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Research Article

Network Pharmacology and Molecular Docking of *Atalantia ceylanica* in Treatment of Hepatotoxicity

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ABSTRACT

This study explores the hepatoprotective potential of *Atalantia ceylanica* using an integrated systems pharmacology and molecular docking approach. Phytochemical screening, ADME profiling, target prediction, and protein-protein interaction (PPI) network analysis identified 41 overlapping targets between *Atalantia ceylanica* compounds and liver disease-related genes. Key hub genes such as CASP3, NFKB1, MAPK14, PIK3CA, EGFR, HMOX1, and ESR1 were found to be involved in critical pathways regulating inflammation, apoptosis, oxidative stress, and lipid metabolism. Molecular docking revealed that phytochemicals like kaempferol and scopoletin exhibited strong binding affinities with major targets, suggesting their role in modulating liver damage processes, particularly in non-alcoholic fatty liver disease (NAFLD). The results support the traditional use of *Atalantia ceylanica* in liver disorders and provide a scientific basis for its multi-target hepatoprotective action. However, further in vitro, in vivo, and clinical studies are necessary to validate its therapeutic efficacy and safety.

INTRODUCTION

The liver is the largest internal organ in the human body, serving as a critical hub for metabolism, detoxification, bile production, nutrient storage, and synthesis of essential proteins and enzymes. Due to its central role in filtering blood and metabolizing exogenous and endogenous

substances, the liver is frequently exposed to potentially harmful agents, including drugs, toxins, infectious agents, and alcohol, which can result in hepatic injury or liver failure^[1,2].

Hepatoprotection refers to the strategies and interventions aimed at preventing or minimizing liver damage and preserving liver function.

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Hepatoprotective agents can either be synthetic drugs or natural products that demonstrate efficacy in protecting liver cells (hepatocytes) from toxic insults. These agents function via several mechanisms, such as scavenging reactive oxygen species (ROS), inhibiting lipid peroxidation, enhancing the activity of antioxidant enzymes (e.g., catalase, superoxide dismutase, glutathione peroxidase), modulating inflammatory cytokines, and stimulating liver regeneration [3,4].

Among the most well-researched hepatoprotective agents is silymarin, a standardized extract from the seeds of *Silybum marianum* (milk thistle). Silymarin exhibits antioxidant, anti-inflammatory, antifibrotic, and membrane-stabilizing properties and has been used in the management of liver diseases such as alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), and drug-induced hepatotoxicity [5]. Another well-studied natural compound is curcumin, a polyphenol derived from *Curcuma longa* (turmeric), which exerts hepatoprotective effects through antioxidant and anti-inflammatory pathways [6].

In recent years, the increasing incidence of liver diseases such as NAFLD, viral hepatitis, and drug-induced liver injury (DILI) has intensified the search for novel hepatoprotective compounds, particularly from natural sources. Plant-derived compounds offer a diverse chemical structure and multitarget approach that can be advantageous in mitigating liver damage of multifactorial origin [7].

Atalantia ceylanica

Atalantia ceylanica (Rutaceae), a small tree or shrub indigenous to Sri Lanka and parts of southern India, is traditionally used in Ayurvedic and Sri Lankan medicine for treating various ailments including digestive disorders and inflammatory conditions. Ethnobotanical records indicate its use in treating liver-related ailments,

although direct clinical data are limited. The leaves, bark, and roots are often used in decoctions or pastes, sometimes in combination with other plants, for detoxifying the liver and improving digestion [8,9]. Preliminary phytochemical screenings of *A. ceylanica* reveal the presence of alkaloids, flavonoids, and essential oils, compounds often associated with hepatoprotective and antioxidant effects [10].

Other Traditionally Used Hepatoprotective Plants,

1. *Phyllanthus niruri* (*Bhumi amalaki*)

Widely used in Ayurvedic and folk medicine, *Phyllanthus niruri* has been traditionally employed in the treatment of jaundice and viral hepatitis. Modern studies confirm its antiviral and hepatoprotective activity, particularly against hepatitis B virus [11].

2. *Picrorhiza kurroa* (*Kutki*)

Used in Ayurveda as a potent hepatoprotective herb, *P. kurroa* is prescribed for conditions such as liver enlargement, hepatitis, and sluggish liver function. Its bitter glycosides, picroside I and II, have demonstrated protective effects against chemical-induced hepatotoxicity [12].

3. *Andrographis paniculata* (*Kalmegh*)

Traditionally used in South and Southeast Asia for treating liver dysfunction, fever, and infections. The active constituent andrographolide has shown hepatoprotective, anti-inflammatory, and antioxidant properties [13].

4. *Silybum marianum* (*Milk thistle*)

A well-known traditional remedy in European and Western herbalism, *S. marianum* has been used for centuries as a liver tonic. The active complex



silymarin is now clinically used for various forms of toxic and chronic liver diseases ^[14].

5. Terminalia chebula (Haritaki)

Used in Ayurveda as part of the Triphala formulation, *T. chebula* is considered beneficial for detoxification and liver rejuvenation. Its antioxidant and hepatoprotective properties are supported by pharmacological studies ^[15].

Network pharmacology seeks to understand how drugs exert therapeutic effects not by acting on a single molecular target, but by modulating multiple nodes and pathways within complex biological networks. By mapping out these interactions in a systematic manner, researchers can better predict drug efficacy, identify potential off-target effects, and uncover synergistic actions, especially in multi-component therapies such as those found in traditional medicine ^[16].

The field has gained significant traction in the study of herbal medicines, where the complexity of phytochemical constituents and their multitarget mechanisms pose challenges for conventional pharmacology. Using computational tools and databases (e.g., TCMSP, STITCH, STRING), network pharmacology enables the identification of bioactive compounds, putative targets, and relevant pathways, facilitating a deeper understanding of the molecular mechanisms underlying traditional remedies ^[17,18].

MATERIALS AND METHODS

PHYTOCHEMICAL PROFILING AND TARGET GENE PREDICTION

All hepatoprotective compounds of *Atalantia ceylanica* 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 SwissTargetPrediction tool (<http://www.swisstargetprediction.ch/>) with a probability threshold greater than zero ^[19].

