Research Article

Molecular Docking Assessment and ADMET Studies on the Anti-inflammatory Potential of Some Ancient Herbs for the Treatment of Inflammatory Bowel Disorder

Vismaya Achutha[1]*, Kesha M Desai[1], Madhushree K[2], Mahasin Khan[2]
1. Department of Pharmacology, M S Ramaiah University of Applied Sciences, Mathikere, Bangalore, Karnataka, India.
2. Department of Pharmaceutical Chemistry, M S Ramaiah University of Applied Sciences, Mathikere, Bangalore, Karnataka, India.
***Corresponding author's E-mail:** vismayaachutha04@gmail.com

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ABSTRACT

Inflammatory bowel disease (IBD) is a chronic condition that mostly affects the gastrointestinal tract anywhere from the mouth to the anus. The two diseases which make up IBD are: Crohn's disease (CD) and ulcerative colitis (UC). Tumor necrosis factor-alpha (TNF-α) is an inflammatory cytokine mediating the TH1 immune response characteristic of IBD. The prolonged use of marketed drugs was reported to cause various side effects such as diabetes, intestinal flora disturbance. The current study aimed to determine the anti-inflammatory potential of Ashwagandha, Shilajit, Liquorice, Alangi which also had anti-ulcer, anti-oxidant, wound healing abilities and was chosen after literature review. Molecular docking was done using Autodock vina. The Liquorice showed more binding energy values when compared with binding affinity of standard drugs. The selected phytoconstituents are subsequently evaluated for ADMET properties using the PKCSM web server. The results showed that the selected herbs were more potent than standard drugs. The anti-inflammatory potential of these herbs as a natural remedy and as a source of new drugs against IBD is validated. In order to standardize and enhance the traditional herbs for IBD medication, our current work represents a modest first step in that direction.

Keywords: Inflammatory bowel disease, TNF- α, Molecular Docking, ADMET.

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INTRODUCTION

Inflammatory Bowel Disorder is a chronic disorder which causes inflammation of the inner lining of our digestive tract. The disorder consists mainly of two conditions: Crohn's disease (CD) and Ulcerative colitis (UC). However, the signs and symptoms of these two conditions are almost the same and the difference lies in their severity. Crohn's disease causes inflammation of any part of the gastrointestinal tract. The typical symptoms of patients diagnosed with CD include abdominal pain, diarrhea, bloating, nausea, vomiting and weight loss and if left undiagnosed with time it can develop into fibrous fistulas. Malnourishment and growth retardation are the commonly observed symptoms in young patients of CD. Unlike CD, Ulcerative colitis causes severe inflammation of the mucosal and sub mucosal lining of colon and rectum and is restricted to that area only. Bloody stools, mild Diarrhea 4 times a day which might increase up to 6 times with the progression in the severity of disease are the most commonly observed symptoms of UC. Each condition has the potential to raise the risk of colorectal cancer.

IBD has a nearly insurmountable etiology and is now incurable. IBD treatment's primary objectives are to induce and maintain symptom remission, avoid and treat complications, and enhance patients' quality of life. Drugs used for treatment of IBD include cyclosporine, 5-ASA, corticosteroids, and tacrolimus. These drugs produce many adverse events like tacrolimus can cause diabetes and disturbance in the intestinal flora which further worsens the condition. TNF- α, an inflammatory agent which causes inflammation by triggering the release of interleukin-1 and interleukin-6 both being immune system molecules and is responsible for activating diverse signaling events involved in inflammation. TNF- α inhibitors are incapable of treating all the symptoms of IBD. The medication bracket of IBD and depending on the severity if any recommended surgery is very expensive. The prevalence of IBD is increasing year over year throughout Southeast Asia due to dietary changes and environmental changes, industrialization, and other causes. Finding a new medicine that is affordable, efficient, and low-toxic thereby becomes the new goal.[1]

Herbs Selected

The following four herbs have strong anti-inflammatory, anti-ulcer, anti-oxidant, wound healing abilities which was chosen after conducting extensive literature analysis. Although some of these herbs were individually established and scientifically validated for their said activities, there was no evidence of these herbs in treating Inflammatory Bowel Disorder so it was deemed worthwhile to do research on these conventional herbs in order to produce scientific proof.

