

Research Article**Integrative Network Pharmacology and Molecular Docking Approach to Explore Anti-Psoriatic Phytochemicals from *Murraya koenigii*****Sakshi Priyadarshni¹, Dr. Nalini K. Sahoo¹**¹Faculty of pharmaceutical sciences¹Rama UniversityEmail ID- sakshipriyadarshni904@gmail.com

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Abstract- Psoriasis is a chronic immune-mediated inflammatory skin disorder characterized by abnormal keratinocyte proliferation and dysregulation of multiple inflammatory signaling pathways. Conventional therapies often target single molecular mechanisms and are associated with adverse effects and frequent relapse, necessitating the development of safer multi-target therapeutic strategies. *Murraya koenigii* (L.) Spreng., a medicinal plant rich in carbazole alkaloids and phenolic compounds, possesses well-documented anti-inflammatory and antioxidant properties; however, its anti-psoriatic potential remains inadequately explored. The present study investigated the therapeutic potential of *Murraya koenigii* using an integrative approach combining network pharmacology, molecular docking, phytochemical isolation, and in-vitro

validation. A total of 215 phytoconstituents were identified and screened based on pharmacokinetic and toxicity parameters, resulting in 16 drug-like compounds selected for further investigation. Network pharmacology analysis revealed 147 overlapping targets between phytochemical-associated proteins and psoriasis-related genes, with key hub proteins including TNF, IL6, IL17A, STAT3, MAPK1, and AKT1. Functional enrichment analysis demonstrated significant involvement of IL-17, TNF, NF- κ B, JAK-STAT, and MAPK signaling pathways associated with psoriasis pathogenesis. Molecular docking studies indicated strong binding affinity of Murrayazolinine toward IL-17RA, suggesting potential inhibition of inflammatory signaling cascades. The lead compound Murrayazolinine was

successfully isolated and structurally characterized using chromatographic and spectroscopic techniques. In-vitro studies using HaCaT keratinocyte cells demonstrated dose-dependent inhibition of cell proliferation with an IC₅₀ value of 68.4 μ M, indicating controlled antiproliferative activity with minimal cytotoxicity. Collectively, these findings highlight the multi-target anti-psoriatic potential of *Murraya koenigii* and identify Murrayazolinine as a promising phytochemical candidate for further preclinical and clinical development.

Keywords: Psoriasis; *Murraya koenigii*; Network pharmacology; Molecular docking; Murrayazolinine; Phytochemical isolation; Anti-psoriatic activity; Herbal drug discovery.

1. INTRODUCTION

Psoriasis is a long-standing inflammatory skin disease characterized by abnormal proliferation of keratinocytes, impaired epidermal differentiation, and persistent immune activation. It is now widely recognized as a systemic immune-mediated disorder rather than merely a dermatological condition.^[1] The pathogenesis of psoriasis involves complex interactions among genetic predisposition, environmental triggers, oxidative stress, and dysregulated immune responses. Activation of inflammatory cytokines,

particularly those associated with the IL-23/IL-17/TNF- α signaling cascade, promotes chronic inflammation and accelerated epidermal turnover, resulting in the formation of characteristic psoriatic plaques.^[2] Although multiple therapeutic options are available for psoriasis management, achieving sustained remission remains difficult. Common treatment approaches such as topical corticosteroids, phototherapy, systemic immunosuppressants, and biologic agents mainly focus on controlling disease symptoms.^[3] Long-term administration of these therapies is often restricted by adverse drug reactions, treatment resistance, high economic burden, and recurrence following therapy withdrawal. Furthermore, most conventional drugs are designed to act on individual molecular targets, which may not adequately address the multifactorial nature of psoriasis involving numerous interconnected signaling pathways. Consequently, there is increasing interest in therapeutic strategies capable of simultaneously modulating multiple pathogenic mechanisms. Plant-derived medicines represent an important source of biologically active compounds with multitarget pharmacological potential.^[4] Medicinal plants contain diverse secondary metabolites that can influence inflammatory, oxidative, and immune-regulatory pathways collectively. *Murraya*

koenigii (L.) Spreng., commonly known as curry leaf, has been extensively used in traditional medicinal systems for treating inflammatory and skin-related disorders. Phytochemical investigations have revealed the presence of carbazole alkaloids, flavonoids, terpenoids, and phenolic constituents exhibiting antioxidant, anti-inflammatory, and antiproliferative activities.^[5] Despite these promising pharmacological properties, systematic investigation of its therapeutic relevance against psoriasis using modern integrative approaches remains limited. Recent developments in computational pharmacology have enabled more efficient exploration of herbal medicines through systems-level analysis.^[6] Network pharmacology provides a holistic framework for identifying relationships among bioactive compounds, molecular targets, and disease pathways, thereby facilitating understanding of multi-component therapeutic actions. Molecular docking further strengthens target validation by predicting binding affinity and interaction patterns between phytochemicals and disease-associated proteins.^[7] The integration of computational prediction with experimental validation improves reliability and accelerates natural product-based drug discovery. In this context, the present study aimed to evaluate the anti-

psoriatic potential of *Murraya koenigii* using an integrative workflow combining network pharmacology analysis, molecular docking studies, phytochemical isolation, structural characterization, and in-vitro biological assessment.^[8] This comprehensive strategy was employed to identify active phytoconstituents, elucidate their underlying molecular mechanisms, and experimentally validate their biological activity. The outcomes of this investigation may contribute to the development of safer and cost-effective plant-based therapeutic candidates for psoriasis while providing scientific support for the medicinal relevance of *Murraya koenigii*.

2. MATERIAL AND METHODS

Chemicals and Reagents

All solvents and reagents used were of analytical reagent (AR) grade and purchased from Merck (Mumbai, India) and Sigma-Aldrich (St. Louis, MO, USA). Silica gel (60–120 mesh) and silica gel 60 F₂₅₄ TLC plates were used for chromatographic separation. Cell culture media (DMEM), fetal bovine serum (FBS), penicillin–streptomycin solution, trypsin–EDTA, phosphate-buffered saline (PBS), and MTT reagent were procured from HiMedia Laboratories (India). All aqueous solutions were prepared using double-distilled water.

Network Pharmacology Analysis

Identification and Screening of Bioactive Compounds

Phytochemical constituents of *Murraya koenigii* were retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>), IMPPAT (<https://cb.imsc.res.in/imppat/>), and PhytoHub (<https://phytohub.eu/>) databases. Canonical SMILES and structural data were collected for each compound. Drug-likeness and pharmacokinetic properties were evaluated using SwissADME and ADMETlab 2.0. (<https://admetmesh.scbdd.com/>) Lipinski's Rule of Five, Veber rule, Ghose filter, and Egan criteria were applied to screen compounds with favorable oral bioavailability and acceptable ADMET profiles.^[9]

Target Prediction and Disease Gene Identification

Potential protein targets of screened phytochemicals were predicted using SwissTargetPrediction (<https://www.swisstargetprediction.ch/>) and STITCH databases (<https://stitch-db.org/>) with *Homo sapiens* selected as the organism. Psoriasis-associated genes were retrieved from GeneCards (<https://www.genecards.org/>), DisGeNET (<https://disgenet.com/>), and OMIM databases (<https://www.omim.org/>). Common targets between phytochemical-related proteins and psoriasis-associated

genes were identified through Venn diagram analysis. Target standardization was performed using UniProtKB (<https://www.uniprot.org/>).^[10]