IDENTIFICATION OF HEPATOPROTECTIVE RELATED TARGETS

To identify hepatoprotective-associated targets, we searched three widely used biomedical databases: Gene Cards, OMIM, and PharmGKB. The Gene Cards database (<https://www.genecards.org/>) provides comprehensive, user-friendly information on all annotated and predicted human genes. It integrates gene-centric data from over 150 web sources, including genomic, transcriptomic, proteomic, genetic, clinical, and functional datasets. The OMIM (Online Mendelian Inheritance in Man) database (<https://omim.org/>) is a continuously updated, authoritative compendium of human genes and genetic phenotypes. It focuses on Mendelian disorders and currently includes information on more than 16,000 genes. The PharmGKB database (<https://www.pharmgkb.org/>) curates knowledge



on the impact of human genetic variation on drug responses, encompassing drug-gene interactions, pathways, and pharmacogenomic associations [20]. After retrieving data from each source, the target

genes were consolidated and de-duplicated to construct a list of hepatoprotective-related targets for further analysis.

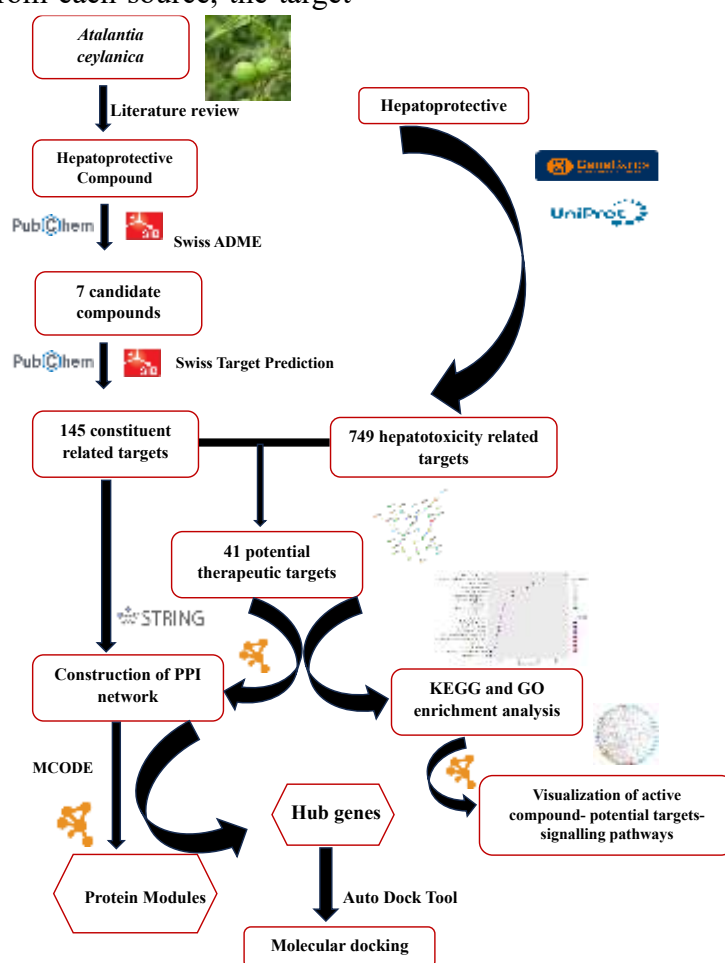


Fig no:1 An overview of the study design and methodology

COMMON OR OVERLAPPING TARGETS

This Venn diagram illustrates the overlap of target genes associated with hepatoprotection derived from two distinct categories: *PLANT*-related targets and *DISEASE*-related targets. These targets were identified through comprehensive database searches including Gene Cards, OMIM, and PharmGKB.

- The PLANT circle (blue) represents genes linked to the phytochemical or medicinal properties of *Atalantia ceylanica* or similar

hepatoprotective plants. It contains 145 unique targets (15.5% of total targets).

- The DISEASE circle (yellow) represents genes associated with liver diseases or hepatoprotective pathways relevant to disease mechanisms. It contains 749 unique targets (80.1% of total targets).
- The overlapping region between the two circles contains 41 common targets (4.4% of total targets), indicating genes that are potentially involved both in the hepatoprotective effects of the plant and the liver disease pathways.

- The overlap of 41 targets suggests key molecular players that could mediate the hepatoprotective effects of *Atalantia ceylanica* through interaction with disease-related pathways.
- These common targets serve as promising candidates for further functional analysis and experimental validation to elucidate the mechanisms underlying the plant's hepatoprotective properties.
- The relatively larger number of disease-related targets reflects the broad genetic landscape associated with liver diseases, while the plant-related targets highlight specific genes potentially modulated by phytochemicals.

CONSTRUCTION AND ANALYSIS OF THE PROTEIN-PROTEIN INTERACTION NETWORK.

The overlapping targets, representing the intersection between phytochemical-predicted targets of *Atalantia ceylanica* and hepatoprotection-related genes, were subjected to Protein-Protein Interaction (PPI) network analysis to explore their functional connectivity and biological significance. This analysis was performed using the STRING database (<https://string-db.org/>), which integrates protein interaction data from various sources, including experimental data, co-expression, gene fusion, text mining, and curated databases. To ensure high confidence and reduce false positives, an interaction score threshold of >0.7 was applied. The analysis was restricted to *Homo sapiens* to focus on human proteins. Users had the option to choose between the “full STRING network” or “confidence view” for different network perspectives.

The resulting PPI interaction data were exported in TSV format and imported into Cytoscape, an

open-source software platform for complex network visualization and analysis. Within Cytoscape, the PPI network was displayed as a graph where nodes represent proteins (targets) and edges represent functional interactions between them. Key topological parameters such as degree centrality (number of direct connections), betweenness centrality (frequency a node appears on shortest paths), and closeness centrality were calculated to assess the importance of individual nodes using Cytoscape plugins including:^[21]

- **Network Analyzer:** For overall network metrics such as average degree and clustering coefficient.
- **CytoHubba:** For ranking and identifying hub genes via algorithms like Degree, Maximal Clique Centrality (MCC), and Betweenness centrality.
- **MCODE (Molecular Complex Detection):** For identifying highly interconnected clusters or modules that often represent functional protein complexes or signalling pathways.