Ashwagandha or Winter Cherry (*Withania Somnifera*, Solanaceae)

Shilajit (*Asphaltum Punjabianum*)

Liquorice (*Glycyrrhiza glabra*, Fabaceae)

Alangi (*Alangium Salviifolium*, Cornaceae)

Activities on individual herbs

Withania Somnifera - Ashwagandha

Withania Somnifera, commonly known as Ashwagandha, is a significant Solanaceae plant that has been used as medicine in the "Ayurveda "system for more than 1500 years.[2]Due to the high accumulation of active ingredients, withanolides, in the plant's roots, they are thought to be the most effective for medicinal purposes .[3]Pawar, P., Gilda, S., Sharma, S., Jagtap, S., Paradkar, A., Mahadik, K., Ranjekar, P. and Harsulkar, A studied about the anti-inflammatory, anti-oxidant and anti-ulcer activity of methanolic extract of *Withania Somnifera* on TNBS induced experimental colitis model (100 mg/kg , TNBS dissolved in 50% ethanol v/v) and proved that W.Somnifera showed cyclooxygenase inhibition and activated tumor necrosis factor alpha. [4]Luvone et al reported that inducible nitric oxide synthase expression is turned on by methanolic extracts of W. Somnifera roots operating at the transcriptional level, leading to enhanced Nitric oxide generation by macrophages, which was associated with anti-inflammatory effects.[5]Bhatnagar M, Sisodia SS, Bhatnagar R. studied the anti-ulcer activity of methanolic extract of W. Somnifera on pylorus ligation induced ulcer model. Treatment with the herb at a dose of 100 mg/kg/day p.o. for 15 days exhibited noticeable lower volume of gastric secretion, free total acidity, and ulcer index Compared to the control group. The antiulcerogenic properties of W. Somnifera extracts were found to be very effective comparable to those of ranitidine hydrochloride. [6]With regard to the above mentioned characteristics, *Withania Somnifera* extract may be able to reduce immune system activity, treat localized inflammation, and treat IBD symptoms.

Asphaltum Punjabianum - Shilajit

Shilajit is a blackish-brown exudation of a herbo-mineral drug obtained as a mineral resin composed of humus and organics compressed by various layers of rock mixed with metabolites of microorganisms during the gradual decay of certain plants.[7] Shilajit compounds of biological importance include dibenzo-alpha pyrones, triterpenes, phospholipids, and low molecular weight phenolic acids, humic acids, and fulvic acids. [8]Most of the natural herbs will be of either plant or animal origin but some obtained from mineral sources like Shilajit can be of pharmaceutical importance. Fulvic acid which comprises almost 60-80% of Shilajit is rapidly absorbed from the intestine because of its lower molecular weight and is proven to exhibit antioxidant properties all of these are attributable to the anti-inflammatory action during IBD. [9] Neelima S, Naresh Babu T, Pradeep Kumar M tested the effect of Shilajit on experimental models of IBD in rats and demonstrated that Shilajit has preventive properties against enterocolitis brought on by indomethacin, and its effects may be comparable to those of sulfasalazine. Shilajit's antioxidant capabilities, which function as a ROS scavenger and an inhibitor of lipid peroxidation, are thought to be responsible for its beneficial effects.[10]