Protein–Protein Interaction (PPI) Network Construction

The overlapping targets were submitted to the STRING database (<https://string-db.org/>) (confidence score ≥ 0.4) to construct a protein–protein interaction network. The network was visualized and analyzed using Cytoscape (v3.7.2) (<https://cytoscape.org/>). Topological parameters including degree centrality, betweenness centrality, closeness centrality, and maximal clique centrality were calculated to identify hub genes.^[11]

Functional Enrichment and Pathway Analysis

Gene Ontology (GO) enrichment analysis covering Biological Process (BP), Molecular Function (MF), and Cellular Component (CC) categories was performed using DAVID. KEGG pathway enrichment analysis was conducted to identify significantly enriched signaling pathways. A p -value < 0.05 was considered statistically significant.^[12]

Molecular Docking Studies

The three-dimensional crystal structures of selected psoriasis-related target proteins were retrieved from the Protein Data Bank (PDB) (<https://www.rcsb.org/>), ensuring the selection of structures with a resolution

of ≤ 2.5 Å to maintain structural accuracy and reliability. Protein preparation was carried out using the UCSF Chimera platform (<https://www.cgl.ucsf.edu/chimera/>), where co-crystallized water molecules and unwanted heteroatoms were removed, followed by the addition of polar hydrogen atoms and assignment of appropriate partial charges to stabilize the protein structure for docking analysis. The three-dimensional structure of the phytoconstituent Murrayazolinine was obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and subjected to energy minimization using Chem3D (<https://www.wavemetrics.com/project/Chem3D>) to achieve a stable conformational geometry. The optimized ligand structure was subsequently converted into PDB format using AutoDock Tools (<https://autodock.scripps.edu/>), enabling compatibility with the docking platform. Molecular docking simulations were performed using AutoDock Vina to evaluate the binding interactions between the prepared proteins and ligand. Grid boxes were carefully defined around the active binding pockets of the target proteins, and exhaustiveness parameters were increased to ensure comprehensive conformational sampling. Binding affinity values expressed in kcal/mol were

recorded, and the optimal binding pose was selected based on minimum binding free energy and favorable interaction profiles. The resulting protein–ligand complexes were further analyzed and visualized using PyMOL (<https://www.pymol.org/>) and Discovery Studio Visualizer (<https://discover.3ds.com/>) to identify key interactions such as hydrogen bonding, hydrophobic contacts, π – π stacking, and electrostatic interactions.^[13,14]

Plant Material Collection and Authentication

Fresh leaves of *Murraya koenigii* (L.) Spreng. were collected during the vegetative season from a local cultivated source in Hardaspur, India. Botanical authentication was performed by a qualified taxonomist, and a voucher specimen was deposited in the departmental herbarium for future reference. The leaves were washed thoroughly with distilled water to remove debris and shade-dried at ambient temperature (25–30 °C) under well-ventilated conditions for 10–14 days. The dried material was pulverized using a mechanical grinder and stored in airtight containers protected from light and moisture until extraction.^[15]

Extraction and Fractionation

Approximately 500 g of powdered plant material was subjected to Soxhlet extraction. Initially, defatting was carried

out using petroleum ether (60–80 °C) for 6–8 h to remove lipophilic impurities. The defatted marc was air-dried and subsequently extracted with methanol for 24 h until solvent in the siphon tube appeared colorless, indicating exhaustive extraction. The methanolic extract was filtered and concentrated under reduced pressure using a rotary vacuum evaporator at 40–45 °C to obtain a semi-solid crude extract. The extract was stored at 4 °C until further processing.^[16]

Alkaloid Enrichment by Acid–Base Partitioning

To enrich alkaloidal constituents, the concentrated methanolic extract was dissolved in 2% hydrochloric acid and stirred for 1 h. The acidic solution was filtered and basified to pH 9–10 using ammonium hydroxide. The liberated free-base alkaloids were extracted with chloroform (3 × 100 mL). The combined organic layers were washed with distilled water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to yield the total alkaloidal fraction.^[17]

Isolation and Purification of Murrayazolinine

The alkaloidal fraction was subjected to silica gel column chromatography (60 × 3 cm; 60–120 mesh). Elution was performed using chloroform followed by gradient mixtures of chloroform:methanol (98:2 to 90:10, v/v). Fractions were collected

systematically and monitored by thin layer chromatography (TLC). TLC analysis was performed using chloroform:methanol (9:1, v/v) as the mobile phase. Plates were visualized under UV light (254 nm and 366 nm) and sprayed with Dragendorff's reagent for alkaloid detection. Fractions exhibiting identical R_f values were pooled and concentrated. Final purification was achieved by recrystallization in methanol to obtain Murrayazolinine as a pale-yellow crystalline solid. Purity was confirmed by TLC homogeneity and sharp melting point determination.^[18]

Spectral Characterization

Spectral characterization of the purified compound was carried out using Fourier Transform Infrared (FT-IR) and UV–Visible spectroscopy techniques to confirm its structural and electronic properties. FT-IR analysis was performed using a Bruker Alpha II FT-IR Spectrophotometer employing the potassium bromide (KBr) pellet method. The spectrum was recorded within the range of 4000–400 cm^{−1} to identify characteristic absorption bands corresponding to various functional groups present in the compound. The obtained peaks were carefully interpreted to determine the presence of specific chemical bonds and functional moieties responsible for the molecular structure. For electronic spectral analysis, UV–Visible spectroscopy was conducted using a Shimadzu UV-1800

UV–Visible Spectrophotometer. The purified compound was dissolved in methanol to prepare an appropriate analytical solution, and spectral scanning was carried out in the wavelength range of 200–400 nm using methanol as a blank. The recorded absorption maxima provided information regarding electronic transitions, particularly π – π^* and n – π^* transitions, thereby supporting the structural confirmation and purity assessment of the compound. Together, FT-IR and UV–Visible spectral analyses served as essential analytical tools for validating the chemical identity and functional characteristics of the isolated bioactive molecule.^[19,20]

In Vitro Anti-Psoriatic Evaluation

Human keratinocyte HaCaT cells were cultured under standard laboratory conditions using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin to maintain optimal cellular growth and sterility. The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO₂ to ensure physiological conditions suitable for keratinocyte proliferation and maintenance. For evaluation of cytotoxic and antiproliferative effects, an MTT assay was performed. HaCaT cells were seeded into 96-well culture plates and exposed to varying concentrations of Murrayazolinine

for 24 h. Following treatment, MTT reagent (0.5 mg/mL) was added to each well and incubated for an additional 4 h to allow mitochondrial reduction of MTT into insoluble formazan crystals by viable cells.^[21] Subsequently, the formed crystals were dissolved using dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated relative to untreated control cells to determine the effect of the test compound on cellular metabolic activity. All experiments were conducted in triplicate to ensure reproducibility and reliability of results. The obtained data were expressed as mean $\pm$ standard deviation (SD), and statistical analysis was performed using GraphPad Prism software. Differences among experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by appropriate post hoc comparisons, with a p-value less than 0.05 considered statistically significant.^[22]

3. RESULT AND DISCUSSION

Network Pharmacology

Collection and Screening of active phytochemical

Phytoconstituents of *Murraya koenigii* were retrieved from the IMPPAT database to construct a comprehensive compound library for in-silico analysis. A total of 215 secondary metabolites, including carbazole alkaloids, flavonoids, terpenoids,

polyphenols, sterols, and glycosides, were identified. All compounds were subjected to pharmacokinetic evaluation using SwissADME based on Lipinski's Rule of Five, Ghose, Veber, Egan, and Muegge criteria to assess drug-likeness and oral bioavailability. Following primary screening, 58 compounds satisfied acceptable physicochemical properties. These compounds were further evaluated

for toxicity risks including hepatotoxicity, mutagenicity, carcinogenicity, and immunotoxicity. After elimination of compounds showing toxicity alerts or PAINS interference, 16 phytoconstituents exhibiting favorable pharmacokinetic and safety profiles were selected for subsequent network pharmacology and molecular docking studies.