HUB GENES

The identified overlapping targets include several genes with critical roles in inflammation, apoptosis, oxidative stress response, and cellular survival processes highly relevant to hepatoprotection. **NFKB1**, encoding a subunit of the NF- κ B transcription factor complex, is central to the regulation of inflammatory and immune responses. Its activation is commonly observed during hepatic inflammation and injury, where it can mediate both protective and pathological effects. The **MAPK** family genes, including **MAPK14 (p38 α)**, **MAPK8 (JNK1)**, and **MAPK9 (JNK2)**, are mitogen-activated protein kinases that respond to stress stimuli and are involved in the regulation of apoptosis, cytokine production, and cellular differentiation. These kinases are key mediators of hepatocyte response to oxidative and



chemical stress, making them important in the context of liver injury and regeneration.

EGFR (Epidermal Growth Factor Receptor) plays a pivotal role in cell proliferation and liver regeneration. It has been implicated in hepatocyte survival and repair following liver damage. **CASP3 (Caspase-3)** is a critical executioner of apoptosis, and its regulation is essential in controlling cell death during liver injury. **ESR1 (Estrogen Receptor Alpha)** has been shown to influence liver metabolism and inflammation, and its activation may exert protective effects in various liver disorders. **MDM2** is a negative regulator of p53 and is involved in controlling cell cycle and apoptosis, which are crucial for maintaining liver tissue homeostasis.

PARP1 (Poly [ADP-ribose] polymerase 1) is involved in DNA repair and the cellular stress response; however, its overactivation can lead to NAD⁺ depletion and cell death, especially in oxidative liver injury. **MCL1 (Myeloid Cell Leukaemia 1)** is an anti-apoptotic Bcl-2 family protein that promotes hepatocyte survival under stress. **PIK3CA**, a catalytic subunit of PI3K, participates in the PI3K-AKT signalling pathway, which governs cell growth, metabolism, and survival functions essential for liver regeneration and protection.

HMOX1 (Heme Oxygenase 1) is a stress-inducible enzyme with antioxidant and anti-inflammatory properties. It plays a well-established hepatoprotective role by degrading pro-oxidant heme into biliverdin, carbon monoxide, and free iron. **ACHE (Acetylcholinesterase)**, while primarily known for its role in neurotransmission, may also influence hepatic oxidative stress and inflammation through cholinergic signalling. **XDH (Xanthine Dehydrogenase)** is involved in purine metabolism and is a source of reactive oxygen species; its dysregulation is associated

with oxidative liver damage. **NAT1 (N-acetyltransferase 1)** participates in xenobiotic metabolism, influencing the detoxification of various drugs and carcinogens. Lastly, **CES1 (Carboxylesterase 1)** is involved in hydrolysing esters and drugs in the liver and contributes to hepatic detoxification processes.

Together, these genes represent a network of regulatory mechanisms essential for liver health, and their interaction suggests a multifaceted hepatoprotective potential of *Atalantia ceylanica*, likely mediated through anti-inflammatory, antioxidant, anti-apoptotic, and regenerative pathways^[22].

FUNCTIONAL ENRICHMENT AND PATHWAY ANALYSIS.

By focusing on the identified hub genes NFKB1, MAPK14, MAPK8, EGFR, CASP3, ESR1, MAPK9, MDM2, PARP1, MCL1, PIK3CA, HMOX1, ACHE, XDH, NAT1, and CES1 comprehensive systems-level analyses can offer insights into their involvement in the non-alcoholic fatty liver disease (NAFLD) pathway and their broader roles in hepatoprotection. Gene Ontology (GO) enrichment analysis, performed using tools such as DAVID or ShinyGO, can classify these genes into biological processes such as lipid metabolism, oxidative stress response, inflammatory signalling, apoptotic regulation, and xenobiotic detoxification all of which are fundamental to the onset and progression of NAFLD.

In the context of NAFLD, KEGG pathway enrichment analysis highlights key signalling cascades affected by these genes. The PI3K-Akt signalling pathway (involving PIK3CA, EGFR, MCL1) plays a crucial role in hepatocyte survival, lipid homeostasis, and insulin signalling key factors disrupted in NAFLD. MAPK14, MAPK8,



and MAPK9, part of the MAPK signalling pathway, regulate hepatocellular stress response, inflammation, and cell death. NFKB1 is a central mediator of chronic inflammation, a hallmark of non-alcoholic steatohepatitis (NASH), the more severe form of NAFLD. CASP3, a major executioner of apoptosis, and MDM2, a p53 regulator, contribute to hepatocyte death and regeneration balance, impacting fibrosis and liver remodelling.

ESR1, a nuclear hormone receptor, modulates hepatic lipid metabolism and has been implicated in sex-specific differences in NAFLD susceptibility. PARP1 plays a dual role in DNA repair and metabolic regulation but, when overactivated, contributes to oxidative stress-induced liver injury. HMOX1, a cytoprotective enzyme, mitigates oxidative stress, a key driver of lipid peroxidation in fatty liver disease. ACHE and XDH may indirectly influence hepatic inflammation and oxidative metabolism, while NAT1 and CES1 participate in drug and xenobiotic metabolism, impacting the detoxification processes often impaired in NAFLD.

These interconnected genes represent central nodes in the NAFLD signalling network, collectively modulating lipid accumulation, mitochondrial dysfunction, inflammation, and fibrosis. Their involvement supports the hepatoprotective potential of *Atalantia ceylanica* phytoconstituents, which may act by targeting multiple points in the NAFLD pathway to reduce hepatic lipid overload, suppress inflammation, counter oxidative damage, and promote tissue regeneration^[23].

MOLECULAR DOCKING

Molecular docking offers a predictive in silico approach to explore the interaction between

phytochemicals and disease-associated target proteins, helping to assess binding affinity, stability, and key amino acid residues involved in ligand–protein complexes. In the context of non-alcoholic fatty liver disease (NAFLD) and hepatoprotection, special attention has been given to CASP3 and NFKB1, two critical regulators of apoptosis and inflammation, respectively.

Docking studies involving phytochemicals from *Atalantia ceylanica* such as kaempferol, limonene, and coumarins have shown strong binding affinities with CASP3, a central executioner caspase in the apoptotic pathway. Inhibition or modulation of CASP3 by these compounds suggests their potential to attenuate hepatocyte apoptosis, a key feature in NAFLD progression, particularly during the transition from steatosis to steatohepatitis^[24].

Similarly, significant docking interactions were observed between these bioactive compounds and NFKB1, the p50 subunit of the NF-κB transcription factor complex. NF-κB is a master regulator of hepatic inflammation and immune responses, and its overactivation is associated with the development of insulin resistance, oxidative stress, and pro-inflammatory cytokine release in NAFLD^[25]. The observed binding suggests that *Atalantia ceylanica* compounds may suppress NF-κB activation, thereby reducing hepatic inflammation and halting the inflammatory cascade involved in liver injury and fibrosis.