Glycyrrhiza glabra – Liquorice

One of the herbal remedies that is used extensively in several parts of the world is Liquorice extract, which is made from the dried roots of *Glycyrrhiza glabra.* Licorice roots and rhizomes have been used clinically for ages in traditional medicine due to their anti-inflammatory, antitumor, antiviral and antimicrobial activities. [11-14] According to modern studies, the most important bioactive compounds in licorice are glycyrrhizin and glycyrrhetinic acid. M A Takhshid , Davood Mehrabani , Jafar Ai, M Zarepoor demonstrated the healing effect of Liquorice extract in acetic-acid induced ulcerative colitis in rat models which was confirmed by histological investigation. Colitis histological and macroscopic scores were dramatically improved by licorice extract. It was clear from the macroscopic and histological data that the healing effect of licorice extract was dose dependent.[15]Additionally, chemicals produced from licorice may raise the concentration of prostaglandins in the digestive tract, which stimulates the release of mucus from the stomach and may have therapeutic effects.[16]

Alangium Salviifolium – Alangi

Alangium Salviifolium is a deciduous tree belonging to the family of Alangiaceae whose various parts have been scientifically determined to determine the presence of phytochemical constituents.[17]Different active compounds were isolated from different portions of the plant which showed diverse pharmacological activity. 1-Methyl-1H-pyrimidine-2,4-dione and 3-O-beta-D-glucopyranosyl-(24 beta)-ethylcholesta-5,22,25-triene, isolated from flowers, possessed antimicrobial activity. Non alkaloid components like betulinic acid, betulin aldehyde, betulin and lupeol, 3-desoxy betulonic acid (III) and hydroxy lactone A of betulinic acid & β-sitosterol were isolated from seed kernels of the plant.[18] Salviifosides like Salviifosides A,B and C were reported to exhibit anti-inflammatory activities.[19] Alangium salviifolium leaves have been said to have the ability to heal wounds. A variety of animal models, including incision, excision, and dead space (granulation) wound models, were utilized to examine the ability of an ethanolic extract to treat wounds.[20] Zahan R, Nahar L , Nesa ML also studied the anti-inflammatory activities of the flower extract of *Alangium Salviifolium* in carrageenan and formalin induced paw edema in mice. They significantly reduced the paw edema caused by carrageenan and formalin (by almost 50%). The inhibitions resembled those brought on by oral indomethacin. [21] With a view to treating the aforementioned traits, *Alangium Salviifolium* extract was chosen because studies have

revealed that it has potent therapeutic wound healing and anti-inflammatory properties.

MATERIALS AND METHODS

The macromolecules used in this study were interleukin-1-beta converting enzyme (Caspase-1), beta-2 adrenergic receptor (ADRB2), cyclooxygenase-2 (COX-2) and tumor necrosis factor-alpha (TNF-α) with PDB ID: 1ICE, 3NYA, 5W58 and 2AZ5 respectively. The ligands used were downloaded from PubChem Database. Molecular docking was done using Autodock vina. The results of docking are then analyzed using Autodock Tools suits software, and the interaction is visualized using Biovia Discovery Studio Visualizer. The selected phytoconstituents are subsequently evaluated for ADMET properties using the PKCSM web server.

Molecular Docking

Ligand preparation

LigPrep was used to produce a single, low energy, 2D structure with proper chiralties with input structure processed successfully.

Protein preparation

An immediate structure file from PDB is not suitable for molecular modeling calculations. A typical PDB structure file consists of heavy atoms which includes a co-crystallized ligand, water molecules, metal ions and cofactors. Some structures are multimeric which can be reduced into a single unit. Whereby due to limited restrictions of X-ray experiments it becomes difficult to distinguish between NH and O, and the positions of this groups has to be verified. The PDB structure may have missing information based on its connectivity, which has to be assigned along with bond order and formal charges. This was rectified using Protein preparation Wizard.[22]

Receptor grid generation

Receptor Grid Generation requires a "prepared" structure: all atom structure with appropriate bond orders and formal charges. AutoDock looks for favorable interactions between one or more ligand molecules and a receptor molecule, mostly protein. The shape and the properties of the receptor are depicted on a grid by various sets of fields which provide accurate scoring of the ligand poses.[23] The choice in each tab of Receptor Grid Generationpanel allows receptor structure by excluding its co-crystallized ligand which may present to determine the position and size of the active site in the receptor grids and set up the AUTODOCK constraints. A grid area is mostly generated around the binding site of the receptor.[24]

