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Targeting ICAM1- driven immune dysfunction in ulcerative colitis: Integrative evidence from *in-vivo* and *in-silico* studies

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Abstract: **Background**: Ulcerative colitis is chronic inflammatory bowel disease defined by aberrant immune response and recurrent intestinal mucosal inflammation. Anti-inflammatory and antioxidant sesquiterpene alpha-cubebene is thought to ameliorate UC pathogenesis via down-regulation of ICAM1 expression, inhibition of NFκB and MAPK-mediated inflammatory pathways. **Objectives**: To investigate ICAM1 as a biomarker and therapeutic target for cell adhesion and leukocyte migration, and to explore alpha-cubebene as a potential ICAM1 inhibitor for the first time**.** **Methods**: ICAM1 structure was retrieved from RCSB PDB database and docked by CB-dock2. ADMET and drud likeness analysis of alpha-cubebene were evaluated by SwissADME, pkCSM and Deep-PK softwares. Stereochemistry of ICAM1 was assessed through Ramachandran plot and hydrophobicity analysis. Protein-protein interaction was established by STRING analysis and Gene Ontology (GO) followed by MD simulation. For *in-vivo* validation, Ulcerative colitis was induced in 30 male Albino Wistar rats using 2% DSS administered orally for four weeks, randomized into six groups (n = 5/group) and then treated with different concentrations of α-cubebene and Mesalamine for one month. Therapeutic efficacy was evaluated by assessing disease activity and experimental parameters and histopathological analysis. **Results**: Molecular docking and MD simulation of ICAM1 and alpha-cubebene revealed Vina score of -6.1kcal/mol showing it a stable protein. Network pharmacology, protein-protein interactions, STRING and GO enrichment analysis revealed therapeutic targeting of ICAM1 interactions can be a potential therapeutic approach for alleviating UC. In *in-vivo* studies, DSS administration significantly increased the disease activity score compared with normal control ($P = 0.001$), while α-cubebene treatment markedly and dose-dependently attenuated disease severity. The highest α-cubebene dose (100 µL, 10 mg/kg/day) restored the disease activity score of ICAM1 towards normal and demonstrated greater therapeutic efficacy than Mesalamine. Histopathological results further confirmed it. **Conclusion**: Alpha-cubebene could serve as an effective candidate for phytotherapy of UC via specifically affecting inflammatory response mediated by ICAM1.

Keywords: Alpha-Cubebene; ADMET analysis; GO enrichment; ICAM1; Molecular docking; Protein-protein interaction

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INTRODUCTION

Chronic inflammation of the GI (gastrointestinal) tract known as ulcerative colitis or UC exclusively impacts the mucosal tissues of the rectum and colon. As a chronic, relapsing inflammatory condition and a prominent form of inflammatory bowel disease (IBD), characterized by recurrent diarrhea, abdominal discomfort, rectal bleeding and subsequent epithelial loss (Kobayashi *et al*., 2020; Gros *et al*., 2023). The global rates of incidences and prevalence of UC have significantly risen, leading it to be a momentous public health concern worldwide (Ungaro R *et al*., 2017). Whilst no definitive cause of UC has yet been identified, current scientific evidence indicates that the disease arises due to a complicated interplay of environmental factors, genetic predispositions, dysbiosis of the microbiome, impaired epithelial barrier function and aberrant host responses. Uncontrolled inflammation leads to reduced quality of life for patients and increases the risk of colorectal cancer and various extra-intestinal complications (Liang *et al*., 2024; Wangchuk *et al*., 2024).

Pathogenesis in UC is induced by a breakdown of the epithelial barrier that separates the interior of the gut lumen from the intestinal epithelium. This breach leads to influx of contents including microbiota, microbes, dietary components and pathogens from the gut luminal content. These ingredients initiate the activation of host innate immune system through interaction with pattern-recognition receptors such as Toll-like receptor (TLR) and NOD-like receptor of the immune cells, macrophages, dendritic cells and neutrophils, to secrete massive array of pro-inflammatory cytokines such as TNFα, IL-1, IL-6, IL-8, IL-13 IL-17 and IFNγ which fuel subsequent colonic inflammation and perpetuate tissue injury (Neurath, 2024; Long *et al*., 2024). Adaptive

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immune response is then characterized by dysregulation of Th2 and Th17 mediated immunity. Intercellular adhesion molecule-1 (ICAM1) has emerged as a crucial regulatory component of leukocyte infiltration and inflammation within UC mucosa, it is a transmembrane glycoprotein expressed predominantly on a range of cell types in human body like Endothelial cells, epithelial cells, fibroblast and immune cell, but under certain condition due to activation of pro-inflammatory mediator the up-regulation expression of ICAM1 occurs via NFκB and MAPK signaling pathways. Expression of ICAM1 is observed to be increased on inflammatory colonic epithelia and correlates with severity of the disease (Zhang *et al*., 2024; Haydinger *et al*., 2023; Singh *et al*., 2023).

Immunologically, ICAM1 mediates the firm recruitment of leukocytes across endothelial cells through its two receptor integrins LFA-1 and MAC-1, this is facilitate the transmigration of neutrophils, monocytes and T-cells into colonic epithelial tissue leading to the liberation of excess of reactive oxygen species (ROS), proteases and the subsequent release of inflammatory factors in a manner that drives epithelium damage and loss (Barreiro and Sanchez-Madrid, 2009). In addition, ICAM1 is important for immune synapses formation and T-cells activation *in-vivo* thereby linking immune system response in both innate and adaptive immunity in UC pathogenesis, because of the fact that the ICAM1 expression has shown increase expression on colon; hence ICAM1 can be a possible Therapeutic Target for UC management (Muller, 2019).

Nowadays Natural Products have garnered the attentions as alternative therapeutics in treating diverse chronic inflammation based on their diversified targets, reasonable cost and few or without side effects (Atanasov *et al*., 2021; Newman and Cragg, 2020). Natural products which possess potent anti-inflammatory and radical scavenging effects such as alpha-cubebene a type of sesquiterpene isolated from some plants, has showed great potential in inflammatory based disease management. Various natural sesquiterpenes extracted from diverse medicinal plants including Piper cubeba, Schisandra chinensis and several aromatic herbs have showed potent antioxidant and immune regulatory effects, which may make these natural products as one potential drug to target chronic inflammatory conditions, including UC (Ramotowski *et al*., 2022). *In-vitro*, the protective and immune regulatory effect of alpha-cubebene seems to arise from inhibition of oxidation and inhibition of downstream inflammatory signals through NFκB and MAPK pathway thereby reducing cytokines levels, such as IFNγ and IL-8 which reduce the invasion and activation of innate and adaptive immune system in inflamed tissue (Subudhi *et al*., 2024). Furthermore, the structural analogue α-iso-cubebene reduces significantly TNFα induced production of Reactive oxygen species and vascular cell adhesion molecules thus inhibiting monocytes migration into activated endothelial cells (Choi *et al*., 2009).

ICAM1-mediated leukocyte adhesion and migration are central to the inflammatory cascade in UC making the ICAM1 a promising therapeutic target. Therefore, the current project aims to investigate whether alpha-cubebene (reported as ICAM1 potential inhibitor for the first time in this study) could interact with and modulate ICAM1 associated inflammatory pathways and alleviate DSS-induced UC through integrated in-silico and *in-vivo* approaches.

MATERIALS AND METHODS

Target protein selection

The various search engines; that include google, yahoo and google scholar have been employed to carry out comprehensive search on the molecular and biochemical biology on ulcerative colitis and a large number of proteins was reported to have been involved in UC and then the relevant genes was confirmed on KEGG pathway using an online server https://www.kegg.jp/pathway/map05321 (Ivan *et al*., 2022; Hopkins, 2008; Wang *et al*., 2025; Jalali *et al*., 2025). ICAM1 protein was shortlisted on basis of having strongest association with onset and proliferating stages of inflammation in ulcerative colitis.

Retrieved target proteins

The Protein Data Bank (PDB) was searched for the information on the selected protein, ICAM1. Its structure was retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) PDB database (https://www.rcsb.org/) (Li *et al*., 2011).

Dataset of compounds

3D structures of alpha-cubebene (compound CID: 442359) was downloaded for docking in SDF format from PubChem (https://pubchem.ncbi.nlm.nih.gov/). SMILES of alpha-cubebene (Computed by OEChem 2.3.0 (PubChem release 2024.12.12)) were acquired from PubChem for ADMET analysis (Xiong *et al*., 2021).

Protein preparation

ICAM1 (PDB ID: 1P53), 3D structures was downloaded in legacy PDB format. PDBsum (https://www.ebi.ac.uk/thorntonsrv/databases/pdbsum/index.html) database was utilized to get the pictorial database of 3D structure of protein along with their Ramachandran Plot using PROCHECK(https://www.ebi.ac.uk/thorntonsrv/databases/cgibin/pdbsum/GetPage.pl?pdbcode=6e3k&template=procheck_summary.html) for validation of the structure of proteins.

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Molecular docking analysis

PDB format of the downloaded protein was taken for docking analysis using CB-dock2 (http://183.56.231 .194:8001/cb-dock2/index.php) (Luo *et al*., 2020). The protein was uploaded into CB-dock2 and was automatically prepared (water molecules and hetatoms, if any gets removed from the protein molecule). Ligand (alpha-cubebene) was then uploaded to the CB-dock2 web-server (the saved SDF file). After uploading of ligand and protein, auto blind dockings was performed (the search for possible docking cavities was set to 5 pockets).

Drug-likeness, pharmacokinetics and toxicity profile of ligands

SwissADME (https://www.swissadme.ch/), pkCSM (https://biosig.lab.uq.edu.au/pkcsm/) and Deep-PK (https://biosig.lab.uq.edu.au/deeppk/) online services were utilized to procure the ADMET (absorption, distribution, metabolism, excretion and toxicity) characteristics, as well as the physicochemical properties of alpha-cubebene. The selected ligand display prominent biological characteristics (PDB, 2019; Yang *et al*., 2020; Luo *et al*., 2020).

Swiss target prediction

The pharmacological properties of alpha-cubebene via *in-silico* target prediction and drug-likeness evaluation was analysed using SwissADME Swiss drug design tool under Swiss target prediction page (https://www. swisstargetprediction.ch/predict.php) and selecting Homo sapiens as a selection of species (https://www.swiss targetprediction.ch/result.php?job=1978465581&organism =Homo_sapiens).

Network pharmacology analysis

Integrative data mining was conducted across multiple databases including Comparative Toxicogenomics Database (CTD; http://ctdbase.org/), HERB (http://herb.ac.cn/) and Encyclopedia of Traditional Chinese Medicine (ETCM; http://www.tcmip.cn/ETCM) to predict the potential binding targets of alpha-cubebene. With the search query of "Ulcerative Colitis" and "Homo sapiens", the UC relevant genes were downloaded from GeneCards (https://www.genecards.org) and Online Mendelian Inheritance in Man (OMIM; https://omim.org/). After the data collection and screening and disease targeting mapping, network construction and analysis was done through STRING analysis, Gene ontology (GO) and KEGG pathway enrichment analysis.

STRING analysis of ICAM1

ICAM1was added to the STRING database (https://string-db.org/) to build protein-protein interaction (PPI) networks for organisms of H. Sapiens and the score more than 0.9 was considered.

Gene ontology analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were implemented using Shiny GO 0.85.1 (https://bioinformatics.sdstate.edu/go/) with coverage for all KEGG pathways and all GO (Ivan *et al*., 2022; Hopkins, 2008; Wang *et al*., 2025; Jalali *et al*., 2025; Huang *et al*., 2009).