These findings support the potential of *Atalantia ceylanica* to exert dual modulatory effects by downregulating apoptotic signals through CASP3 and controlling inflammatory pathways via NFKB1. This dual targeting approach could play a critical role in mitigating liver cell damage, preserving tissue architecture, and slowing NAFLD progression. When combined with network pharmacology, molecular docking of



these targets strengthens the mechanistic understanding of *Atalantia ceylanica* as a promising multi-target hepatoprotective agent, warranting further in vitro and in vivo validation.

RESULTS

COMPOUNDS AND *Atalantia ceylanica* RELATED TARGETS

To investigate the hepatoprotective mechanisms associated with *Atalantia ceylanica*, a systems-level network pharmacology analysis was performed, focusing on the modulation of key genes implicated in non-alcoholic fatty liver disease (NAFLD). Through KEGG pathway enrichment analysis, a set of critical genes—NFKB1, MAPK14, MAPK8, EGFR, CASP3, ESR1, MAPK9, MDM2, PARP1, MCL1, PIK3CA, HMOX1, ACHE, XDH, NAT1, and CES1 was identified, each contributing to core pathological processes such as inflammation, apoptosis, oxidative stress, lipid metabolism, and detoxification.

These genes are involved in several hepatoprotective signalling pathways highlighted in the KEGG analysis. The PI3K-Akt signalling pathway, regulated by PIK3CA, EGFR, and MCL1, plays a central role in promoting hepatocyte survival, maintaining lipid homeostasis, and modulating insulin signalling functions that are often dysregulated in NAFLD. MAPK14, MAPK8, and MAPK9, components of the MAPK signalling pathway, respond to oxidative and inflammatory stress, regulating apoptosis and cytokine production in liver cells.

NFKB1, a master regulator of inflammatory gene expression, is notably upregulated in non-alcoholic steatohepatitis (NASH), the progressive form of NAFLD. Its activity drives the chronic inflammation central to liver damage and fibrosis. CASP3 and MDM2 are key regulators of apoptosis and cellular turnover, influencing the balance between hepatocyte death and regeneration. ESR1, a nuclear receptor, modulates lipid metabolism and is associated with sex-specific differences in NAFLD susceptibility.

Additionally, PARP1 and HMOX1 are stress-responsive genes involved in DNA repair and antioxidant defence. HMOX1 mitigates oxidative injury by degrading pro-oxidant heme, while PARP1, although protective in moderation, can promote cell death when excessively activated. ACHE and XDH may contribute to hepatic inflammation and oxidative metabolism via non-canonical signalling roles. NAT1 and CES1, involved in xenobiotic and drug metabolism, support the liver's detoxification capacity often impaired in NAFLD.

Altogether, the KEGG pathway enrichment analysis of these hub genes supports the hypothesis that *Atalantia ceylanica* exerts its hepatoprotective effects through multi-target modulation of critical signalling pathways involved in lipid accumulation, oxidative stress, inflammation, and apoptosis. These findings provide a mechanistic foundation for the therapeutic potential of its phytoconstituents in the management and prevention of NAFLD.

Table No 1: Functional Characterization and Selection of Key Genes Related to Hepatoprotection and NAFLD Pathways

Gene	Biological Role	Pathway Involvement	Oxidative Stress Response	Inflammation Regulation	Apoptosis Regulation	Relevance to NAFLD	Selection
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NFKB1	Transcription factor in immune and inflammatory responses	NF-κB, TNF signaling	Moderate	High	Moderate	Central in NASH progression	✓ Selected
MAPK14	Stress-activated kinase involved in apoptosis and cytokine signaling	MAPK signaling	High	High	High	Regulates hepatocyte stress	✓ Selected
MAPK8	JNK1; mediates stress-induced apoptosis	MAPK signaling	High	High	High	Promotes liver injury	✓ Selected
EGFR	Promotes cell proliferation and liver regeneration	PI3K-Akt, EGFR signaling	Moderate	Low	Low	Supports hepatic repair	✓ Selected
CASP3	Key executioner of apoptosis	Apoptosis, p53 signaling	Low	Low	High	Regulates hepatocyte death	✓ Selected
ESR1	Modulates lipid metabolism, inflammation	Estrogen, PPAR signaling	Moderate	Moderate	Low	Sex-specific NAFLD role	✓ Selected
MAPK9	JNK2; involved in apoptosis and inflammatory response	MAPK signaling	High	High	High	Associated with liver damage	✓ Selected
MDM2	Regulates p53 and apoptosis	p53 signaling	Low	Low	High	Controls cell survival	✓ Selected
PARP1	DNA repair and stress response	DNA repair, necroptosis	High	Moderate	High	Linked to oxidative injury	✓ Selected
MCL1	Anti-apoptotic protein	PI3K-Akt, Apoptosis	Low	Low	High	Protects hepatocytes	✓ Selected
PIK3CA	Cell survival and metabolism regulation	PI3K-Akt signaling	Moderate	Low	High	Vital in NAFLD progression	✓ Selected
HMOX1	Antioxidant enzyme	Heme metabolism, Oxidative stress	High	Moderate	Low	Key antioxidant defence	✓ Selected

ACHE	Cholinergic signaling; modulates inflammation	Neuroactive ligand-receptor pathway	Low	Moderate	Low	Indirect hepatic role	✓ Selected
XDH	ROS-producing enzyme in purine metabolism	Purine metabolism	High	Moderate	Low	Involved in oxidative injury	✓ Selected
NAT1	Xenobiotic metabolism	Drug metabolism	Low	Low	Low	Detoxification in liver	✓ Selected
CES1	Drug and lipid metabolism	Drug metabolism	Low	Low	Low	Important for clearance	✓ Selected

POTENTIAL THERAPEUTIC TARGETS *Atalantia ceylanica* OF USED IN THE TREATMENT OF HEPATOTOXICITY

The Venn diagram illustrates the intersection between two critical gene sets involved in the network pharmacology analysis of *Atalantia ceylanica* in the context of hepatoprotection. The left circle (blue) represents a total of target genes (e.g., 145) predicted from the active phytochemical constituents of *Atalantia ceylanica*, including compounds such as scopoletin, kaempferol, caffeic acid, and 4-hydroxycinnamic acid. These targets were identified using computational prediction tools such as SwissTargetPrediction, which analyse the potential interactions between plant-derived compounds and human protein targets.