Ligand Docking

Carried out by using AUTODOCK where it looks for the favorable interactions between one or more ligand molecules and a receptor molecule (protein). Each ligand acts as a single molecule while the receptor can bind with more than one molecule, i.e., a protein and a cofactor. AUTODOCK runs in rigid and flexible docking modes, which then automatically generates conformations for each input ligand.[25] The combination of position and orientation of a ligand to the receptor along with its conformation in flexible docking.[26] The ligand poses to AUTODOCK generates pass through a series of hierarchical filters and evaluates the ligand's interaction with the receptor. The filters test the spatial fit of the ligand to the active site and examine the complementary ligand-receptor interactions using a grid-based method pattern after the empirical ChemScore functions.[27] The ligand pose which passes initial screens enters the final stage of the algorithm involving evaluation and minimization of grid approximation to OPLS-AA non bonded ligand receptor interaction energy. Final scoring is carried out by energy minimized poses.

Docking Procedure

Withaferin A, Fulvic acid, Glycyrrhizin and Salviifoside B, the active principles of Ashwagandha, Shilajit, Liquorice and Alangi respectively were downloaded from the PubChem database. Docking studies of selected compounds were performed using interleukin-1-beta converting enzyme (Caspase-1), beta-2 adrenergic receptor (ADRB2), cyclooxygenase-2 (COX-2) and tumor necrosis factor-alpha (TNF-α) obtained from RCSB Protein Data Bank.

Experiments were performed using AUTODOCK software. The ligand structures in SDF formats were optimized and converted to PDB formats. Energy minimization, the addition of charges (to correct ionization), and polar hydrogens were combined to create the ligands. By assigning bond angles, bond orders, and topology, the structure was optimized. The proteins were prepared by removing ligands, irrelevant ions, and heteroatoms (water molecules). The PDB files for the protein and ligand were then uploaded to Pyrex software. E-negative values were used to represent the docking results. Higher negative e-values represent higher ligand-protein binding affinities, which correspond to higher phytochemical efficacy.[28]

In silico ADMET prediction

ADMET profiles were analyzed using the pkcsm web tool. For this analysis, the Simplified Molecular Input Line Entry System (SMILES) formats of all the ligands were obtained from the PubChem database. Lipinski's rule of 5 was applied towards the drug-likeness of all the ligands, to check if all the properties fall within the accepted range. All of the ligands' Simplified Molecular Input Line Entry System (SMILES) formats were obtained for this analysis from the PubChem database. To determine whether each ligand's drug-likeness falls within the acceptable range, Lipinski's rule of five was applied. Lipophilicity levels were analyzed based upon the atom-based logarithm of the partition coefficient (ALogP). The blood-brain barrier (BBB) was examined in relation to drug distribution. Drug metabolism was estimated based upon the Cytochrome P450 (CYP) models (CYP1A2, CYP2C19, CYP2C9, CYP2D6,

and CYP3A4) for substrate or inhibition. In addition to these, drug toxicity was examined, with a focus on hepatotoxicity, AMES toxicity, and inhibition of the human ether-a-go-go-related gene (hERG). In order to identify a potential drug candidate, all relevant ADMET parameters of the active ingredients in all herbs were thoroughly estimated and checked for compliance with their standard ranges.[29]

RESULTS AND DISCUSSION

Molecular Docking Analysis

The drug-receptor complexes were studied by targeting the interleukin-1-beta converting enzyme (Caspase-1), beta-2 adrenergic receptor (ADRB2), cyclooxygenase-2 (COX-2) and tumor necrosis factor-alpha (TNF-α) by AUTODOCKVina and the docking results are shown in Table 1.