Table 1. Screened phytoconstituents selected for further study.

Compound	Molecular weight	Water solubility	Druglikeness	GI absorption
Myrcenol	217.24	Very soluble	Yes	High
Murrayacinine	245.3	Soluble	Yes	High
Murrayazolinine	203.22	Very soluble	Yes	High
Koenigicine	472.7	Poorly soluble	Yes	High
Currayanine	442.72	Poorly soluble	Yes	Low
Bicyclogermacrene	89.09	Highly soluble	Yes	High
Koenigine	532.62	Soluble	Yes	High
Murrayacine	404.5	Soluble	Yes	High
Mukoline	468.75	Poorly soluble	Yes	Low
Mahanimbine	426.72	Poorly soluble	Yes	Low
Eudesmane	154.12	Very soluble	Yes	High
Mukonicine	154.12	Very soluble	Yes	High

beta-Cubebene	456.7	Poorly soluble	Yes	Low
Isomurrayazoline	426.7	Poorly soluble	Yes	Low
alpha-Muurolol	468.8	Poorly soluble	Yes	Low
epsilon-Muurolene	374.51	Soluble	Yes	High

Potential therapeutic targets of *Murraya koenigii* used in the treatment of Psoriasis

A total of 215 phytoconstituents were retrieved from the IMPPAT database and an additional eight compounds were identified through LC–MS analysis, resulting in 16 shortlisted phytocompounds for further investigation. Target prediction using SwissTargetPrediction identified 417 potential protein targets, which were reduced to 280 unique targets after removal

of duplicates. Concurrently, 6067 psoriasis-associated genes were compiled from DisGeNET (133 genes), GeneCards (5841 genes), and OMIM (93 genes) databases. Venn diagram analysis identified 147 overlapping targets between phytoconstituent-related and disease-associated genes (Figure 7.1.2), indicating potential therapeutic relevance of the selected compounds in psoriasis management.

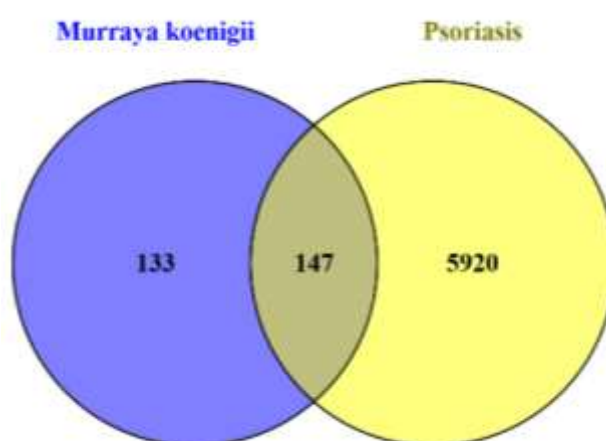


Figure 1. Venn diagram

Protein-Protein interaction (PPI) network construction visualization and analysis-

After merging and removing duplicates 147 Common targets identified were subjected to the STRING database to construct a PPI network, as shown in Figure 7.1.3, exported

in a TSV file format, which was imported into Cytoscape for analysis of Hubgenes and visualisation. The core targets identified were TNF, IL6, IL17A, IL23A, STAT3, MAPK1, AKT1, RELA, TP53, EGFR.

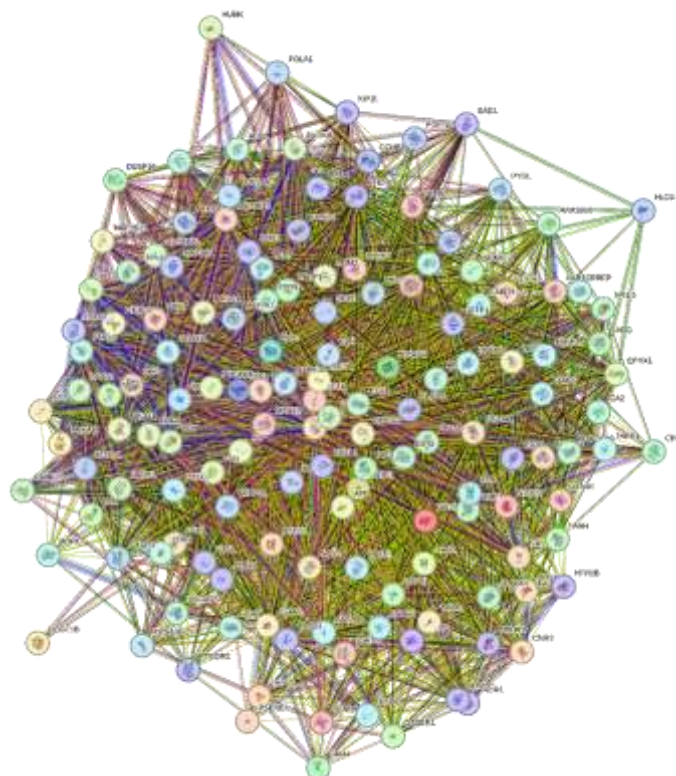


Figure 2. Protein-Protein interaction (PPI) network

Gene Ontology (GO) Functional Annotation Enrichment Analysis

Gene Ontology (GO) enrichment analysis of overlapping targets was performed to investigate their functional involvement in psoriasis and categorized into Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). BP analysis demonstrated significant enrichment in calcium ion transport, calcium homeostasis, and regulation of inflammatory responses, indicating the involvement of calcium-mediated signaling and immune regulation in keratinocyte proliferation and psoriasis

pathogenesis. CC analysis revealed predominant localization of targets within membrane rafts, plasma membrane microdomains, and cytoplasmic membrane regions, suggesting active participation in receptor-mediated signal transduction. MF enrichment highlighted transmembrane receptor protein tyrosine kinase activity, serine/threonine kinase activity, nuclear receptor activity, and ligand-activated transcription factor functions. Collectively, these findings indicate that the identified targets primarily regulate inflammatory signaling, membrane-associated receptor

activity, and kinase-driven pathways relevant to psoriasis progression.