Molecular pathway and interaction of alpha-cubebene

Molecular pathway of ICAM1 was generated using AI by adding prompt, also inhibiting and stimulatory effects of alpha-cubebene in this pathway are generated.

MD simulation analysis

Desmond package in Schrödinger suite was employed for the MD simulation to check the dynamic stability of the protein-ligand complexes. Solvated complexes were simulated for 100 ns in TIP3P explicit solvent using TIP3P (10 buffer). TIP3P water box surrounded with counter ions Na+ /Cl− to obtain physiological ionic strength 0.15M. Energy minimized system was equilibrated using NPT ensemble under 310K, 1atm. Used OPLS_2005 force fields with Nose-Hoover thermostat and Martyna-Tobias-Klein barostat for the control of temperature and pressure respectively. RMSD and RMSF, protein-ligand interaction and contact analysis, secondary structure element analysis, radius of gyration and MM-GBSA-binding free energy were computed from MD simulation trajectory to determine structure dynamic properties and binding strength (Karunarathne *et al*., 2026; Othman *et al*., 2025).

Experimental animals

Albino Wistar rats (*Rattus norvegecus*) (n=30) weighing 100-150grams, each group containing 5 rats were acclimatized first for a 12-hour day and night cycle at a standard temperature of 25^0C for two weeks.

Experimental design

Groups	Treatment
G1	Control
G2	2% DSS solution
G3	2% DSS solution + mesalamine
G4	2% DSS solution + 50µl alpha-cubebene
G5	2% DSS solution + 75µl alpha-cubebene
G6	2% DSS solution + 100µl alpha-cubebene

No of albino rats in each group (n) = 5
DSS: Dextran sodium sulphate: 1ml per rat per day
Mesalamine: 50 mg/kg b.wt per day per rat
Alpha-Cubebene: 10 mg/kg b.wt per day per rat

Induction of ulcerative colitis by dextran sodium sulphate (DSS)

For induction of the ulcerative colitis, Wistar albino rats were given 2% DSS solution in 1/3 dilutions, 1ml per rats through oral gavage for one week. Then 2% DSS solution in ½ dilutions was given 1ml per rat for two weeks

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through oral gavage. Finally, 1ml of 2% DSS solution was given for one week to each rat. Albino Wistar rats were continuously assessed for disease symptoms like lethargy, loose stools and finally bloody stools. The groups were then given treatment according to the experimental design given below with different concentrations of alpha cubebene intraperitoneally for one month.

Statistical analysis

Data obtained was expressed as the mean±SD. Statistical evaluation was carried out by using Duncans Multiple Range Test (DMR), by the use of One Way ANOVA at SPSS Version 27.0 for windows. Significant differences between control and treatment animals were detected at $p<0.05$.

RESULTS

Physicochemical properties of ICAM1

The physicochemical characteristics of the Intercellular Adhesion Molecule-1 (ICAM1) were determined and investigated to assess its structural traits and physical and chemical stability. ICAM1 is a moderate size transmembrane glycoprotein involved in the cell adhesion and the regulation of immune responses with a sequence length of 532 amino acids, the molecular weight of the ICAM1 was identified as 57,825.22g/mol, with the molecular formula $C_{2550}H_{4121}N_{713}O_{778}S_{19}$ as enlisted in table 1. ICAM1 molecular 3D structure was obtained from PDB with an ID number: 1P53 and also the ICAM1 protein sequence was retrieved from the Uniprot (Uniprot: ID: P05362).

Physicochemical properties of ICAM1 were also investigated (index instability: 36.47, which is less than 40 that means it is stable) using software to study and validate the molecule using this index. Because it is a stable protein ICAM1 has been classified and selected to conduct docking analysis for the study of protein-ligand in Ulcerative colitis (table 1).

Table 1: Physicochemical properties of ICAM1.

Sr #	Properties	ICAM1
1	No of amino acid	532
2	Mol weight (g/mol)	57825.22
3	Molecular formula	$C_{2550}H_{4121}N_{713}O_{778}S_{19}$
4	PDB ID	1P53
5	Uniprot ID	P05362
6	Numerical instability index	36.47
7	Stability index	Stable

ADMET and physicochemical analysis of alpha-cubebene

The physicochemical, drug-likeness and ADMET properties of alpha-cubebene were explored in an effort to identify it as a potential therapeutic candidate. Alpha-cubebene is a relatively small sesquiterpene molecule weighing 204.35 g/mol and with a molecular formula of $C_{15}H_{24}$. It is largely hydrophobic, exhibiting very low aqueous solubility of 0.12 g/L at 25C but much higher solubility in organic solvents like ethanol (80.87 g/L) and acrylic acid (40.9 g/L) and a boiling point of 245°C at 760 mmHg confirming good physicochemical stability. The compound satisfied all Lipinski's (Lipinski *et al*., 1997), Ghose's (Ghose *et al*., 1999), Veber's (Veber *et al*., 2002) and Egan's (Egan *et al*., 2000) rules for drug-likeness, with a high oral bioavailability score of 0.55, although it failed the Muegge rules (Muegge *et al*., 2001) (table 2). Analysis of absorptive, distributive, metabolic and excretive (ADMET) properties revealed that alpha-cubebene is efficiently absorbed across gastrointestinal and intestinal barriers, demonstrating excellent absorption, with 95.96% Human Intestinal Absorption (HIA), high Oral Bioavailability (82.9%), high Caco-2 permeability (1.389 log Papp 10cm/s) and an inability to pass as substrates/inhibitors of P-glycoprotein. The molecule's ability to permeate skin is moderate, 3.12 log Kp. It is permeable across the Blood Brain Barrier (BBB). The predicted volume of distribution of 0.77 logs L/kg and substantial free fraction in plasma indicate the propensity of alpha-cubebene to bind plasma proteins to a high extent. Clearance values of 12.78 log ml/min/kg and short half-life (<3h) predict rapid *in-vivo* excretion from the body (table 2).

Cytochrome P450 metabolism data indicate that alpha-cubebene may be a substrate for CYP1A2, CYP2C9, CYP2D6 and an inhibitor for CYP2C9; however, its profile indicates a non-inhibitory role in CYP1A2, CYP2C19 and CYP2D6 mediated drug metabolism (Oda *et al*., 2003). In terms of toxicity assessment, alpha-cubebene has the potential to produce AMES toxicity, but the model shows negative results in terms of toxicity against hERG1, hERG2 and liver. With oral LD50 value predicted as 1.568 mol/kg (table 2), it represents a good safety margin for human consumption. Overall, the computed physicochemical properties, the oral drug-likeness and ADMET properties along with the predicted non-cardiotoxic and non-hepatotoxic profiles suggest that alpha-cubebene as a viable candidate for pre-clinical investigation as a treatment option in patients with ulcerative colitis.

Brain and intestinal estimated permeation analysis

BOILED-EGG (Brain and Intestinal Estimated Permeation Method) model was developed to predict the Passive Gut Absorption and Blood Brain Barrier Penetration of alpha-cubebene from its logP (WLOGP) and topological polar surface area (TPSA). In figure 1A, the white area represents the physicochemical property which shows good probability of high Human Intestinal Absorption (HIA) whereas yellow area (yolk) shows that the molecule has a good potential for the Penetration of the BBB.

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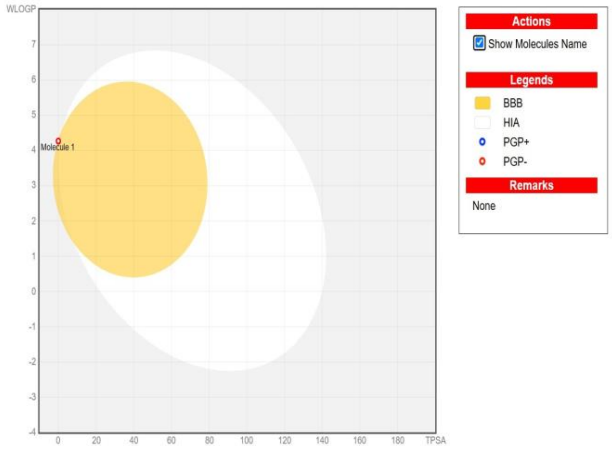
Table 2: Physicochemical and ADMET analysis of alpha-cubebene.

Sr #	Properties	Alpha-cubebene
A	*Physicochemical properties*	
	Molecular weight	204.35 g/mol
	Molecular formula	$C_{15}H_{24}$
	PubChem ID	442359
	CAS number	17699-14-8
	Water solubility	0.12 g/L at 25°C
	Ethanol solubility	80.87 g/L at 25°C
	Acrylic Acid solubility	40.9 g/L at 25°C
	Boiling point	245°C @ 760mmHg
B	*Drug likeness properties*	
	Lipinski	Yes
	Ghose	Yes
	Veber	Yes
	Egan	Yes
	Muegge	No
	Bioavailability score	0.55
C	*ADMET profile*	
ADSORPTION	Water solubility	-5.968 log mol/L
	Caco-2 permeability	1.389 log Paap10^{-6} cm/s
	Intestinal absorption (human)	95.964 % absorbed
	Oral bioavailability	82.9%
	Skin permeability	-3.12 log Kp
	P-glycoprotein substrate	No
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No
DISTRIBUTION AND EXCRETION	Blood Brain Barrier (CNS)	-2.45 log PS
	Blood Brain Barrier	Penetrable
	Fraction unbound (human)	1.25 free proportion
	Plasma protein binding	50.38 TI
	Steady state volume of distribution (VDss)	0.77 log L/kg
	Total clearance	12.78 log (ml/min/kg)
	Renal OCT2 (organic cation transporter 2) substrate	Inhibitor
	Half-life of drug	< 3hr
TOXICITY	AMES toxicity	Toxic
	Max. tolerated dose (human)	-0.18 log mg/kg/day
	hERG I inhibitor	Safe
	hERG II inhibitor	Safe
	Oral Rat Acute Toxicity (LD50)	1.568 mol/kg
	Oral Rat Chronic Toxicity (LOAEL)	1.364 log mg/kg_bw/day
	Hepatotoxicity	No
METABOLIC	CYP 1A2 inhibitor	Non-inhibitor
	CYP 1A2 substrate	Substrate
	CYP 2C19 inhibitor	Non-inhibitor
	CYP 2C19 substrate	Non-substrate
	CYP 2C9 inhibitor	Inhibitor
	CYP 2C9 substrate	Substrate
	CYP 2D6 inhibitor	Non-inhibitor
	CYP 2D6 substrate	Substrate

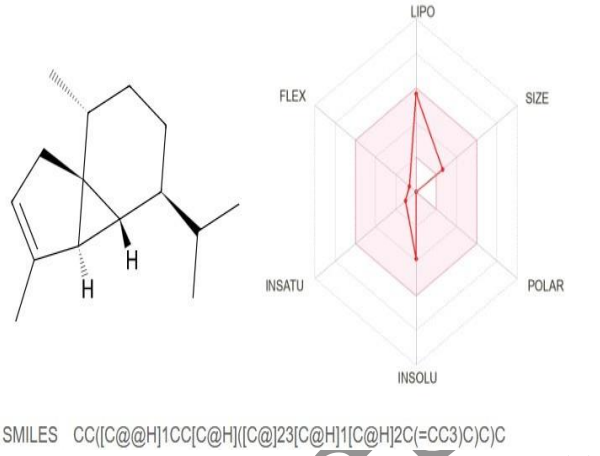
It can be seen from the figure 1A, that Molecule 1 (alpha-cubebene) which is represented by a red circle is falling in the yellow yolk region which means that this drug molecule has good properties for both Intestinal Absorption and BBB Penetration.