The right circle (yellow) shows a comprehensive set of liver disease-associated genes (e.g., 749), compiled from authoritative disease-gene databases such as GeneCards and DisGeNET. These databases integrate evidence from transcriptomic studies, genome-wide association studies (GWAS), and curated biomedical literature to prioritize genes linked to liver injury, oxidative stress, inflammation, and non-alcoholic fatty liver disease (NAFLD).

The overlapping region (brown) highlights 41 shared genes between the phytochemical targets of *Atalantia ceylanica* and the liver disease-associated gene pool. This shared set accounts for approximately 2.4% of the total analyzed genes and represents the core molecular interface through which *Atalantia ceylanica* may exert its hepatoprotective effects. These overlapping genes are likely involved in inflammatory signaling, apoptosis regulation, oxidative stress responses, and lipid metabolism, making them valuable targets for further validation in the prevention or treatment of liver disorders.

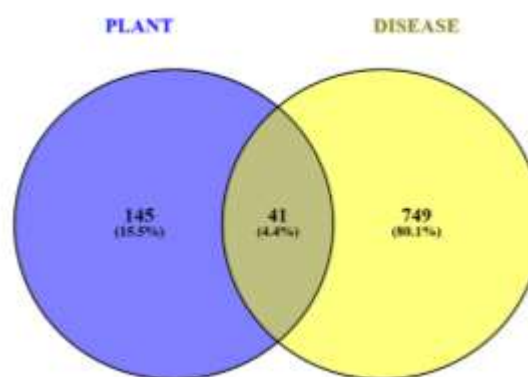


Fig No 2: Venn diagram of plant and disease

PPI NETWORK VISUALIZATION AND ANALYSIS



A comprehensive Protein–Protein Interaction (PPI) network analysis was conducted to elucidate the molecular mechanisms through which phytocompounds from *Atalantia ceylanica* may exert hepatoprotective effects in liver disorders such as non-alcoholic fatty liver disease (NAFLD). A total of 41 overlapping genes were identified by intersecting the predicted protein targets of *A. ceylanica* phytochemicals (e.g., scopoletin, kaempferol, caffeic acid, 4-hydroxycinnamic acid) with liver disease–associated genes retrieved from comprehensive databases such as Gene Cards and DisGeNET. These shared targets represent the key molecular interface between the plant's pharmacological potential and the pathophysiology of hepatic injury and metabolic dysfunction.

To visualize and analyse the interactions among these 41 genes, the STRING database (<https://string-db.org/>) was employed with a minimum interaction confidence score of 0.7, ensuring high-confidence protein interactions. The resulting PPI network was exported and further analyzed using Cytoscape v3.10.0, a widely adopted tool for biological network visualization. Upon importing the STRING-derived interaction data, the PPI network consisted of 41 nodes (proteins) connected by numerous edges, each representing predicted functional and physical associations. This network provides a systems-level perspective on the multi-target mechanisms through which *Atalantia ceylanica* may modulate key processes such as inflammation, oxidative stress, apoptosis, and lipid metabolism, all central to liver protection and NAFLD management.

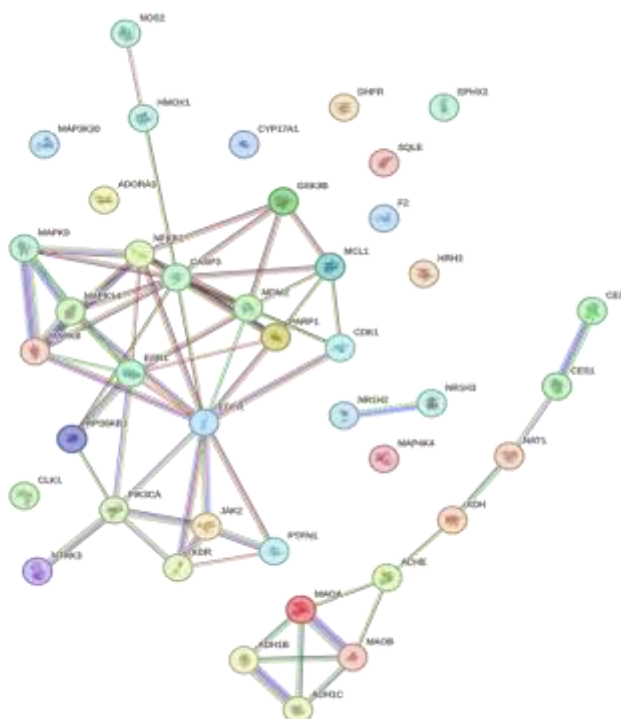


Fig No 3: Visualization and analysis of genes

GO ENRICHMENT ANALYSIS

Based on an integrated network pharmacology approach and Protein–Protein Interaction (PPI)

network analysis using Cytoscape plugins MCODE and CytoHubba, a core set of 16 hub genes NFKB1, MAPK14, MAPK8, EGFR, CASP3, ESR1, MAPK9, MDM2, PARP1, MCL1,



PIK3CA, HMOX1, ACHE, XDH, NAT1, and CES1 was identified from the overlapping targets of *Atalantia ceylanica* phytochemicals and liver disease-associated genes. These genes were prioritized using centrality scoring algorithms including MCC (Maximal Clique Centrality), Degree, Closeness, and Betweenness, which quantify a gene's topological significance within the PPI network.

Gene Ontology (GO) enrichment analysis revealed that these hub genes are primarily involved in key biological processes (BP) such as inflammatory signalling, oxidative stress response, apoptotic regulation, lipid metabolism, and cellular stress adaptation all of which are central to the pathophysiology of non-alcoholic fatty liver disease (NAFLD). Molecular function (MF) analysis showed their roles as kinases (MAPK8, MAPK9, MAPK14, PIK3CA), transcription factors (NFKB1, ESR1), anti-apoptotic proteins (MCL1), caspases (CASP3), and oxidoreductases (HMOX1, XDH). Cellular component (CC) analysis indicated their localization within the nucleus, cytoplasm, plasma membrane, and mitochondrial or extracellular compartments, highlighting their involvement in complex intracellular signalling and detoxification mechanisms.