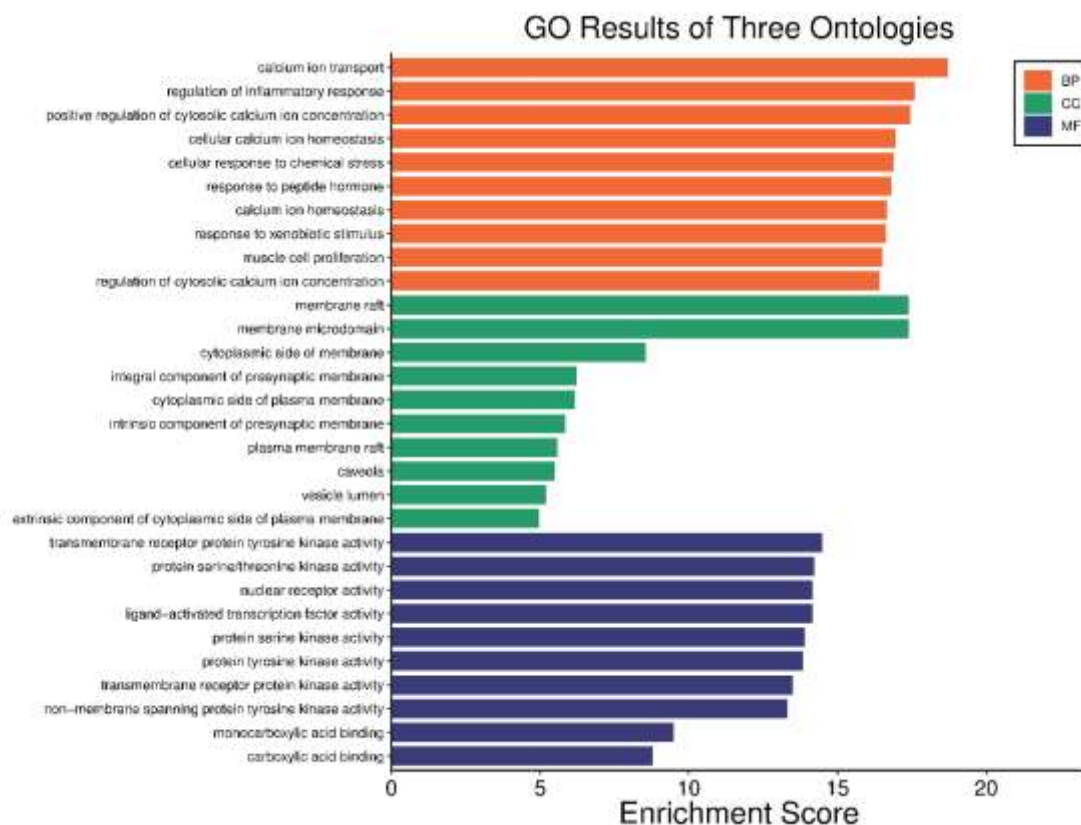


Figure 3. Bar plot of Biological Process (BP), Cellular Components (CC), and Molecular Function (MF).

KEGG signalling pathway enrichment analysis-

KEGG pathway enrichment analysis identified 186 significantly enriched pathways associated with immune and inflammatory signaling mechanisms relevant to psoriasis. Prominent pathways included IL-17, TNF, NF- κ B, JAK-STAT, MAPK signaling, and Th1/Th2 cell differentiation, which are critically involved in keratinocyte hyperproliferation, epidermal inflammation, and immune dysregulation.

Key enriched genes such as *TNF*, *IL6*, *IL17A*, *STAT3*, *JAK2*, *AKT1*, and *MAPK1* indicated active regulation of cytokine signaling, T-cell activation, and inflammatory responses. Additional enrichment in PD-1/PD-L1 checkpoint, insulin resistance, and neurotrophin signaling pathways suggested interactions between immune, metabolic, and stress-related mechanisms. Overall, the findings support the multi-target and multi-pathway therapeutic potential of the investigated phytochemicals against psoriasis.

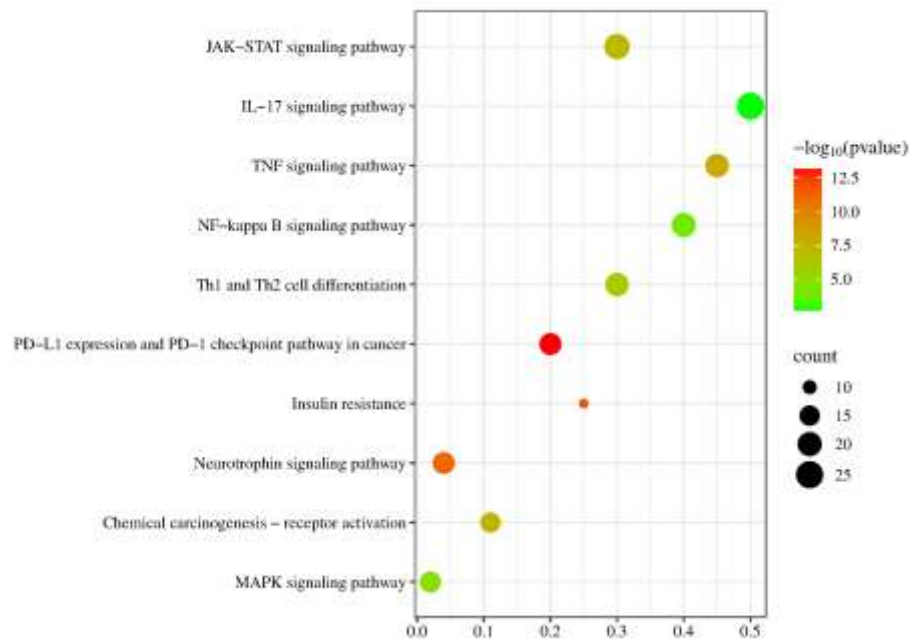


Figure 4. Bubble plot of the top 10 KEGG enrichment pathways

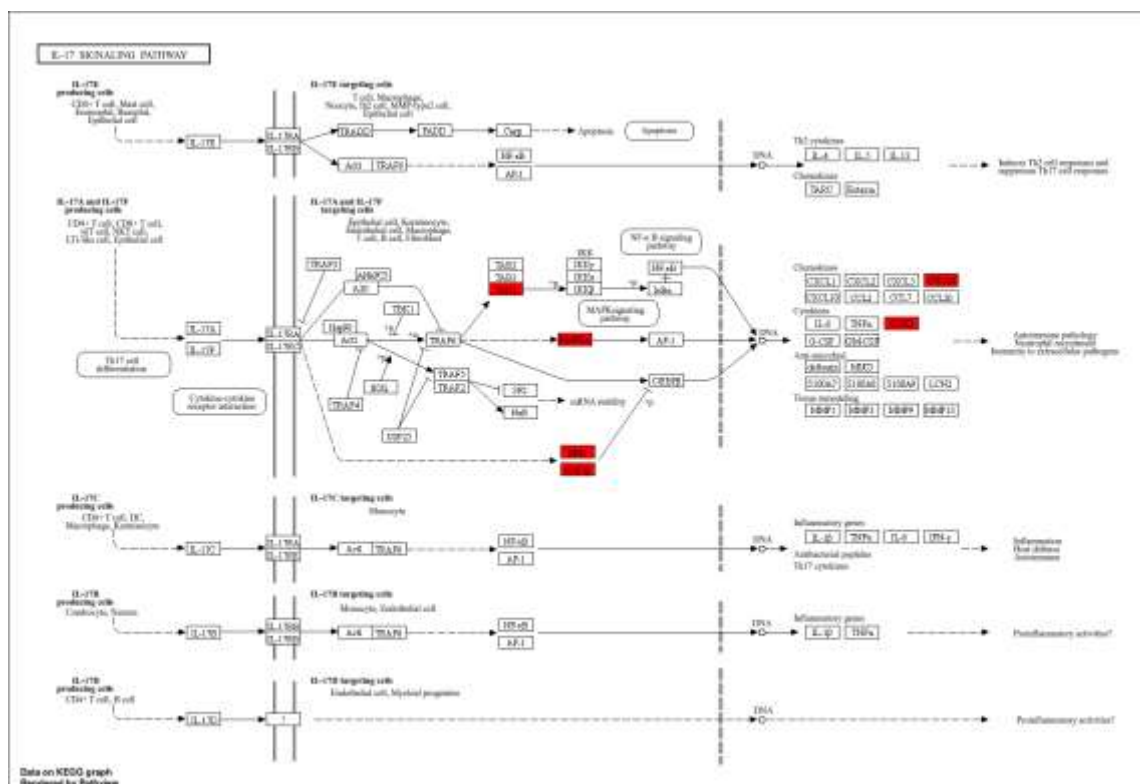


Figure 5. Signaling pathway associated with psoriasis.