The compounds demonstrated varying levels of binding affinities for the protein targets, some giving docking scores higher than those of the standard ligands. Liquorice showed the highest binding affinity for caspase-1, ADRB 2, COX-2 and TNF-α. and the molecular interactions of the amino acid residues of caspase-1 with liquorice is shown in Figure 1. The molecular interactions of the amino acid residues of ADRB-2 with liquorice is shown in Figure 2. The molecular interactions of the amino acid residues of COX-2 with liquorice is shown in Figure 3. The molecular interactions of the amino acid residues of TNF-α with liquorice is shown in Figure 4.

Table 1: Binding affinities of the compounds for the protein targets

Compounds	PubChem ID	ΔG energy (Kcal/mol) Caspase-1	ΔG energy (Kcal/mol) ADRB2	ΔG energy (Kcal/mol) COX-2	ΔG energy (Kcal/mol) TNF-α
Withaferin A	265237	-8	-9.1	-9.1	-7.1
Fulvic acid	5359407	-8.3	-8	-7.7	-6
Glycyrrhizin	3495	-9.5	-9.9	-9.3	-8.3
Salviifoside B	45271726	-7.6	-9.5	-7.3	-6.8
Standard Mesalamine	4075	-6.1	-6.2	-6.4	-5.1

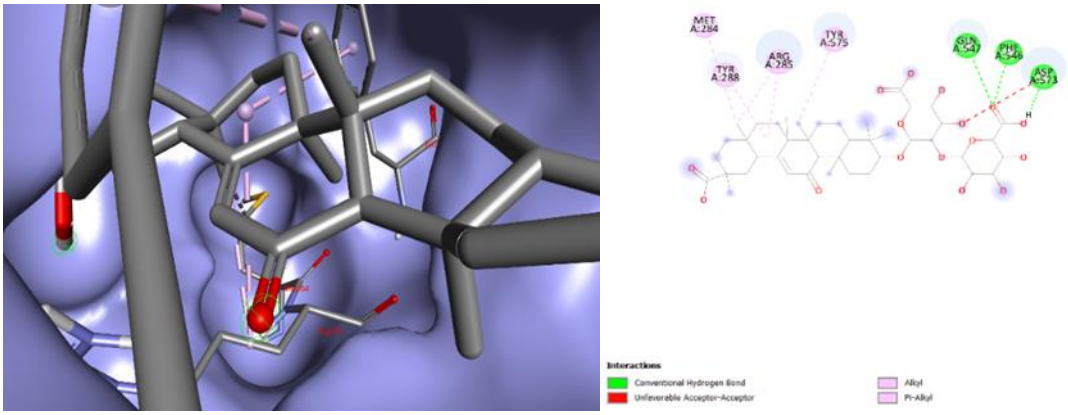

Figure 1: 3D (left) and 2D (right) views of the molecular interactions of amino-acid residues of caspase-1 with Liquorice

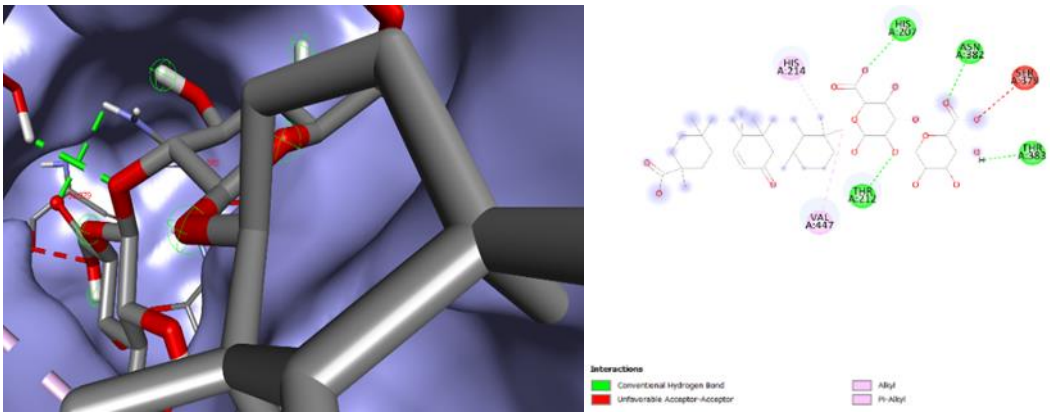

Figure 2: 3D (left) and 2D (right) views of the molecular interactions of amino-acid residues of beta-2 adrenergic receptor (ADRB2) with Liquorice