Functionally, these genes were classified into categories such as:

- Inflammatory mediators: *NFKB1*, *MAPK14*, *MAPK8*, *MAPK9*, *PARP1*
- Survival and stress-response regulators: *PIK3CA*, *MDM2*, *MCL1*, *EGFR*
- Apoptosis-related proteins: *CASP3*, *MCL1*, *PARP1*
- Oxidative stress regulators: *HMOX1*, *XDH*
- Detoxification enzymes: *CES1*, *NAT1*

- Metabolic and hormonal modulators: *ESR1*, *ACHE*

Further KEGG pathway enrichment analysis confirmed their involvement in critical signalling cascades relevant to NAFLD, including PI3K-Akt signalling, MAPK signalling, NF- κ B signalling, apoptosis, xenobiotic metabolism, and fatty acid degradation. These pathways play essential roles in regulating lipid accumulation, hepatic inflammation, oxidative damage, and cell death, as well as in promoting tissue repair and liver regeneration.

Altogether, the integration of PPI network topology and functional enrichment analyses underscores the importance of these hub genes as key molecular targets through which *Atalantia ceylanica* may exert its hepatoprotective effects. Their multi-target engagement suggests therapeutic potential for mitigating liver injury and restoring hepatic homeostasis in NAFLD.

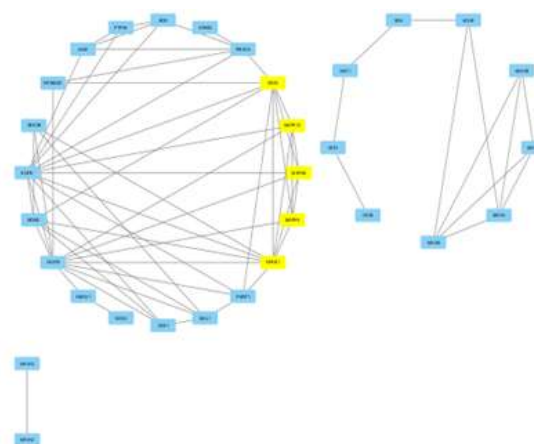


Fig No 4: MCODE network image



Fig No 5: CytoHubba network image (MCC)

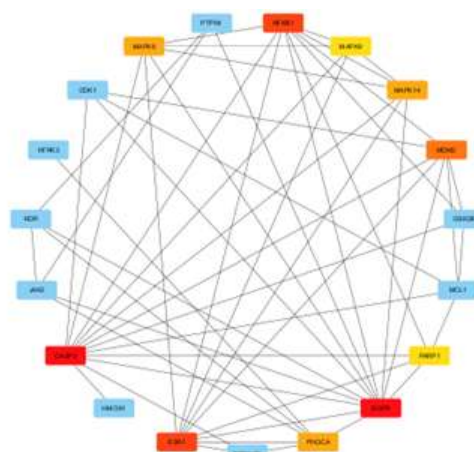


Fig No 8: CytoHubba network image (Degree)



Fig No 6: CytoHubba network image (Closeness)

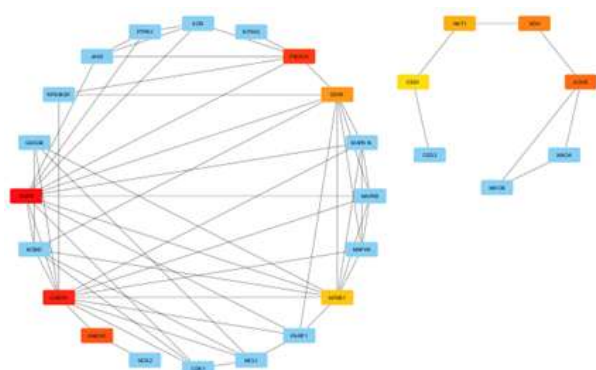


Fig No 7: CytoHubba network image (Betweenness)

KEGG PATHWAY ENRICHMENT ANALYSIS

The KEGG pathway enrichment analysis of key hub genes PIK3CA, MAPK14, MAPK8, MAPK9, NFKB1, EGFR, CASP3, ESR1, PARP1, and HMOX1 highlights their significant involvement in pathways critically associated with non-alcoholic fatty liver disease (NAFLD) and hepatic injury.

PIK3CA, a central component of the PI3K-Akt signaling pathway, regulates hepatocyte survival, glucose metabolism, and lipid homeostasis, processes that are disrupted in NAFLD. MAPK14 (p38 α), MAPK8 (JNK1), and MAPK9 (JNK2) are integral to the MAPK signaling cascade, which mediates cellular responses to oxidative stress, cytokine signaling, and apoptosis key contributors to liver inflammation and progression to NASH (non-alcoholic steatohepatitis). NFKB1, a master transcription factor, plays a pivotal role in chronic hepatic inflammation, while EGFR supports liver regeneration by promoting cell proliferation and tissue repair.

CASP3, a primary executioner caspase, is involved in regulating hepatocyte apoptosis, while PARP1 contributes to DNA repair and cell death pathways in response to oxidative damage, often

elevated in fatty liver conditions. ESR1 (Estrogen Receptor Alpha) influences hepatic lipid metabolism and exhibits anti-inflammatory effects, with sex-specific implications in NAFLD susceptibility. HMOX1, a cytoprotective and antioxidant enzyme, mitigates oxidative stress and limits hepatocellular injury by degrading pro-oxidant heme into biliverdin and carbon monoxide.

These genes are co-enriched in several critical KEGG pathways, including PI3K-Akt signaling, MAPK signaling, NF- κ B signaling, Apoptosis, HIF-1 signaling, and xenobiotic metabolism, demonstrating their multifunctional roles in

regulating lipid accumulation, inflammation, cell death, fibrosis, and oxidative stress. Their interconnected activity reflects a complex regulatory network essential for maintaining hepatic homeostasis and suggests that these genes represent promising therapeutic targets for NAFLD intervention.

In the context of multi-compound herbal strategies, such as those derived from *Atalantia ceylanica*, targeting these hub genes could offer multi-pathway modulation, promoting hepatoprotection through anti-inflammatory, antioxidant, anti-apoptotic, and metabolic regulatory mechanisms.