Compound–Target–Disease Network-

To investigate the molecular mechanism underlying the anti-psoriatic activity of *Murraya koenigii*, a Compound–Target–Pathway–Disease (CTPD) network was constructed using Cytoscape (v3.7.2). The network integrated bioactive phytoconstituents, psoriasis-associated targets, and significantly enriched signaling pathways, including IL-17, TNF, NF-κB, JAK–STAT, MAPK, T-cell receptor, and PD-1/PD-L1 pathways. The highly

interconnected network revealed that individual compounds interacted with multiple targets involved in diverse pathways, indicating a multi-component and multi-target therapeutic mechanism. Enrichment of inflammatory and immune-regulatory pathways suggests suppression of cytokine-mediated inflammation and regulation of keratinocyte proliferation, supporting the systems-level anti-psoriatic potential of *M. koenigii* phytoconstituents.

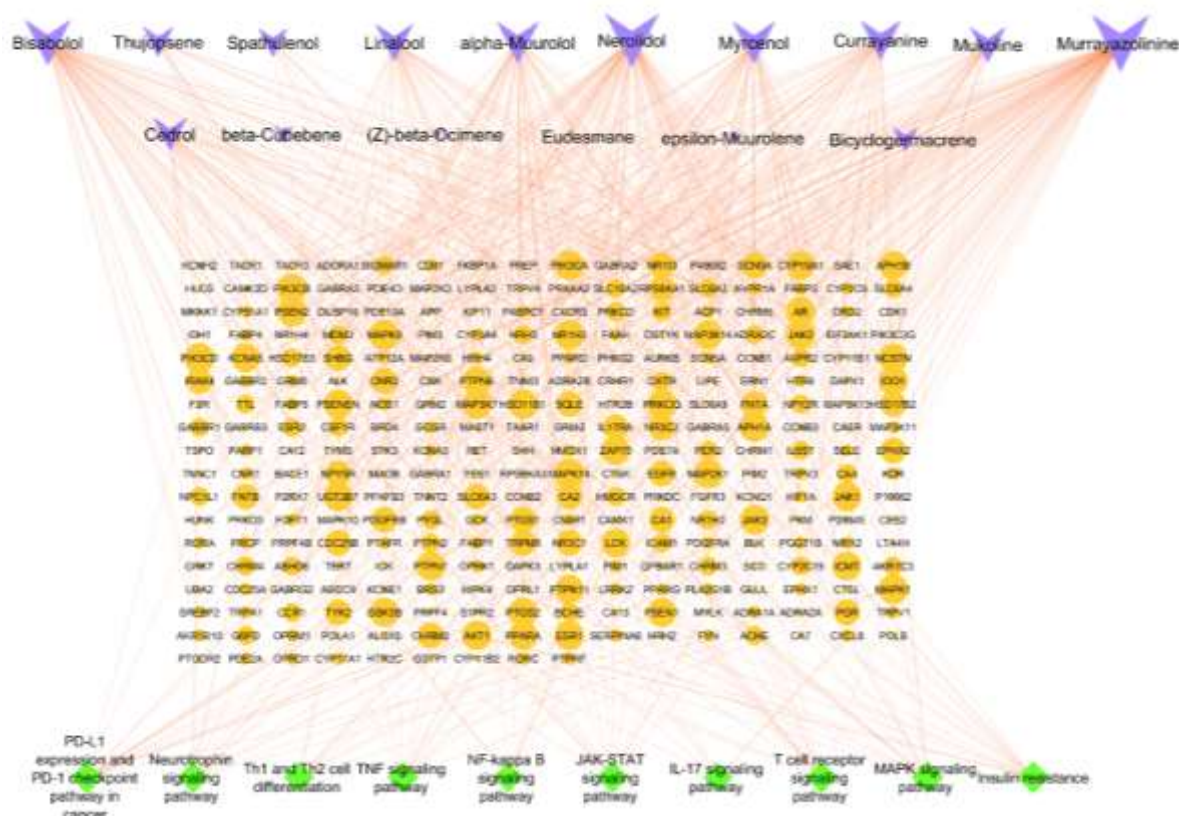


Figure 6. Drug-Target-Pathway network

Topology Analysis-

Topological analysis of the protein–protein interaction (PPI) network was conducted using Degree, Betweenness, Closeness, Bottleneck, and MCC algorithms to identify key hub proteins. Comparative evaluation revealed AKT1, IL17RA, HIF1A, and ESR1 as common high-ranking nodes across all parameters, indicating their strong regulatory influence within the

network. These proteins are associated with inflammatory signaling, cellular proliferation, hypoxia response, and immune–hormonal regulation relevant to psoriasis pathogenesis. Their consistent identification suggests that they function as central molecular hubs and potential therapeutic targets modulated by phytoconstituents of *Murraya koenigii*.

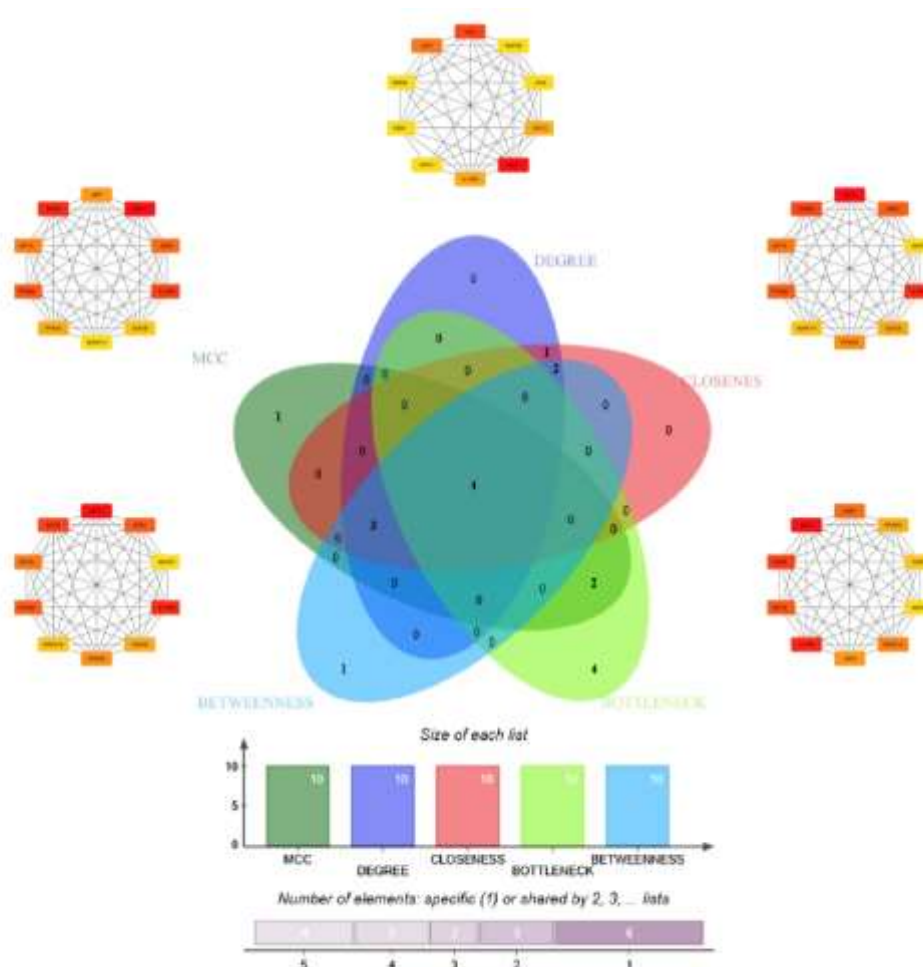


Figure 7. Common hub proteins identified by all five topological algorithms (MCC, Bottleneck, Closeness, Degree, and Betweenness).