The position of molecule toward the left indicates that it has the low topological polar surface area (TPSA 0) and moderately high lipophilicity (WLOGP 4.2), this is also favourable for good oral administration and penetration through biological membranes like BBB.

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(A) Boiled egg model of alpha-cubebene



(B) Spider web model of alpha-cubebene

Fig. 1: (A) Boiled egg model of alpha-cubebene describing that it can cross blood brain barrier (BBB) and has passive gut permeation (PGP); (B) Spider web radar for oral bioavailability studies of alpha-cubebene.

Moreover, alpha-cubebene, which is indicated by a red circle (PGP) (figure 1A), indicates that the drug has no PGP substrates; thus, it may enhance cell penetration, thereby improving the oral bioavailability and intestinal absorption. To conclude based on these observations, it can be inferred that alpha-cubebene has demonstrated that it possessed some desirable drug like properties that enable good oral absorption, blood brain barrier penetration and it does not have a liability toward the P-gp efflux transporter and thus could be used therapeutically for inflammatory disorders such as ulcerative colitis.

The oral bioavailability features of alpha-cubebene have also been determined by employing the SwissADME bioavailability radar that considers the Six physiochemical parameters- Lipophilicity (LIPO), molecular size (SIZE), polarity (POLAR), water solubility (INSOLU), flexibility (FLEX), saturation (INSATU) that affect oral absorption of oral active substances. The orange shaded zone in the radar plot in figure 1B is indicative of the optimum physicochemical parameter ranges for orally active substances and the red line represents the physicochemical features of the compound, alpha-cubebene. Almost all the physicochemical parameters of alpha-cubebene have come closer to or within the optimal range showing good drug-like characteristics. Alpha-cubebene has optimal lipophilicity and size required for better oral absorption via better diffusion through various membranes. Furthermore, it is characterized by a low value for polarity, a property which explains its hydrophobic nature that favors passive transport through the biological membranes. In spite of being having a poor value for water solubility that is quite common for terpenoid nature of phytochemicals, its properties showed excellent suitability for its oral administration as drug candidate for the target of interest in the current studies and other applications also.

Swiss target prediction

Pharmacological properties of alpha-cubebene were further analyzed via *in-silico* target prediction and drug-likeness evaluation. The result showed in the target class prediction chart (figure 2), that alpha-cubebene may has wide spectrum of potential biological functions by targeting numerous classes of proteins, which may also contribute its multitarget therapeutic potential. The first class of predicted targets of alpha-cubebene was nuclear receptors (28%), which implied that alpha-cubebene might regulates cellular signaling transduction pathways related to inflammation, metabolism and immune systems through nuclear receptors mediated cellular signal transcription and regulation. Unclassified protein class was the second highest of the class, accounted for 16% of the target of alpha-cubebene. The next class is Oxidoreductases (12%), followed by Phosphatase, Family A G protein-coupled receptors and secreted proteins class, each accounted for 8%. Then electrochemical transport, Hydrolases and voltage-gated ion channels accounted for 4% of the targets, respectively and additionally Membrane receptor and Cytochrome P450 each is 4% of predicted targets. All the above analysis indicates alpha-cubebene might act as a promising therapeutic agent for the treatment of inflammatory diseases like UC because it is characterized with multi-target drug acting mode.

The stereochemical quality of the protein structure ICAM1 (PDB ID: 1P53) was assessed with a Ramachandran plot obtained from PROCHECK.

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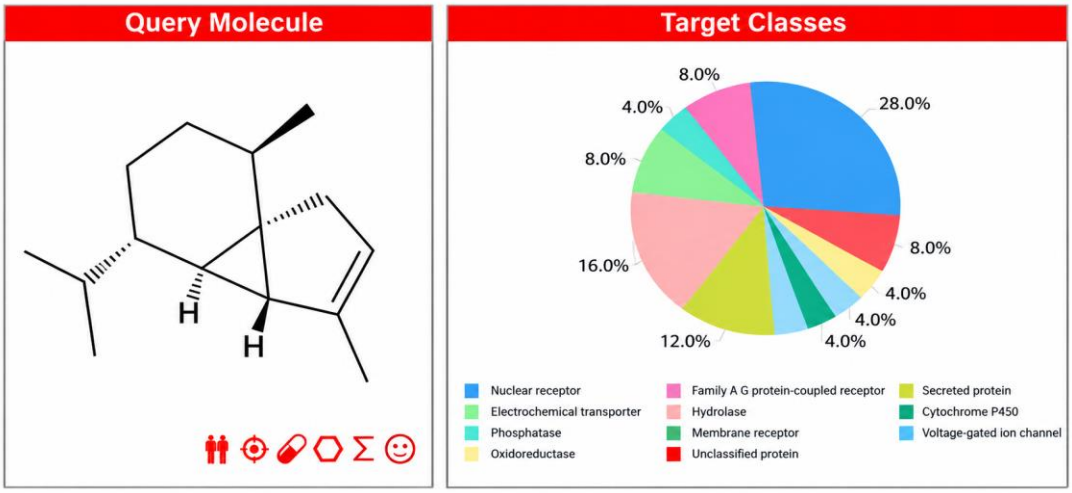


Fig. 2: Target class prediction of alpha-cubebene for pharmacological and drug likeness properties.

Ramachandran plots describe the stability of protein conformation through an analysis of the backbone dihedral angles (φ and ψ) of the amino acid residues of proteins, identifying residues which fall within energetically allowed regions. As shown in the plot in figure 3, the vast majority of residues fell into the most favored regions, where 380 residues (90.0%) occupy energetically favorable regions of the space. An additional 41 residues (9.7%) lie in the additionally allowed regions, with 1 residue (0.2%) situated in the generously allowed regions. There are 0 residues (0.0%) in the disallowed regions of the Ramachandran plot. A total of 508 residues were plotted, 422 of which were non-glycine/proline residues, 44 were end residues and 40 were proline residues. The Ramachandran plot analysis has showed a good correlation for residues in allowed and favored regions and no residues in disallowed regions, which clearly indicates excellent quality of ICAM1. Thus, ICAM1 protein structure is well refined, conformationally stable and good for any further studies, molecular docking and molecular dynamics simulations.

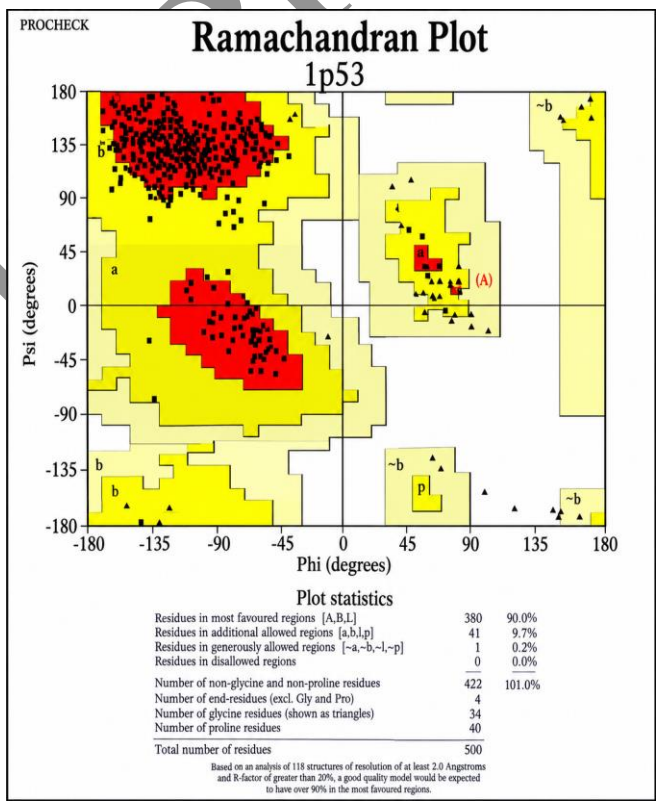
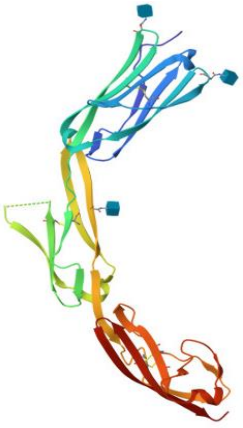


Fig. 3: Ramachandran plot of ICAM1 for assessing its stereochemical quality.

ICAM1, a type I transmembrane glycoprotein from the immunoglobulin superfamily, is mainly expressed on the cell surface of endothelial cells, epithelial cells, macrophages, T cells and other immune cells. ICAM1 protein is composed of the extracellular, transmembrane and intracellular regions. It contains an extracellular region with five immunoglobulin-like domains (D1–D5), one transmembrane span and an intracellular C-terminal region that plays important roles in signalling pathways (figure 4A). ICAM1 acts primarily by binding to lymphocyte function-associated antigen 1 (LFA-1; CD11a/CD18) and macrophage-1 antigen (Mac-1; CD11b/CD18) on leucocytes. Specifically, the first immunoglobulin domain (D1) is the major LFA-1 binding site to mediate firm adhesion and transmigration of leucocytes across the endothelium during inflammation.

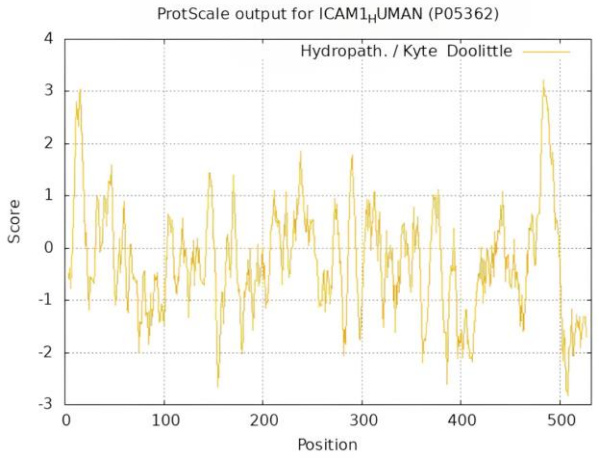
Available high-resolution structures of ICAM1 greatly facilitate the characterization of its protein-protein interaction and identification of potential target for treatment of inflammatory conditions. Hydrophobicity analyses has revealed the prevalence of a largely hydrophilic surface throughout the extracellular immunoglobulin-like domains of ICAM1 as a potential site for interaction with its ligands and its association of low hydrophobicity in the intracellular segment, reflecting

interactions with cytoplasmic proteins (figure 4B). As depicted in the 3D structure, there is a single hydrophobicity patch on the protein and this patch mainly localizes near the C-terminal region as it corresponds to its transmembrane region.



(A)

3D Structure of ICAM1



(B)

ICAM1 hydrophobicity chart

Fig. 4: (A) 3D structure of ICAM1 showing 5 immunoglobulin-like domains (D1-D5); (B) Hydrophobicity chart of ICAM1 revealing it to be a transmembrane protein.

