therapeutic potential of the plant. The compound–target and protein–protein interaction networks revealed TNF, PTGS2, MMP9, EGFR, PPARG, HIF1A, and MAPK14 as important hub genes associated with inflammatory regulation. KEGG pathway enrichment analysis further indicated the involvement of TNF, MAPK, NF-$_K$B, and cytokine-mediated signaling pathways.

Molecular docking studies demonstrated favorable binding interactions of quercetin, luteolin, apigenin, and kaempferol with the selected target protein (PDB ID: 2AZ5), with luteolin exhibiting the strongest binding affinity. The observed hydrogen-bonding and hydrophobic interactions support the stability of the ligand–protein complexes and suggest potential inhibitory activity against inflammation-related targets.

Overall, the findings provide scientific support for the traditional medicinal use of *Dichrocephala integrifolia* and suggest that its anti-inflammatory activity may arise from the synergistic actions of multiple phytochemicals acting on interconnected molecular targets and pathways. Further in vitro and in vivo studies are required to validate these findings and confirm the therapeutic potential of the identified compounds.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are including in this manuscript. Additional datasets are available from the corresponding author upon reasonable request.

ETHICAL STATEMENT

The present study does not involve human participants or experimental animals. All analyses were performed using publicly available databases and computational tools.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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