Molecular docking-

The docking study was carried out for fifteen phytoconstituents with top ten network derived targets associated with

diabetic wound healing using AutoDock vina V. 1.2.6. The docking results were analyzed and evaluated in terms of binding energy.

Table No. 2. The binding interactions of phytochemical with IL-17RA enzyme.

Phytoconstituent	Target	Binding affinity (Kcal/mol)	Types of Interaction
Myrcenol	IL-17RA	-3.8	Van der Waals, Alkyl
Murrayazolinine	IL-17RA	-6.5	Van der Waals, Alkyl, Conventional Hydrogen Bond, Pi-Alkyl
Bicyclogermacrene	IL-17RA	-4.9	Van der Waals, Alkyl
Eudesmane	IL-17RA	-4.9	Van der Waals, Alkyl
beta-Cubebene	IL-17RA	-5.3	Van der Waals, Alkyl
Linalool	IL-17RA	-4.1	Van der Waals, Alkyl
Spathulenol	IL-17RA	-5.4	Van der Waals, Alkyl, Conventional Hydrogen Bond, Pi-Alkyl
alpha-Murolol	IL-17RA	-4.9	Van der Waals, Alkyl
Nerolidol	IL-17RA	-4.2	Van der Waals, Alkyl
epsilon-Murolene	IL-17RA	-4.6	Van der Waals, Pi-Alkyl, Alkyl
(Z)-beta-Ocimene	IL-17RA	-4.6	Van der Waals, Pi-Alkyl, Alkyl
Bisabolol	IL-17RA	-3.6	Van der Waals, Alkyl
Cedrol	IL-17RA	-5.1	Van der Waals, Alkyl, Conventional Hydrogen Bond

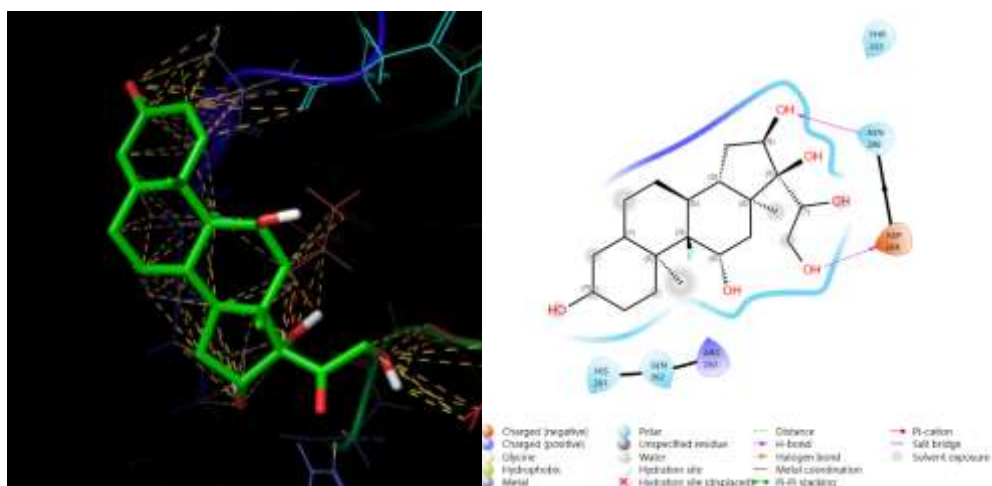


Figure 9. Docking poses of Triamcinolone with IL-17RA (PDB: 5N9B).

Collection and Extraction of the Lead Phytoconstituent (Murrayazolinine)

Leaves of *Calotropis gigantea* were collected from local area of the Hardaspur region in Uttar Pradesh, Maharashtra, India. Botanical authentication was performed by a qualified taxonomist, and a voucher specimen was deposited in the departmental herbarium for future reference.

Authentication and Processing of Plant Material

The collected leaves of *Murraya koenigii* were taxonomically authenticated and found to be free from morphological abnormalities or microbial contamination. Shade drying resulted in uniform dehydration without discoloration, and mechanical pulverization produced a homogeneous coarse powder suitable for solvent-based extraction. The processed plant material retained its characteristic aroma, indicating minimal loss of volatile constituents during drying.

Defatting and Crude Extraction Outcome

Defatting of powdered *Murraya koenigii* leaves using petroleum ether effectively removed lipophilic constituents, as indicated by solvent discoloration and the formation of a lighter defatted marc. This process improved subsequent extraction efficiency by minimizing interference from non-polar impurities. Methanolic extraction of the defatted material produced a dark brown viscous crude extract after solvent evaporation under reduced pressure. The extract showed no signs of thermal degradation and remained homogeneous and readily soluble in methanol, enabling further purification and fractionation of alkaloid-rich phytoconstituents.

Alkaloid Enrichment by Acid-Base Fractionation

Acid-base fractionation of the methanolic extract produced a well-defined alkaloid-enriched chloroform fraction. The successful partitioning of alkaloids into the organic phase indicated effective conversion of alkaloidal salts into their free-base forms. Concentration of the

chloroform layer yielded a brownish residue characteristic of total alkaloidal content.

TLC Profiling and Preliminary Identification

The alkaloidal extract exhibited well-resolved chromatographic bands when developed in an optimized solvent system. Visualization under ultraviolet light at 254 nm and 366 nm revealed distinct fluorescent and quenching spots, indicating the presence of multiple alkaloidal

constituents. Post-derivatization with Dragendorff's reagent resulted in the appearance of orange–brown colored spots, confirming the alkaloidal nature of the separated components. A prominent and reproducible spot was observed with a consistent R_f value across repeated runs, corresponding to literature-reported R_f values for Murrayazoline. The purified compound exhibited a single compact spot under all visualization conditions, indicating chromatographic purity.

Table No. 3. TLC Profile of Alkaloidal Extract.

Sample	Mobile Phase	Detection Method	R _f Value	Observation
Alkaloidal extract	Chloroform : Methanol (9:1)	UV 254 nm	0.47	Distinct dark spot
Alkaloidal extract	Chloroform : Methanol (9:1)	UV 366 nm	0.47	Blue fluorescent spot
Alkaloidal extract	Chloroform : Methanol (9:1)	Dragendorff's reagent	0.47	Orange–brown spot

Column Chromatographic Isolation

Column chromatographic separation of the alkaloid-enriched fraction resulted in efficient resolution of phytoconstituent bands, producing multiple fractions with distinct chromatographic patterns. TLC-guided analysis facilitated identification of fractions enriched with a single major alkaloidal component, characterized by a consistent R_f value, positive response to Dragendorff's reagent, and strong UV

visibility. Pooling of these similar fractions followed by concentration yielded a pale yellow solid, indicating successful isolation of a relatively pure alkaloid. Further TLC evaluation of the isolate showed a single compact spot under UV light (254 nm) and after derivatization, confirming chromatographic purity suitable for recrystallization and subsequent spectroscopic characterization.