Molecular docking investigation of ICAM1 with alpha-cubebene

Molecular docking simulation of alpha-cubebene against ICAM1 showed five putative pockets that possessed varied binding affinities, cavity volumes and interacting amino residues. Amongst them, the pocket C3 exhibited maximum binding affinity having a score of -6.1 kcal/mol and a cavity volume of 343 A^{03} which likely shows maximum favorability towards the interaction with alpha-cubebene on ICAM1 protein. The interaction at this site included GLU373, ARG374, ASP375, PRO377, GLY378, ASN379, TRP380, MET391 and GLN393amino acid residues, which likely contribute substantially towards stabilization of the ligand through favorable hydrophobic and polar contacts. The second highest binding affinity was recorded at C2 having binding energy of -5.1 kcal/mol and a cavity volume of 445 $A^{03}$and formed stable protein-ligand interactions with the residues LYS304, CYS305, GLU306, ALA307, GLY324, PRO325, ARG326 and ALA327 of the protein (table 3). The C1 had a significantly large cavity volume (589 A^{03}) that accommodate ligand efficiently however the recorded binding energy is -4.9 kcal/mol. Pocket C4 and C5 had comparatively weaker binding affinity of -4.8 and -4.6 kcal/mol respectively having smaller volume of 194 $A^{03}$and 129 A^{03}. Both C4 and C5 were primarily composed of extracellular residues and likely a secondary binding site of alpha-cubebene (table 3). Hence the results indicate that alpha-cubebene has favourable interactions with the protein ICAM1 which may in part elucidate the mechanism of its action as anti-inflammatory agent.

Structure of ICAM1 in PDB format was docked to a phytocompound, alpha-cubebene, for analysis of interactions. In this simulation, the three-dimensional structure of ICAM1 was successfully docked to the phytocompound alpha-cubebene in order to investigate the possible inhibitory potential of alpha-cubebene against ICAM1 related inflammatory pathways. The docking produced a reasonable binding pocket interaction resulting in a stable protein–ligand complex where the phytocompound, alpha-cubebene was well situated in the predicted binding site of the protein ICAM1. The docking indicated good affinity between alpha-cubebene and binding site amino residues of the protein. This interaction possibly changes the structural and dynamic of ICAM1 (figure 5). This binding of alpha-cubebene and ICAM1 is important since ICAM1 mediates the leukocyte binding and migration and also serves for amplification of inflammatory signals, ultimately contributing to leukocyte recruitment and mucosal inflammation and ICAM1 serves a very important function in recruiting and initiating inflammation via enhancing leukocyte activities that drive inflammation.

STRING analysis

A protein–protein interaction network was constructed via STRING to identify the functional associations of Intercellular Adhesion Molecule-1 (ICAM1) with other proteins playing important roles in immune-related processes. The network revealed ICAM1 as a core protein associated with a considerable number of immune-related partners and demonstrated the significance of the central position of ICAM1 in managing leukocyte adhesion, emigration and inflammation-associated events (Fig. 6).

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Table 3: Binding energies, cavity volume and contact residues of ICAM1 with Alpha-Cubebene during docking analysis.

Protein	Pocket #	Binding vina score	Cavity volume ($A^{\circ 3}$)	Contact residues
ICAM1	C1	-4.9	589	Chain B: ALA221 GLN222 VAL223 HIS224 LEU225 ALA226 ASP229 GLN230 ARG231 LEY232 ASN233 PRO234 THR235 VAL236 TYR238 THR262 ALA264 GLU273
	C2	-5.1	445	Chain A: LYS304 CYS305 GLU306 ALA307 GLY324 PRO325 ARG326 ALA327 GLN328 Chain B: CYS305 ALA307 GLY324 PRO325 ARG326 ALA327
	C3	-6.1	343	Chain A: GLU373 ARG374 ASP375 CYS376 PRO377 GLY378 ASN379 TRP380 THR389 MET391 Chain B: GLU373 ARG374 ASP375 PRO377 GLY378 ASN379 TRP380 MET391 GLN393
	C4	-4.8	194	Chain A: GLU401 LEU402 LYS403 CYS404 LEU405 ASP407 GLY408 PRO411 LEU412 PRO413 ILE414 GLU416 LEU429 AR431 ARG433
	C5	-4.6	129	Chain A: GLU297 GLY298 GLU300 THR302 LEU330 LEU331 LYS332 ALA333 THR334 GLU336 Chain B: GLU336 ASN338 GLY339 ARG340 SER341 PHE342 SER343 SER345 ASN358 THR360

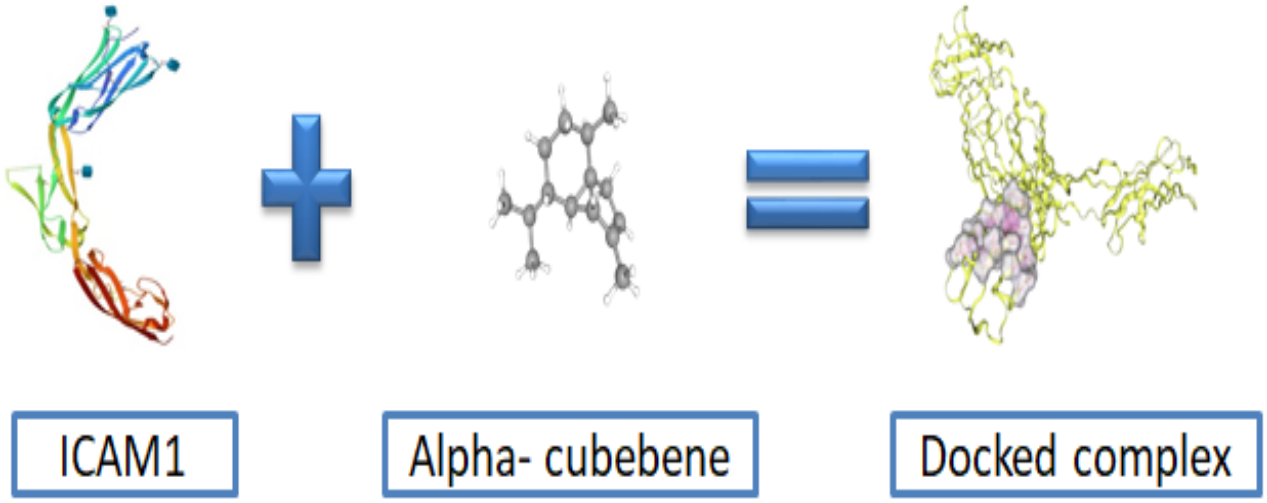


Fig. 5: Docked complex of ICAM1 with Alpha-cubebene.

Binding of ICAM1 to integrin-based adhesion complexes like LFA-1 (ITGAL/ITGB2) and Mac-1 (ITGAM/ITGB2) facilitates firm adhesion of leukocytes including neutrophils, monocytes and lymphocytes onto endothelial cells, leading to infiltration into the inflamed colon.

The network showed robust binding of ICAM1 to VCAM1 (Vascular Cell Adhesion Molecule-1), another surface adhesion molecule on the surface of endothelial cells involved in leukocytes migration across the endothelium, thereby cooperating with ICAM1 in sustaining the ongoing inflammation in the context of chronic inflammatory disease, such as UC.

Hence, it is likely that ICAM1 not only participates in leukocytes adhesions but also the inflammatory signaling cascades affecting T cell mediated inflammation and immune responses. The interactions of ICAM1 with MUC1 a heavily glycosylated mucin produced by epithelial cells and involved in maintenance of epithelial lining integrity and in regulation of both innate and adaptive immunity, suggests possible interaction during inflammatory processes and possibly its role in tissue damages during inflammation, which is observed to be altered in UC patients. Therefore, ICAM1-interacting network suggested ICAM1 being a master mediator that bridges with integrins, adhesion molecules, cytoskeletal-associated elements and other immune-modulating molecules orchestrating inflammation in the intestine. This finding accentuates that therapeutic targeting toward the interactions of ICAM1 can be a potential therapeutic approach for alleviating UC.

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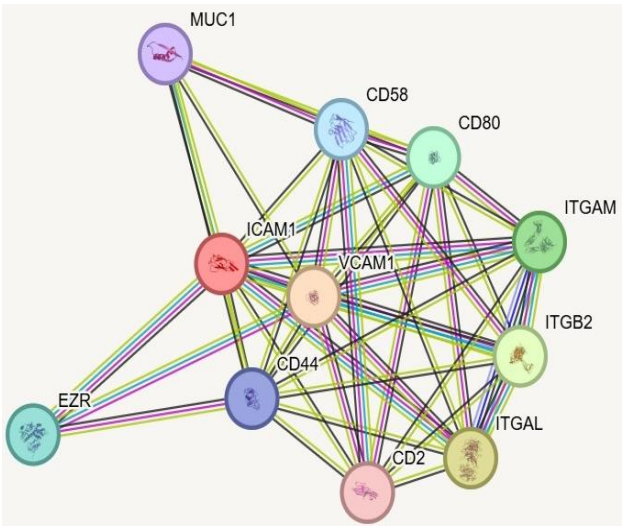


Fig. 6: Protein–protein interaction network to identify the functional associations of Intercellular Adhesion Molecule-1 (ICAM1) with other proteins playing important roles in immune-related processes via STRING.

Gene ontology enrichment analysis

Protein-protein interaction network analysis predicted that ICAM1 is a central hub protein involved in the regulation of inflammation and immune response. ICAM1 exhibited a strong correlation with adhesion molecules and integrins such as VCAM1, ITGAL (CD11a), ITGAM (CD11b) and ITGB2 (CD18), all of which plays important roles in leukocyte adhesion, migration and transmigration upon inflammation. ICAM1 interacts with LFA-1 (ITGAL/ITGB2) and Mac-1 (ITGAM/ITGB2) and contribute to the firm adhesion of leukocytes to the endothelium, promoting leukocyte transmigration into the inflamed colon tissue. Also, ICAM1 interacts with CD44, CD2, CD58, CD80, thus suggesting a crucial role in the process of activation of immune cells, presented by immune cells and in adaptive immunity; moreover, it shows association with EZR and MUC1 that support cytoskeletal organization and intracellular signaling and maintenance of mucus barrier integrity. Together, the analysis indicates ICAM1 plays significant roles in the immune cell recruitment and maintenance of the inflammatory colonic inflammation in UC.

Biological processes GO enrichment

Gene Ontology (GO) biological process enrichment analysis was performed to determine the functional significance of the identified target genes of ICAM1 and related proteins. Enrichment results indicated that most significantly enriched biological processes were predominantly related to cell adhesion, leukocyte trafficking and immune cell activation, highlighting their crucial involvement in inflammation and immunity. Most enriched term "leukocyte cell-cell adhesion" has highest enrichment signal and lowest FDR, which suggests strong association with the analyzed gene set (figure 7A).

The enrichment of membrane-to-membrane ensures that proteins interact at the cell surface for cell-cell communication for Immune Synapse formation and signaling transduction. "Cellular extravasation", significantly enriched, suggests migration of leukocytes from the circulation into target organs/tissues, a primary event in chronic inflammation associated with Ulcerative Colitis. Significantly enriched term "cell-matrix adhesion" regulates cell-extracellular matrix interactions and has important functions in tissue modeling, disruption of epithelial barrier and ongoing inflammation in IBD. Furthermore, the term "cell-cell adhesion via plasma membrane adhesion molecules" implies specific role of cell-cell adhesion molecules including ICAM1, VCAM1 and integrin in cell adhesion and inflammation (figure 7A). The identified pathways are important in the underlying mechanisms of ulcerative colitis with excessive activation and recruitment of inflammatory and immune cells, resulting in persistent tissue damage and ongoing inflammation in the mucosa. Regulation of these pathways may have therapeutic implication for reducing chronic inflammation in the gut.