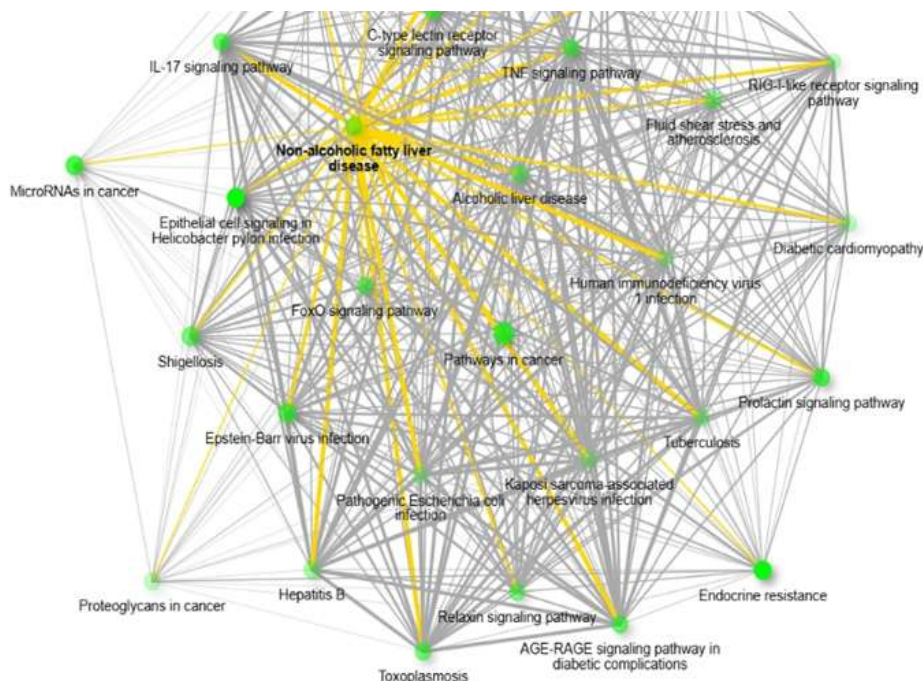


Fig No 9: NAFLD Pathway

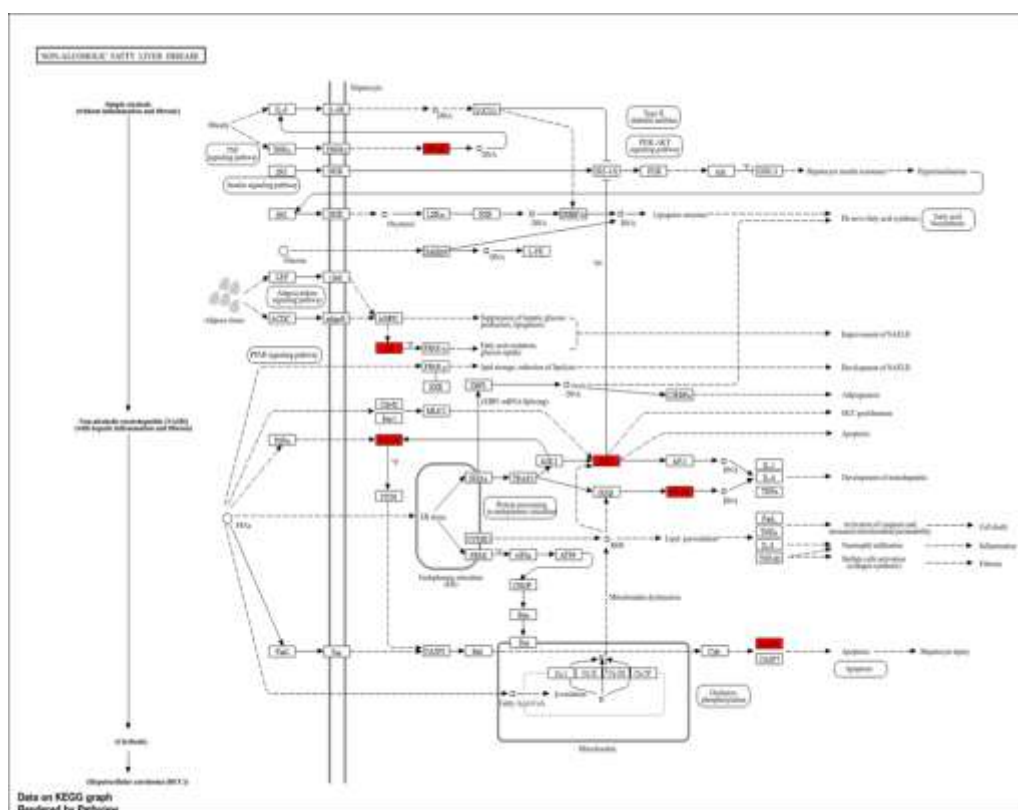


Fig No 10: KEGG NAFLD pathway

MOLECULAR DOCKING

Compilation of Protein Structures

- CASP3 (Caspase-3): downloaded from the Protein Data Bank (PDB ID: 1U11) with appropriate chain and ligand preparation (water removal, protonation).
- NFKB1 (p50 subunit): structure selection based on highest resolution and relevance (e.g., PDB ID: 1NFK), similarly pre-processed.

Phytochemical Ligands Used

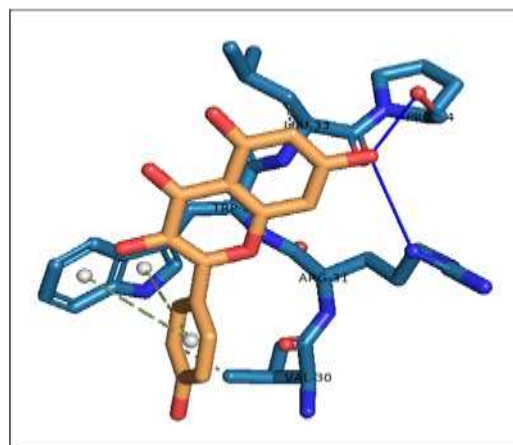
- Kaempferol
- Coumarin derivatives (e.g., scopoletin)