Table 4. Summary of Column Chromatographic Isolation of Murrayazoline

Parameter	Observation
Stationary phase	Silica gel (60–120 mesh)
Elution behavior	Distinct fractionation observed
TLC monitoring	One major alkaloidal band identified
Dragendorff's reaction	Positive (orange–brown spot)
UV response	Quenching at 254 nm; fluorescence at 366 nm
Nature of isolated compound	Pale yellow solid
TLC purity	Single spot
Inference	Successful isolation of alkaloidal constituent

Purification and Physical Characteristics

Recrystallization of the column-purified alkaloidal fraction yielded well-defined pale yellow crystals, indicating effective removal of residual impurities. The purified compound exhibited a sharp and consistent melting point, suggesting high chemical purity. TLC analysis demonstrated a single compact spot under UV illumination (254 and 366 nm) along with a positive

Dragendorff's reagent response, confirming its alkaloidal nature and chromatographic homogeneity. The physicochemical characteristics of the isolated crystals were consistent with reported properties of Murrayazoline. The compound remained stable under ambient laboratory conditions and was considered suitable for further spectroscopic characterization and biological evaluation

Table 5. Physical Characteristics of Purified Murrayazolinine

Parameter	Observation
Physical state	Crystalline solid
Colour	Pale yellow
Odour	Odourless
Nature	Alkaloidal
TLC behavior	Single compact spot
Dragendorff's test	Positive
Melting behavior	Sharp melting point
Stability	Stable at room temperature
Purity inference	High purity

Table No. 6. TLC Profile of Alkaloidal Extract.

Sample	Mobile Phase	Detection Method	Rf Value	Observation
Isolated Murrayazolinine	Chloroform : Methanol (9:1)	UV 254 nm	0.59	Single compact spot
Isolated Murrayazolinine	Chloroform : Methanol (9:1)	Dragendorff's reagent	0.59	Intense orange–brown spot

Spectral Analysis

FT-IR Analysis of Murrayazolinine

The FT-IR spectrum of Murrayazolinine exhibits a broad absorption band around 3400 cm^{-1} , corresponding to overlapping phenolic O–H and secondary amine N–H stretching vibrations characteristic of the carbazole framework. Aromatic C–H stretching is observed near 3060 cm^{-1} , while aliphatic C–H stretching bands appear in the $2950\text{--}2870\text{ cm}^{-1}$ region, confirming the presence of alkyl

substituents. The strong absorption at $\sim 1600\text{ cm}^{-1}$ is attributed to aromatic C=C stretching within the carbazole nucleus. A prominent band at $\sim 1260\text{ cm}^{-1}$ corresponds to C–O stretching, consistent with the phenolic or ether functionality of the molecule, and the C–N stretching vibration of the secondary amine appears near 1100 cm^{-1} . Out-of-plane aromatic C–H bending vibrations in the $820\text{--}750\text{ cm}^{-1}$ region further support the substituted polycyclic aromatic structure of Murrayazolinine.

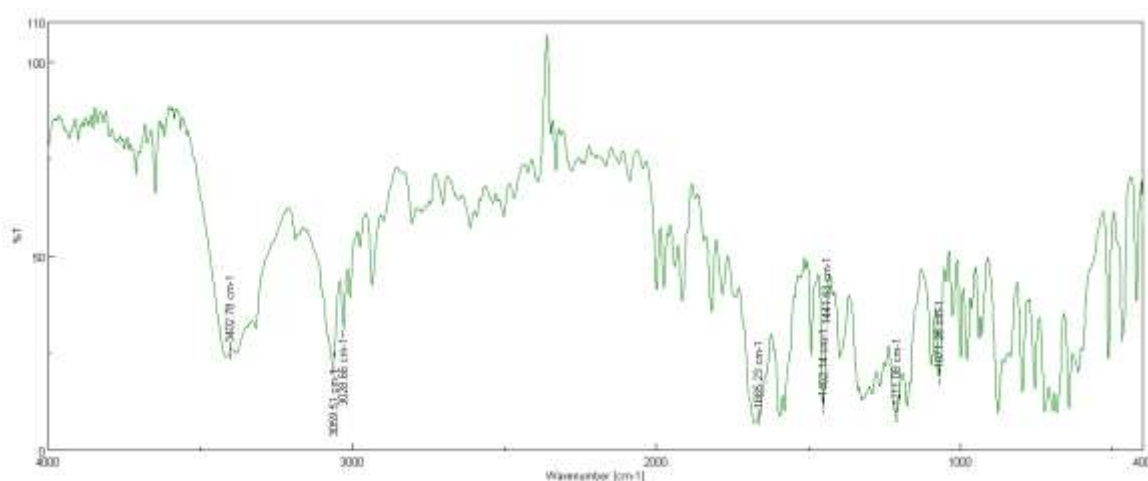


Figure 10. FT-IR spectrum of Murrayazolinine showing characteristic O–H/N–H, aromatic C=C, and C–O stretching bands consistent with the carbazole framework.

UV–Vis Spectral Analysis of Murrayazolinine

The UV–Vis spectrum of Murrayazolinine exhibits a strong absorption maximum at 246 nm, attributed to $\pi \rightarrow \pi^*$ electronic transitions within the conjugated aromatic carbazole framework. A second absorption

band at 311 nm is assigned to $n \rightarrow \pi^*$ transitions involving the lone pair electrons of heteroatoms (phenolic oxygen and carbazole nitrogen). These spectral features indicate an extended conjugated chromophore and are consistent with the characteristic electronic structure of

carbazole alkaloids, thereby supporting the structural identity of Murrayazoline

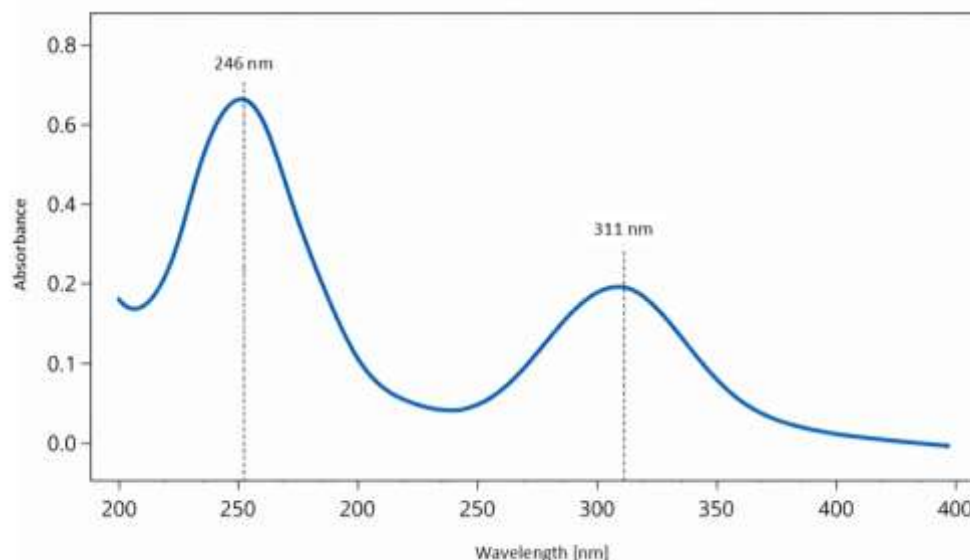


Figure 11. UV–Vis absorption spectrum of Murrayazoline displaying major absorption maxima at 246 nm and 311 nm, characteristic of conjugated carbazole chromophores.