Molecular functions GO enrichment

GO molecular function enrichment analysis indicated that most of the enriched targets participate in binding integrin, cell adhesion molecule binding and signaling receptor mediated by cell adhesion molecule. Binding integrin became the most significant enriched term that has essential implication for leukocytes' transmigration; in addition,ICAM1 receptor binding, protein-containing complex binding, signaling receptor binding and signaling receptor activity were among top ranking enriched functions that are implicated in intracellular signaling and cell communications between the cells; complement component C3b binding function was enriched, suggesting an important role in complement-mediated inflammation. Overall, the enrichment analysis indicated that the molecular function terms are of key significance to recruit leukocytes, stimulate cells and transmit signaling pathways that mediate inflammation, which are crucially related to IBD and UC (figure 7B).

Cellular component GO enrichment

To understand the subcellular locations and structure features of the identified target proteins, further gene ontology (GO) cellular component enrichment analysis was performed. It suggests the central role of these proteins to connect two cells in inflammation processes. Enrichment of the external side of plasma membrane, cell surface and side of membrane also reveals that these proteins are likely positioned in cell surface to control leukocyte adhesion, signaling and communication with the environment.

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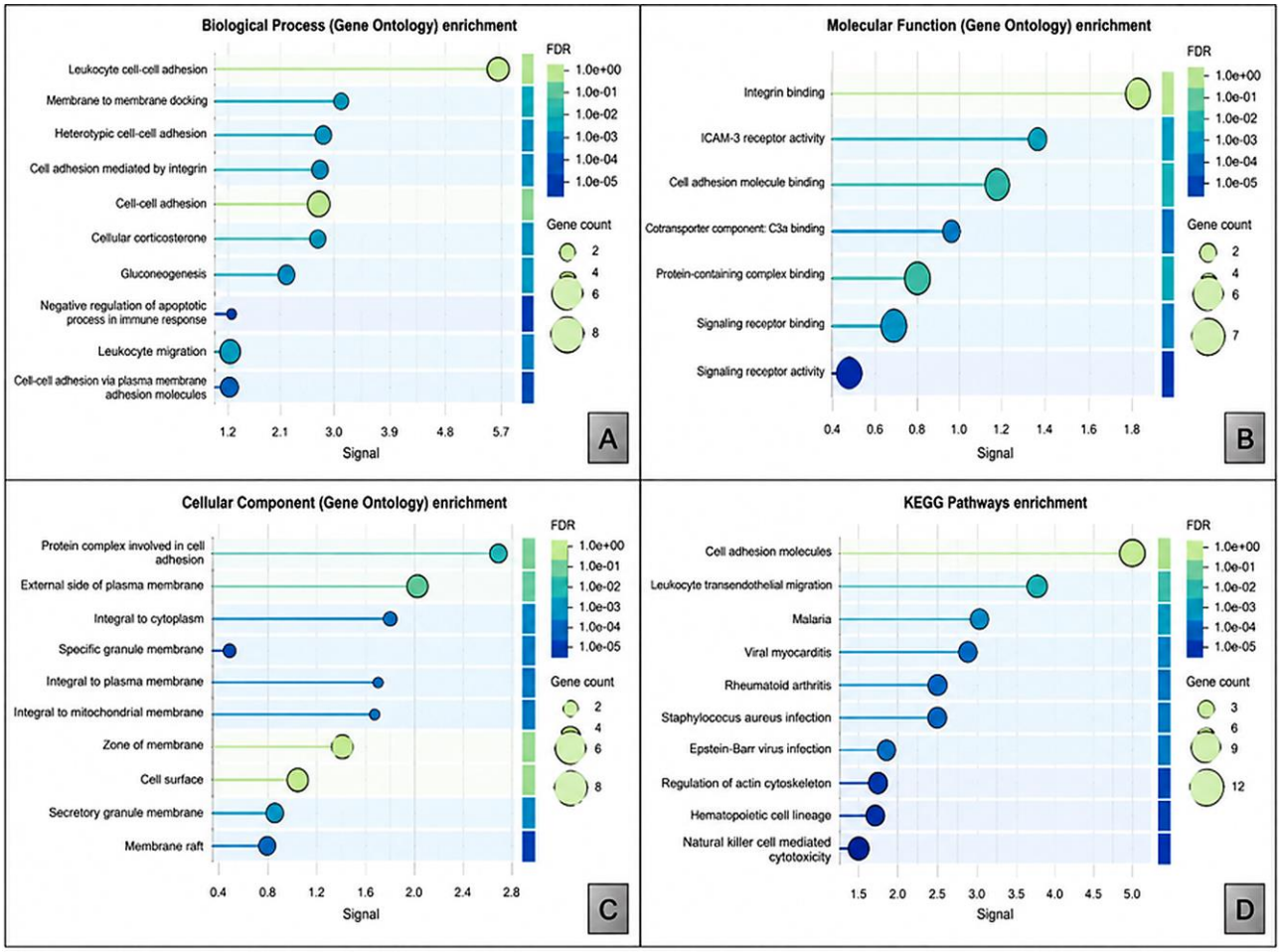


Fig. 7: Gene ontology (GO) enrichment analysis; (A) Biological process enrichment; (B) Molecular function enrichment; (C) cellular component enrichment; (D) KEGG pathway enrichment.

Furthermore, the enrichment of secretory granule membrane, specific granule membrane and membrane raft suggests potential role in internal signaling and transport pathways and immune activation (figure 7C). Collectively, based on cellular component enrichment, it can be concluded that the identified target proteins mainly contribute to adhesion and cell surface structures and facilitate leukocyte adhesion and immune signaling.

KEGG pathways GO enrichment

KEGG pathway enrichment analysis was performed to map these target genes to biologically relevant pathways and determine their associated functions. The results in figure 7D suggest a strong enrichment in pathways related to cell adhesion, immune cell trafficking and inflammation, among others. In particular, cell adhesion molecules (CAMs) presented with the strongest signal for enrichment, coupled with the lowest false discovery rate (FDR), demonstrating that adhesion molecules are crucial in modulating immune cell adhesion and host inflammatory responses.

The leukocyte trans endothelial migration pathway was significantly enriched, implying an essential role for the identified target genes in mediating the recruitment of immune cells across the endothelium and their entrance into the inflammatory site, a crucial process in ulcerative colitis development and pathogenesis. Furthermore, enrichment in the regulation of actin cytoskeleton pathway signals for the potential implication of the target genes in cell adhesion, movement and other functional processes by means of cell membrane modifications. Also, the enrichment of hematopoietic cell lineage and natural killer cell-mediated cytotoxicity points to a potential involvement of ICAM1 in immune cell generation and function, as well as in adaptive immune responses against targeted threats. Therefore, on the whole, the KEGG pathway analyses clearly demonstrate that these target molecules primarily play an important part in adhesive molecule functions, as well as in leukocyte transport, immunity regulation, inflammation and other common biological pathways. This may present an effective means of tackling ulcerative colitis due to their crucial role in the pathogenesis.

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MD simulation of ICAM1 with alpha-cubebene
RMSD (Root Mean Square Deviations):

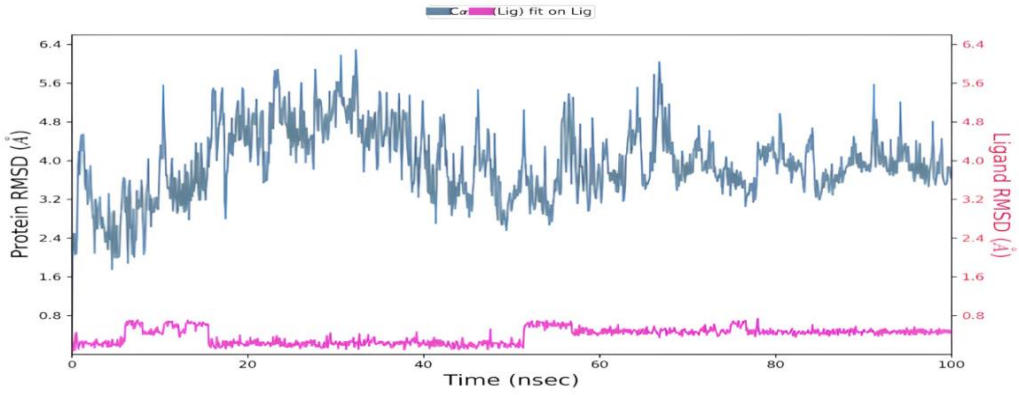


Fig. 8: The root means squared deviations profiles for the protein and ligand in the molecular dynamic's simulations of the protein-ligand complexes. Structural stability of the protein and positional stability of the ligand during the simulation were assessed by using the RMSD trajectories.

To monitor the structural integrity of the ICAM1 (PDB ID: 1P53) with alpha-cubebene complex, a root mean square deviation (RMSD) analysis was performed across the 100 ns simulation. After an initial equilibration period, the protein backbone RMSD quickly assumed a stable value between ~3.0-4.5 Å with transient deviations only occasionally reaching 6.0 Å during the middle of the simulation (fig 8). These oscillations are more indicative of local rearrangements of mobile loop structures and accommodation/refinement of interactions with the ligand and should not necessarily be interpreted as overall protein instability. The plot then levels off towards the end of the simulation reaffirming that the ICAM1-α-cubebene complex is in dynamic equilibrium and that the tertiary structure of the ICAM1 is well maintained within the structure during the simulation period.

The ligand α-cubebene RMSD remained below 0.8 A throughout the entire simulation. This indicates that the orientation of α-cubebene in the binding pocket of ICAM1, remained quite constant and that the ligand did not show any significant positional deviations throughout the simulation. These binding characteristics would be attributed to strong hydrophobic and van der Waals interactions between the non-polar sesquiterpene structure of α-cubebene and its binding pocket on ICAM1. Overall, the RMSD values suggest that both the protein and the ligand structure was stable throughout the simulation and thereby supporting the finding from docking analysis that the ICAM1-α-cubebene complex was indeed held together via tight and stable binding.

The root mean square fluctuation (RMSF) analysis was conducted to reveal the residue-level flexibility in the ICAM1 (PDB ID: 1P53)-α-cubebene complex along 100 ns molecular dynamics trajectory. The higher RMSF values (~6.4 Å) were found among C-terminal residues, which are well known to harbor flexibility due to solvent accessibility and relaxed constrain of termini. Moderate fluctuation was also presented in residue segment of ~50-60, around residue 180 and between residue 220-230, which is believed corresponding to some flexible loop regions or surface regions allowing large conformational changes compared to the rigid secondary structure (fig 9). The mobility of such regions might provide structural flexibility to accommodate required movement for interaction. Importantly, the amino residues belonging to the α-cubebene binding pocket showed considerably low RMSF in entire MD simulation of 100 ns after binding, suggesting stable structure of the pocket in the bound state and proper protection of the binding site.

The protein−ligand interactions analysis of ICAM1 (PDB ID: 1P53)–α-cubebene complex over 100 ns MD simulation shows that the hydrophobic interaction is responsible for the complex stabilization. The binding pocket has the presence of interactions of ligand with residues like PHE216, VAL288, VAL303, ALA327, LEU329, PHE342, LEU448 and PRO450 and the interaction creates a consistent hydrophobic pattern in the pocket and forms a tight hydrophobic interaction network (fig 10). The three amino acids namely ALA327, LEU329 and PHE342 have the maximum interaction occupancies, which indicate the significance for the ligand identification, orientating and stabilizing within the pocket. The dominance of hydrophobic, van der Waals interaction in the pocket and complete absence of hydrogen bonding consistent with the nature and non-polarity of α-cubebene; these also supported the low RMSF (root mean square fluctuation) values of the binding site residues and constant RMSD of the binding-site which clearly indicate a highly stable complex formed by the α-cubebene.