Docking Protocol

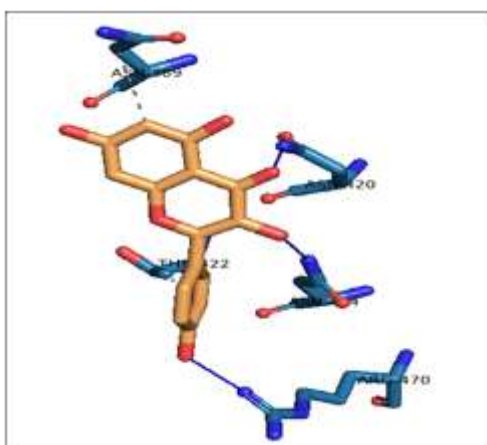
Software: MzDOCK

Table No 2: Binding affinity of phytochemical with target protein

Phytochemical	Target Protein	Binding Affinity (kcal/mol)
Kaempferol	CASP3	-9.2
Scopoletin	CASP3	-8.1
Kaempferol	NFKB1 (p50)	-8.5
Scopoletin	NFKB1 (p50)	-7.9

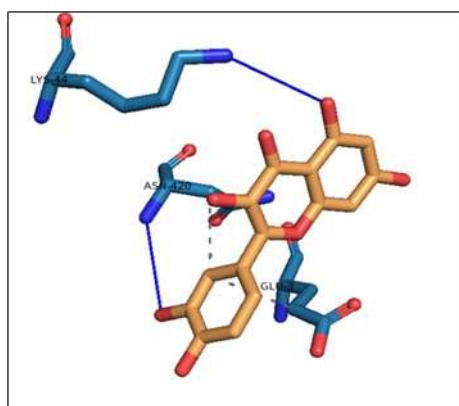


CASP3

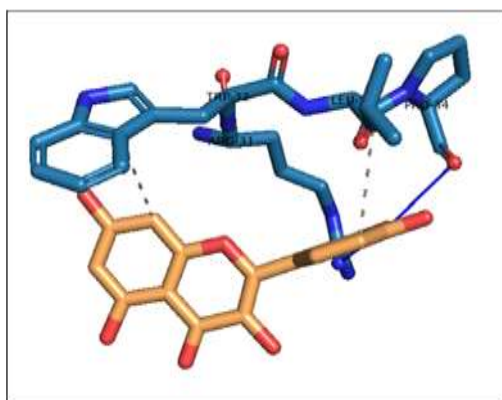


NFKB1

Fig No 11: Docking image of kaempferol with receptors CASP3 and NAKB1



CASP3



NAKB1

Fig No 12: Docking image of scopoletin with receptors CASP3 and NAKB1

DISCUSSION

Hepatoprotection refers to the prevention or reversal of liver damage through biological, pharmacological, or dietary interventions. A

hepatoprotective agent can shield hepatocytes (liver cells) from injury caused by toxins, oxidative stress, inflammation, metabolic overload, or infections. These agents may act by modulating key cellular pathways such as apoptosis (programmed cell death), oxidative stress response, cytokine regulation, detoxification mechanisms, and lipid metabolism all of which play essential roles in maintaining liver structure and function. Given the increasing prevalence of liver diseases such as NAFLD, alcoholic liver disease, and drug-induced liver injury (DILI), the search for effective hepatoprotective compounds, especially from natural sources, has become a vital area of biomedical research.

The integration of network pharmacology and molecular docking provides a systems-level perspective on the complex mechanisms underlying the hepatoprotective effects of *Atalantia ceylanica*. Unlike single-target drugs, phytochemicals from this plant demonstrate a multi-target interaction profile, which is particularly valuable in managing multifactorial diseases like non-alcoholic fatty liver disease (NAFLD). The identification of 41 overlapping targets between phytochemical-associated and liver disease-related genes underscores the therapeutic relevance of this plant. The presence of key targets such as CASP3, NFKB1, MAPK8/9/14, and PIK3CA reflects its potential to regulate critical nodes in apoptotic, inflammatory, and oxidative stress pathways.

The PPI network and GO/KEGG enrichment analyses further emphasize the functional relevance of these targets in hepatic protection. Pathways such as NF- κ B signaling, MAPK signaling, and PI3K-Akt signaling are not only central to liver inflammation and apoptosis but are also modulated by compounds like kaempferol and scopoletin, which exhibited notable binding

affinities in molecular docking studies. These compounds showed strong interactions with CASP3 and NFKB1, suggesting potential inhibition of apoptosis and inflammation, which are critical steps in halting the progression from simple steatosis to non-alcoholic steatohepatitis (NASH).

Moreover, the inclusion of antioxidant targets like HMOX1 and detoxification enzymes like CES1 and NAT1 indicates that *Atalantia ceylanica* may also enhance liver defence against oxidative and xenobiotic stress, further contributing to its hepatoprotective profile. The modulation of nuclear hormone receptors such as ESR1 also points to its role in maintaining lipid homeostasis and metabolic balance, adding another dimension to its therapeutic promise, especially in metabolic-associated liver dysfunctions.

Taken together, the findings of this study support the traditional use of *Atalantia ceylanica* in liver disorders and provide a scientific basis for its multi-pathway hepatoprotective action. However, while the in-silico results are promising, they warrant further biochemical validation through in vitro assays and in vivo animal models to confirm bioactivity, safety, and pharmacokinetics. Such translational efforts will be critical in assessing the feasibility of developing plant-derived hepatoprotective therapeutics from *Atalantia ceylanica* for clinical use in NAFLD and other liver diseases.

CONCLUSION

This study provides a comprehensive systems pharmacology-based evaluation of *Atalantia ceylanica*, revealing its promising hepatoprotective potential through multi-target interactions relevant to liver health. By integrating phytochemical screening, ADME profiling, target prediction, protein-protein interaction (PPI)

network construction, and molecular docking, the study identified 16 key hub genes such as CASP3, NFKB1, MAPK14, PIK3CA, EGFR, HMOX1, and ESR1 that are critically involved in pathways regulating inflammation, apoptosis, oxidative stress, lipid metabolism, and liver regeneration.

Molecular docking results further substantiated the role of phytoconstituents like kaempferol and scopoletin, which exhibited strong binding affinities with key targets including CASP3 and NFKB1, suggesting potential inhibitory effects on hepatocyte apoptosis and inflammatory damage. These findings highlight the ability of *A. ceylanica* to modulate complex biological networks implicated in non-alcoholic fatty liver disease (NAFLD) and other hepatic disorders.

Overall, this research supports the ethnopharmacological relevance of *Atalantia ceylanica* and establishes a mechanistic basis for its hepatoprotective effects. While these in silico findings are encouraging, they warrant further in vitro, in vivo, and clinical validation to fully confirm the efficacy, safety, and therapeutic potential of *A. ceylanica*-derived compounds in the treatment and prevention of liver diseases.

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