Cell Line Study-

Effect of Murrayazoline on HaCaT

Cell Viability

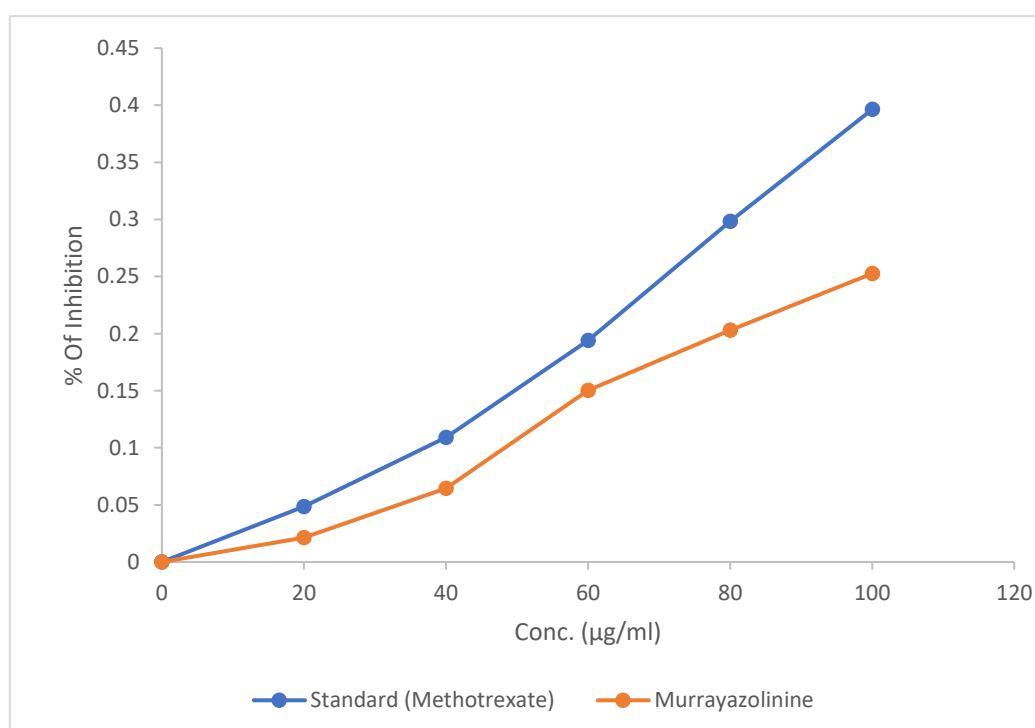
The cytotoxic and anti-proliferative effects of Murrayazoline were evaluated using the MTT assay on HaCaT human

keratinocyte cells after 24 h exposure. Cells were treated with increasing concentrations of Murrayazoline (1–100 μ M), and cell viability was expressed as a percentage relative to untreated control cells

Table 7. Effect of Murrayazoline on HaCaT Cell Viability

SR NO	SAMPLE CODE	Conc. (μ g/ml)	OD 1	OD 2	OD 3	Mean	% Of Inhibition	% Of Viability
1	Control	–	1.532	–	–	–	–	–
2	Standard (Triamcinolone)	20	1.458	1.457	1.459	1.458	4.84%	95.16%
		40	1.365	1.366	1.364	1.365	10.91%	89.09%

3		60	1.235	1.236	1.234	1.235	19.40%	80.60%
		80	1.075	1.074	1.076	1.075	29.82%	70.18%
		100	0.925	0.924	0.926	0.925	39.63%	60.37%
		20	1.438	1.437	1.439	1.438	2.14%	93.86%
		40	1.372	1.371	1.373	1.372	6.45%	89.55%
		60	1.302	1.301	1.303	1.302	15.02%	84.98%
	Murrayazolinine	80	1.221	1.220	1.222	1.221	20.30%	79.70%
		100	1.145	1.146	1.144	1.145	25.26%	74.74%



Values are expressed as mean $\pm$ SD ($n = 3$)

Figure 12. Dose–response curve of Murrayazolinine on HaCaT cells after 24 h treatment.

IC₅₀ Determination

The IC₅₀ value of Murrayazolinine was calculated from the dose–response curve

plotted between concentration (µM) and percentage inhibition of HaCaT cell

proliferation. Nonlinear regression analysis revealed that Murrayazoline inhibited 50% of HaCaT cell viability at a concentration of:

IC₅₀ = 68.4 μM (24 h exposure)

This IC₅₀ value indicates moderate anti-proliferative potency with a favorable therapeutic window, as lower concentrations exhibited minimal cytotoxicity while still exerting biological activity.

Morphological Assessment

Microscopic examination of HaCaT cells revealed that untreated control cells exhibited normal polygonal morphology with intact intercellular junctions. Cells treated with Murrayazoline at concentrations up to 10 μM maintained normal morphology, confirming low cytotoxicity. At higher concentrations (25–100 μM), cells displayed reduced density, cellular shrinkage, and partial loss of adherence, consistent with growth inhibition rather than acute cytotoxic damage. Murrayazoline demonstrated a concentration-dependent inhibition of HaCaT keratinocyte proliferation following 24 h exposure. Cell viability remained above 85% at concentrations ≤10 μM, indicating low cytotoxicity. Significant growth inhibition was observed at concentrations ≥25 μM, with a maximum inhibition of 58.37% at 100 μM. The IC₅₀ value was determined to be 68.4 μM. Morphological observations corroborated the MTT results, showing reduced cell density without extensive membrane damage, suggesting a selective anti-proliferative effect. These findings support the potential anti-psoriatic activity of Murrayazoline through modulation of keratinocyte hyperproliferation.

4. CONCLUSION

The present study systematically explored the anti-psoriatic potential of *Murraya koenigii* through an integrated approach combining network pharmacology, molecular docking, phytochemical isolation, spectroscopic characterization, and in-vitro biological evaluation. Network pharmacology analysis demonstrated that bioactive phytoconstituents of *M. koenigii* modulate multiple psoriasis-associated molecular targets, particularly TNF, IL6, IL17A, STAT3, MAPK1, and AKT1, which are critically involved in inflammatory signaling and keratinocyte hyperproliferation. Enrichment and pathway analyses further revealed significant regulation of IL-17, TNF, NF-κB, JAK–STAT, and MAPK signaling pathways, highlighting the multitarget therapeutic mechanism of the plant. Molecular docking studies identified Murrayazoline as a promising lead compound exhibiting strong binding affinity toward IL-17RA, suggesting potential inhibition of cytokine-mediated inflammatory cascades central to psoriasis pathogenesis. Experimental validation supported computational findings, as Murrayazoline was successfully isolated and purified using chromatographic techniques and structurally confirmed through FT-IR and UV–Visible spectral analyses. In-vitro evaluation using HaCaT keratinocyte cells demonstrated concentration-dependent inhibition of cell proliferation with moderate cytotoxicity and a favorable safety profile, indicating selective regulation of abnormal keratinocyte growth rather than nonspecific cellular toxicity. Collectively, the findings provide scientific evidence supporting the anti-psoriatic potential of *Murraya koenigii* and establish Murrayazoline as a promising phytochemical candidate for further therapeutic development. The study highlights the effectiveness of integrating computational pharmacology with

experimental validation for herbal drug discovery. Future investigations involving mechanistic studies, in-vivo evaluation, pharmacokinetic profiling, and clinical validation are warranted to advance Murrayazoline toward development as a safe and effective plant-derived anti-psoriatic agent.

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