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RMSF (Protein residue fluctuations)

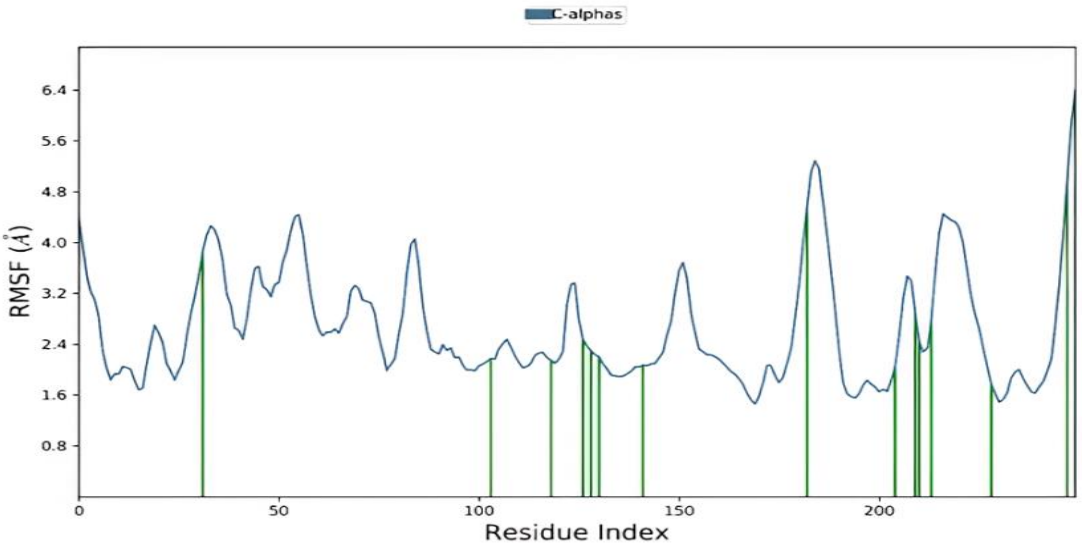


Fig. 9: Protein residue fluctuation of protein residues from the molecular dynamic trajectories. The RMSF profiles show that the residues are flexible residue-wise and that those with large RMSF values are highly mobile, whereas the residues that are involved in binding the ligand are highlighted.

PLI histogram (protein-ligand interaction)

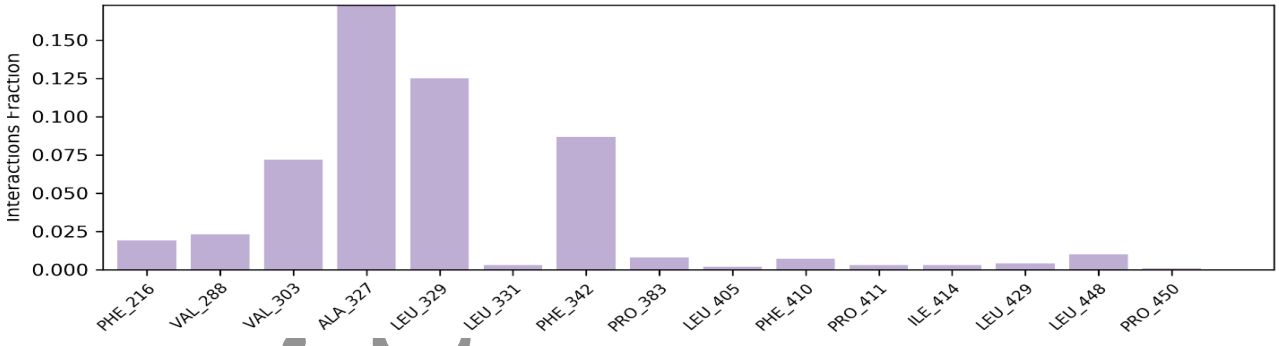


Fig. 10: Protein-ligand interaction histogram plotting the number of contacts between the protein and the ligand during the molecular dynamic simulation. Interaction fractions are good examples of the longer-lasting residues contacts that stabilize the ligand.

By performing MD simulation, In the first few ns, Rog decreases from around 35 to 29 A suggesting that there is an initial compaction in the protein structure just after the protein equilibrates. Then the Rog shows modest fluctuations between 26 and 30 A till ~55 ns that shows normal conformational fluctuation of the protein-ligand complex. The Rog grows till ~34-35 A between 55-70 ns showing transient growth in the protein structure that might be due to local conformational fluctuation instead of unfolding of the protein structure. After 70 ns the Rog drops down to around 24-25 A. From 70 ns until the end of simulation, Rog oscillates between 24 and 25 A indicating a relatively compact and stabilized protein structure (fig 11). Although ICAM1-α-cubebene shows fluctuation during initial and middle phase of simulation, at the end of simulation, Rog shows a stable conformation, so a more rigid and stable state could be achieved with stable binding.

In order to further monitor the effect of α-cubebene binding on the ICAM1 structure, the SASA of the ICAM1-α-cubebene complex was investigated throughout the simulation. Figure 12 shows the SASA as a function of time in the100ns trajectory. The plot is similar to the RMSD and RoG plots. The solvent accessibility for the protein-ligand complex remained fairly consistent during MD trajectory. The SASA stayed between 14,600 and 15,400 A, with little deviation from this value during simulation. This observation suggests a relatively dynamic interaction but without major change of the solvent accessibility and overall protein fold.

RoG (Radius of gyration):

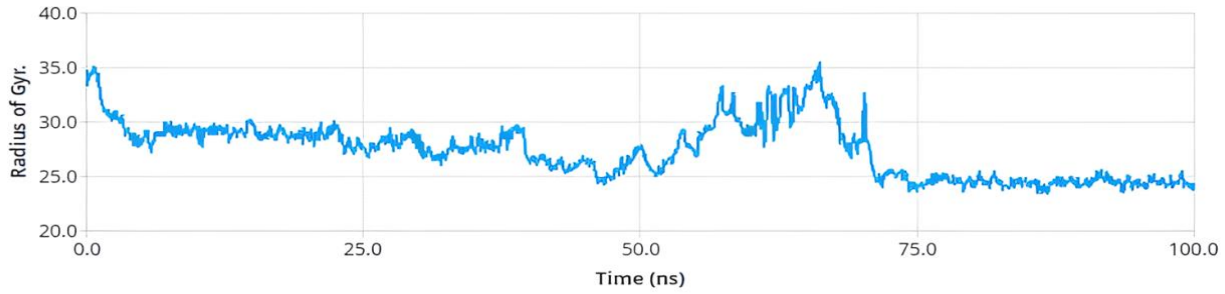


Fig. 11: The radius of gyration (RoG) profile of ICAM1-alpha-cubebene complexes during the molecular dynamic simulation, showing that the structural compactness and overall conformational stability of the complexes change over time.

SASA (solvent accessible surface area)

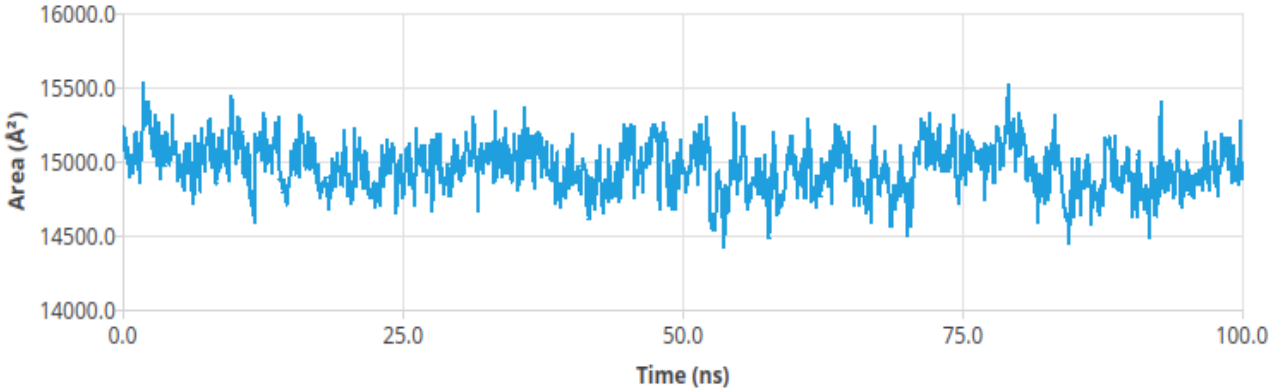


Fig. 12: Complex of ICAM1-alpha-cubebene with solvent accessible surface area (SASA) profile during the 100 ns simulation, showing differences in solvent exposure and protein surface area accessibility during the simulation.

MM/GBSA

Table 4: The binding free-energy components are obtained from representative structures of the dynamic's trajectories of the complexes. ΔG_bind is the binding free energy and the other terms are the individual energetic contributions to the stability of the complexes.

Complexes	1P53-Ligand
ΔG_bind (kcal/mol)	-23.36
ΔG_Lipo	-15.89
ΔG_vdW	-11.13
ΔG_Coulomb	-0.03

MM/GBSA binding free energy analysis shows ICAM1 (PDB ID: 1P53)-α-cubebene complex forms a favourable binding affinity with total binding free energy (Gbind) of 23.36 kcal/mol. Lipophilic interaction (GLipo=15.89 kcal/mol) provides the largest contribution to the binding stability and is dominated by contacts with hydrophobic regions of amino acids within the ICAM1 binding pocket. The van der waals forces (G_vdW=11.13 kcal/mol) contribute significantly to the total binding free energy as it allows the protein binding site and α-cubebene packing together closely to minimize the surface area through interactions between complementary shapes of protein and ligand. This result is in accord with hydrophobic character of α-cubebene and confirmed the docking study with the interactions formed. The simulation reveals frequent contacts between α-cubebene and PHE216, VAL288, VAL303, ALA327, LEU329, PHE342, LEU448, PRO450 residues of ICAM1, suggesting the close contact of α-cubebene with the hydrophobic groups of nonpolar residues in a stable structure. With an appropriate binding free energy value and the favourable terms such as lipophilic and van der waals forces indicate α-cubebene forms an energetically stable complex with ICAM1.

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Biochemical and Pharmacological effects of α-cubebene on ICAM1

Table 5: Biochemical and pharmacological Intercellular Adhesion molecule 1 (ICAM1) ng/mL on hydrogel-loaded α-Cubebene to DSS induced ulcerative colitis in albino Wistar rats.

Groups	Treatments	Mean± S.D
G1	Control	6.23± 0.12
G2	2% DSS solution	15.22 ± 0.02
G3	2% DSS solution + Mesalamine	10.22± 0.53
G4	2% DSS solution + 50µl alpha-cubebene	7.35 ± 0.02
G5	2% DSS solution + 75µl alpha-cubebene	6.85 ± 0.26
G6	2% DSS solution + 100µl alpha-cubebene	6.24 ± 0.65
LSD (0.05)	6.10 ± 0.02	0.001
P-value		0.001

*2% DSS solution (1ml per rat per day)
*Mesalamine (50mg/kg b.wt per day per rat)
*Hydrogel loaded with alpha-cubebene (10mg/kg b.wt per day per rat)

The effects of different concentrations of α-cubebene on the disease activity score in rats with DSS-induced ulcerative colitis are presented in the table 5. The normal control group (G1) exhibited a low disease activity score of 6.23 ± 0.12 ng/mL, indicating the absence of clinical signs of colonic inflammation. In contrast, administration of 2% DSS solution (G2) significantly increased the disease activity score to 15.22 ± 0.02 ng/mL (P = 0.001), confirming the successful induction of ulcerative colitis and the development of severe clinical manifestations, including weight loss, diarrhea and rectal bleeding.

Treatment with the standard anti-inflammatory drug Mesalamine (50 mg/kg body weight/day; G3) significantly reduced the disease activity score to 10.22 ± 0.53 ng/mL, demonstrating a protective effect against DSS-induced colonic injury. However, the disease activity score remained significantly higher than that of the normal control group, indicating only partial recovery. Administration of α-cubebene produced a marked dose-dependent improvement in clinical outcomes. Rats treated with 50 µL α-cubebene (10 mg/kg body weight/day; G4) exhibited a substantial reduction in disease activity score to 7.35 ± 0.02 ng/mL, representing a significant improvement compared with the DSS-treated group. Increasing the treatment volume to 75 µL (G5) further reduced the score to 6.85 ± 0.26 ng/mL, indicating greater attenuation of DSS-induced inflammation. The highest treatment volume, 100 µL α-cubebene (G6), produced the greatest therapeutic response, lowering the disease activity score to 6.24 ± 0.65 ng/mL, which was almost identical to that of the normal control group (6.23 ± 0.12 ng/mL), suggesting nearly complete restoration of normal clinical status as evident from the graph in figure 13.

Statistical analysis demonstrated highly significant differences among all treatment groups (P = 0.001; LSD = 0.001). Overall, these findings indicate that α-cubebene effectively ameliorated DSS-induced ulcerative colitis in a dose-dependent manner, with the 100 µL α-cubebene treatment producing the most pronounced protective effect, surpassing the therapeutic efficacy of the standard drug Mesalamine. The progressive reduction in disease activity scores across the α-cubebene-treated groups suggests that increasing concentrations of α-cubebene enhance its anti-inflammatory and coloprotective effects against DSS-induced ulcerative colitis.

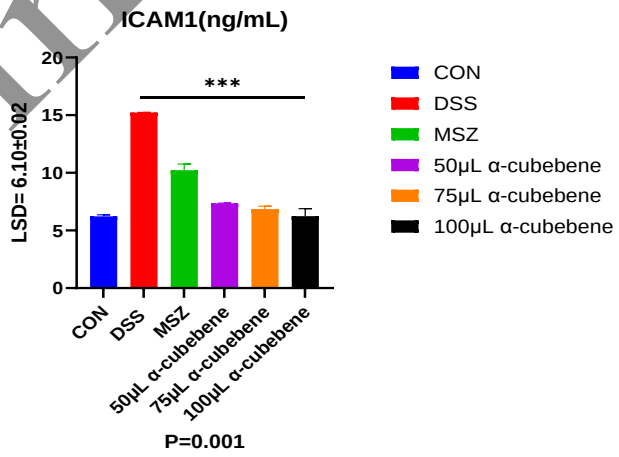
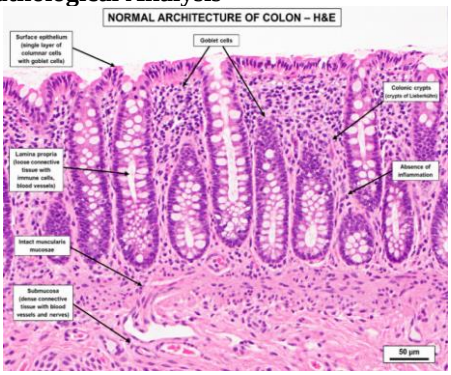


Fig. 13: Graphical representation of different concentrations of α-cubebene on ICAM1.
CON: control; DSS: 2% Dextran Sodium Sulphate; MSZ: mesalamine; ***: significant to P=0.001.

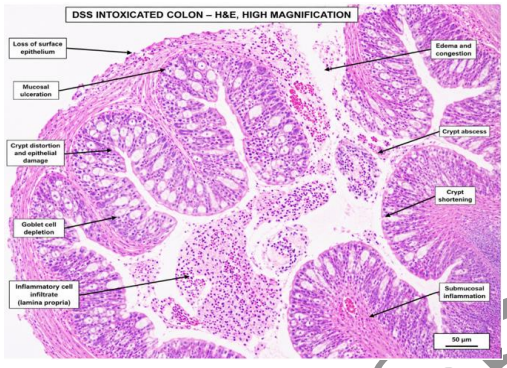
Histopathological examination of colon sections demonstrated marked differences among the experimental groups (figure 14). The normal control group (A) exhibited intact mucosal architecture with well-organized crypts, preserved epithelial lining and an absence of inflammatory cell infiltration. In contrast, the DSS-treated group (B) showed severe colonic damage characterized by extensive epithelial erosion, crypt distortion, mucosal ulceration, edema and dense inflammatory cell infiltration, confirming successful induction of ulcerative colitis.

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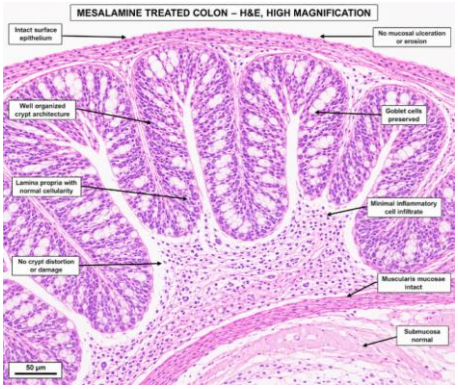
Histopathological Analysis



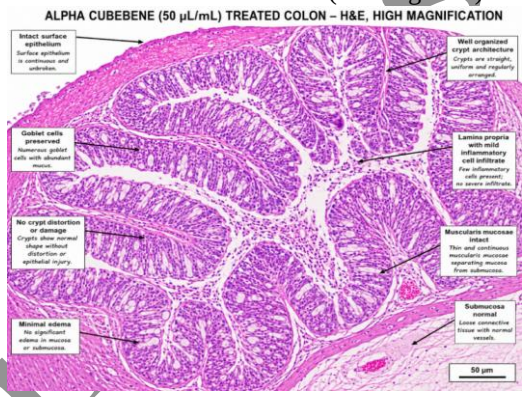
(A) Normal Colon



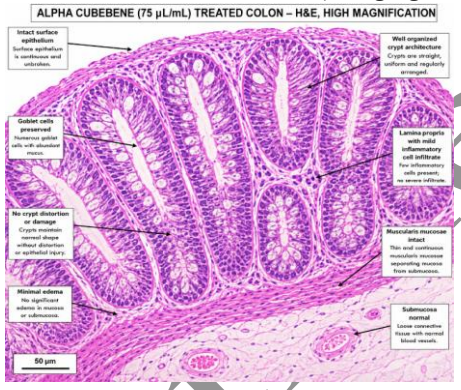
(B) Colon intoxicated with 2% DSS (1mL/kg b.wt)



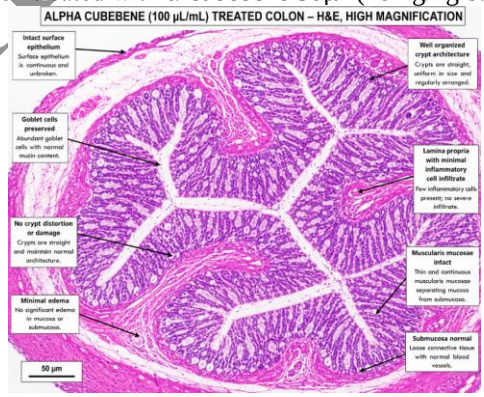
(C) Colon treated with Mesalamine (50mg/kg b.wt)



(D) Colon treated with α-cubebene 50µL (10mg/kg b.wt)



(E) Colon treated with α-cubebene 75 µL (10mg/kg b



(F) Colon treated with α-cubebene 100 µL (10mg/kg b.wt)

Fig. 14: Histopathological examination of colonic tissues stained with hematoxylin and eosin (H&E) (Scale=10X). (A) Normal control group showing preserved colonic mucosal architecture and intact colonic lining. (B) DSS-treated group exhibiting severe epithelial degeneration and mucosal ischemic changes with marked disruption of tissue architecture. (C) Mesalamine treated group demonstrating reduced inflammatory cell infiltration and partial restoration of colonic mucosal architecture. (D) Colon treated with α-cubebene 50µL showing partial restoration of the colonic lining. (E) Colon treated with α-cubebene 75 µL showing less neutrophil infiltration, well organized crypt orientation and intact muscularis mucosa, (F) Colon treated with α-cubebene 100 µL showing complete restoration of the crypt architecture, well preserved goblets and intact surface epithelium Mesalamine-treated rats (C) demonstrated partial restoration of colonic architecture with reduced inflammatory infiltration and improved crypt organization. Treatment with α-cubebene produced dose-dependent histological improvement. The 50 µL group (D) showed moderate recovery with reduced mucosal injury and inflammatory infiltration, while the 75 µL group (E) exhibited further restoration of crypt architecture and marked reduction in inflammatory changes. The highest dose of α-cubebene (100 µL; F) demonstrated near-complete preservation of normal colonic histology, characterized by intact epithelial lining, well-defined crypts, minimal inflammatory cell infiltration and restoration of mucosal integrity, indicating superior colon protective efficacy compared with mesalamine.

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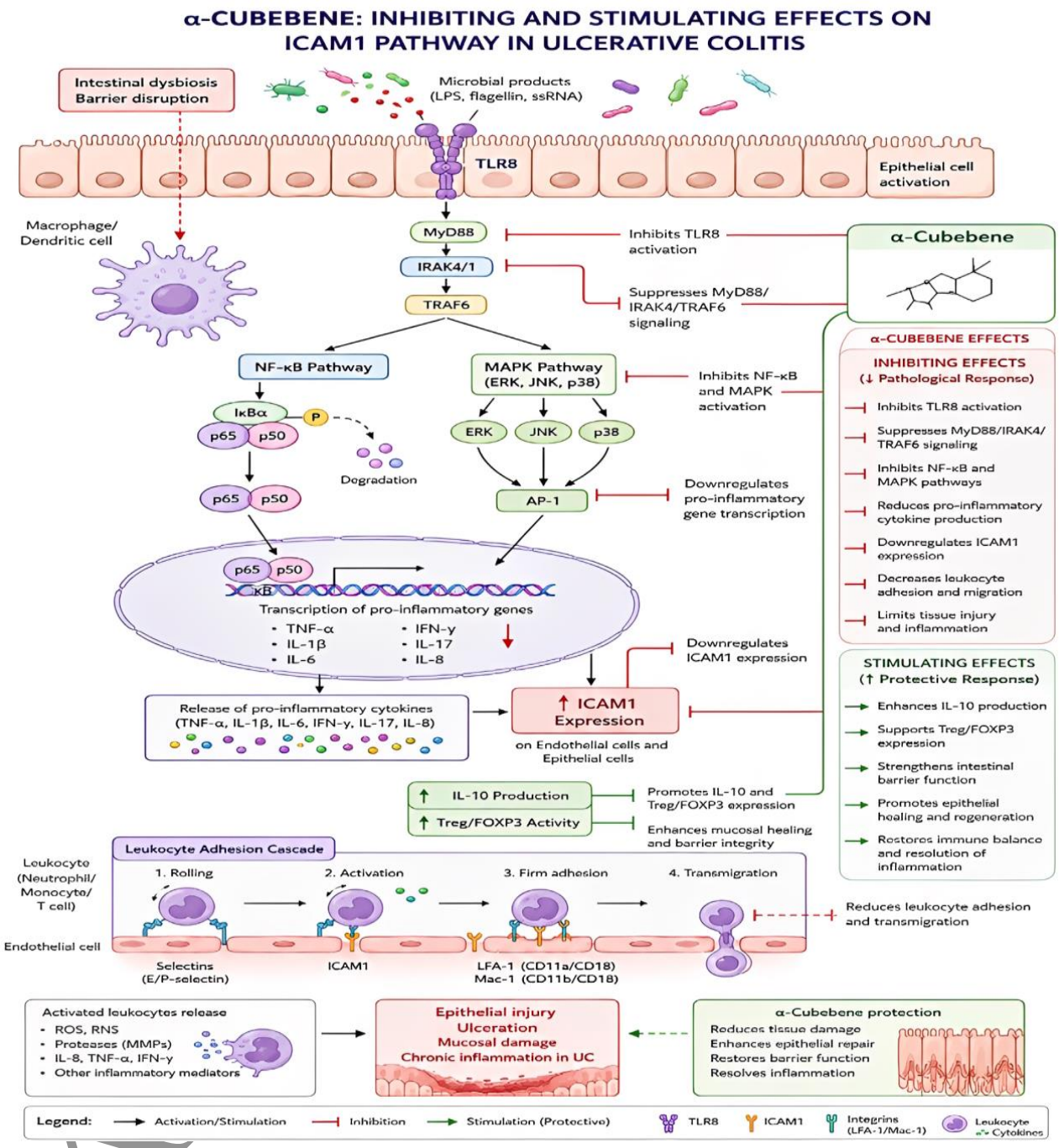


Fig. 15: Proposed schematic molecular mechanism of alpha-cubebene targeting ICAM for the inhibition and treatment of ulcerative colitis.

DISCUSSION

Ulcerative colitis (UC) is a chronic inflammatory disease of the intestinal tract characterized by aberrant immune responses, impaired epithelial barrier integrity and significant infiltration of leukocytes into the colonic mucosa (Kobayashi *et al*., 2020). Among the multiple mediators implicated in UC pathogenesis, Intercellular Adhesion Molecule-1 (ICAM1), a crucial regulator of inflammatory cell recruitment and activation, is increasingly recognized for its contribution. Increased expression of ICAM1 in inflamed colonic tissues in UC patients and its positive association with disease activity indicate ICAM1 as a promising therapeutic target (Bui *et al*., 2020; Haydinger *et al*., 2023; He *et al*., 2023). This study integrates network pharmacology, molecular docking, bioinformatics and ADMET analysis to

elucidate the potential of alpha-cubebene to ameliorate ICAM1-induced inflammation in UC (Laskowski *et al*., 1993; Morris *et al*., 2009). The network analysis via STRING predicts ICAM1 acting as a central node, central in inflammation and immune regulations. Indeed, significant protein-protein interaction (PPI) networks between ICAM1 and various adhesion molecules and integrins including VCAM1, ITGAL, ITGAM and ITGB2 were found to be highly populated networks. VLA4 is well known to be involved in targeting circulating leukocytes into the inflammation site, especially lymphocytes migration to endothelium in UC via adhesion to ICAM-1 or VCAM-1 molecule (Ley *et al*., 2007; Bui *et al*., 2020; Haydinger *et al*., 2023). Excessive leukocyte recruitment into the colon via interaction between ICAM1 and various leukocyte negative transmembrane receptors (e.g., leukocyte integrin 2 beta (ITGA2B/ITGB2, CD11a/CD18, LFA1, or CD11b/CD18 Mac-1) is closely related to constant intestinal mucosa inflammation, tissue erosion and ulceration in UC. In this regard, it was identified that abundant leukocytes accumulate at the intestinal ulceration site of UC and its quantity correlates positively with disease severity.

Molecular Function analysis suggested that "integrin binding" (GO: 0005518) and "cell adhesion molecule binding" (GO: 0097021) to be the most significantly enriched terms, suggesting interactions between ICAM1 and target proteins mediated by the binding of these molecules. Likewise, Cellular Component analysis predict the most abundant target proteins were located at the "external side of the plasma membrane" (GO: 0009986), "integrin complexes" (GO: 0005868) and "cell adhesion molecules binding complexes" (GO: 1990702).Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis identifies that "cell adhesion molecules (CAMs)"(ko04514) and "leukocyte transendothelial migration"(ko04670) were the top enriched pathways, both representing crucial events underlying the pathogenesis of UC (Neurath, 2024; Kobayashi *et al*., 2020; Haydinger *et al*., 2023).

Over the years, natural products are garnering increasing interest as multi-target therapy agents for inflammation-related diseases due to their therapeutic benefits and favorable profiles of safety and effectiveness (Atanasov *et al*., 2021). Alpha-cubebene was chosen due to its reported anti-inflammatory and antioxidant properties (Kim *et al*., 2026), but is first time reported for the possible therapeutic effect in UC. Drug-likeness evaluation revealed that alpha-cubebene fits for good drug-like characteristics according to Lipinski, Ghose, Veber and Egan rule of five. The calculated molecular weight, LogP, HD and HA satisfied most of the drug-likeness rules and the molecular descriptors suggest that alpha-cubebene has agreeable oral drug-likeness properties. ADMET analysis predicts high gastrointestinal absorptions (95.96%), excellent oral bioavailability (82.9%), high intestinal absorption and high permeability (Caco-2) (Daina *et al*., 2017; Pires *et al*., 2015).

Molecular Docking Analysis and MD simulation demonstrates that alpha-cubebene interacted favorably with the ICAM1 binding protein. Among the five calculated docking pockets, pocket C3 of ICAM1 (the region contains of residues GLU373, ARG374, ASP375 ASN379 TRP380 and MET391) had better binding affinity score of -6.1Kcal/mol. Although Vina score of -6.1Kcal/mol could be classified as moderate affinity, this score can possibly be considered significant for a ligand acting through multiple biological targets, which has been shown to lead to biological activity even at moderately moderate interactions (Morris *et al*., 2009; Sliwoski *et al*., 2014). It demonstrates that binding to the pocket C3 can effectively reduce leukocytes adhesion by inhibition of its interaction with leukocyte integrins (Choi *et al*., 2009; Ramotowski *et al*., 2022). It is proposed that alpha-cubebene may act as anti-inflammatory phytoactive compound by modulating inflammatory pathways by interacting with ICAM-1 (Figure 15). Some previous studies revealed that the use of sesquiterpenoid derivatives such as α-iso-cubebene can also inhibit ROS release and activate NFκB signaling pathways which suppress release of inflammation mediators and promote anti-inflammatory activities (Liang *et al*., 2024). Therefore, it was hypothesized that alpha-cubebene exercise anti-inflammatory and UC-remission through interaction with ICAM1 and thus suppress leukocyte influx and reduce tissue inflammatory injury.

The present findings demonstrate that α-cubebene exerts a pronounced protective effect against DSS-induced ulcerative colitis, as evidenced by a significant reduction in ICAM-1 levels, improved clinical disease severity and restoration of normal colonic histo architecture. Dextran sodium sulphate is a well-established experimental model of ulcerative colitis that disrupts the intestinal epithelial barrier, promotes infiltration of inflammatory cells and induces the excessive production of adhesion molecules, particularly intercellular adhesion molecule-1 (ICAM-1), which facilitates leukocyte adhesion and transmigration into inflamed colonic tissue (Pabois *et al*., 2024; He *et al*., 2023). In the present study, DSS administration markedly elevated ICAM-1 concentrations compared with the normal control group, indicating severe inflammatory activation. Treatment with α-cubebene produced a clear dose-dependent reduction in ICAM-1, with the highest dose (100 µL; 10 mg/kg/day) restoring ICAM-1 levels almost to those of the normal control and exhibiting greater efficacy than mesalamine. These biochemical findings were corroborated by histopathological observations, where DSS-treated rats displayed extensive epithelial erosion, crypt destruction, inflammatory cell infiltration and mucosal edema, whereas α-cubebene

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treatment progressively preserved crypt architecture, reduced inflammatory infiltration and promoted mucosal regeneration. The nearly normal histological appearance observed in the 100 µL α-cubebene-treated group confirms its potent coloprotective activity. The concomitant improvement in disease activity and suppression of ICAM-1 suggest that α-cubebene alleviates intestinal inflammation by limiting leukocyte recruitment and preserving epithelial integrity, thereby interrupting the inflammatory cascade responsible for DSS-induced colonic injury. Collectively, these findings indicate that α-cubebene possesses significant anti-inflammatory and mucosal healing properties and may represent a promising phytotherapeutic candidate for the management of ulcerative colitis, although further mechanistic and clinical investigations are warranted to elucidate its molecular targets and translational potential.
Overall, current study demonstrates that alpha-cubebene acts as multi-target agent targeting at inflammatory mediator ICAM1 to ameliorate the colitis symptoms and inflammation by interacting with inflammation mediate ICAM1 through Network pharmacology, bioinformatics, ADMET prediction and molecular docking analyses. Hence, the result in the current study provided promising direction for the development of alpha-cubebene to be a potential novel anti-inflammatory drug candidate for UC treatment. However more scientific investigations will be required to identify the efficacy in terms of *in-vitro*, *in-vivo* and human trials.

CONCLUSION

The present study, using an integrative approach (bioinformatics, network pharmacology, molecular docking and pharmacokinetic predictions) and *in-vivo* experimental model revealed that alpha-cubebene could suppress ICAM1-mediated inflammation in UC. It is found that ICAM1 plays a crucial role in inflammation that involves leukocyte adhesion, immune cell recruitment and trans-endothelial migration, which is a key event that is directly related to the development and progression of UC. Protein-protein interaction and pathway enrichment analyses confirmed that ICAM1 is involved in many cells signaling processes and biological functions that are directly or indirectly linked to the pathogenesis of inflammatory bowel disease, such as cell adhesion, leukocyte migration, integrin-binding cell signaling and immune regulation. Furthermore, molecular docking studies suggested that alpha-cubebene can bind to the active site of ICAM1 protein, thus modulating the downstream inflammation responses mediated by ICAM1 including leukocyte recruitment to the inflammatory sites and downstream signal pathways such as NFκB and MAPK.

Additionally, ADMET parameters indicated that alpha-cubebene is a drug like molecule with high absorption and a reasonable safety profile for oral administration. This work provided insights into the utilization of computational and bioinformatics strategy to explore natural products and target diseases in inflammatory disorders. Furthermore, the study is strengthened by the evidence given by *in-vivo* analysis and restoration of the histopathology. Hence, this study proposed a novel mechanism for the action of alpha-cubebene in the treatment of UC involving negative regulation of ICAM1-induced inflammation pathways.

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Authors' contributions
SR: Software usage, conceptualization, methodology, writing—original draft and Data curation; SZ: Formal analysis, Supervision, writing—review and editing; MF: Analysis, interpretation; MK: Reviewing and editing.

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Data availability statement
The datasets generated and analyzed during the current study are available in the article in whole.

Ethical approval
Ethical approval was obtained from the Institutional Review Board of The University of Lahore (Approval No: UOL/IREB/26/21/01/0001) and animal experiments were performed in accordance with the Institutional Guidelines for the Care and Use of Animals for Scientific Purposes. This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interest
The authors declare no conflict of interest.

Declaration of generative AI
During the preparation of this work, the authors used QuillBot for language refinement and used generative AI tools for the proposed schematic representation of molecular mechanism of alpha-cubebene targeting ICAM for the inhibition and treatment of ulcerative colitis.

Consent to publication
The authors confirm that the submitted manuscript is original, has not been published previously in any language or format and is not currently under review elsewhere.

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