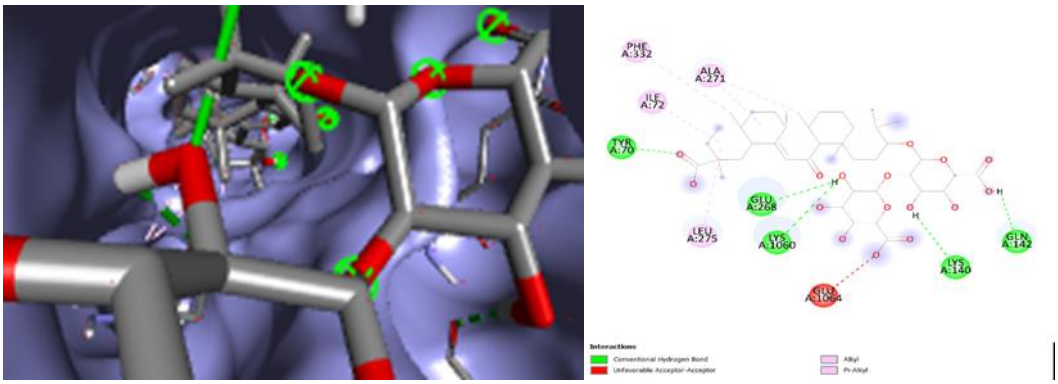

Figure 3: 3D (left) and 2D (right) views of the molecular interactions of amino-acid residues of Cyclooxygenase 2 (COX-2) with Liquorice

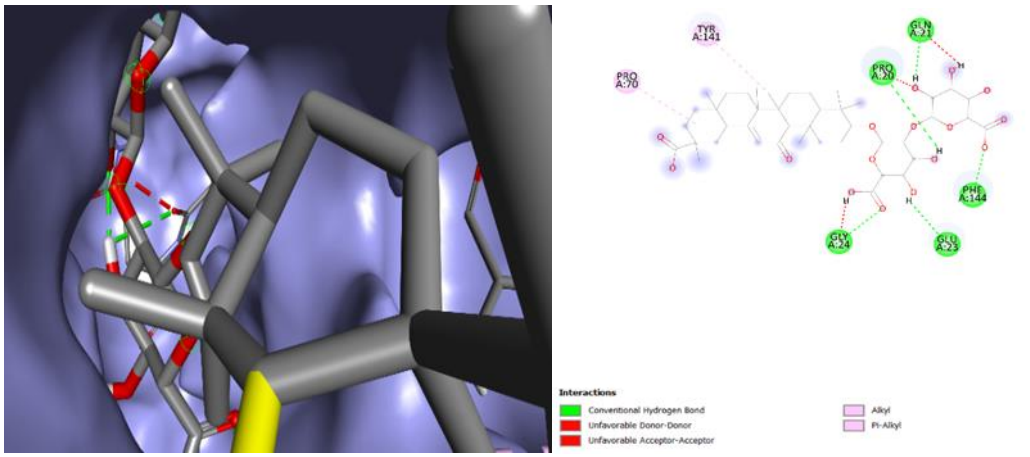

Figure 4: 3D (left) and 2D (right) views of the molecular interactions of amino-acid residues of TNF-α with Liquorice

ADMET prediction

ADMET profile of Ashwagandha:

Through the SwissADME analysis it was observed that Ashwagandha follows Lipinski's rule of five towards drug-likeness with molecular weight 470.6 (less than 500 g/mol) with two H-bond donor (not more than 5), six H-bond acceptor (not more than 10), AlogP value of 3.24 (not more than 5), 3 rotatable bonds (not more than 10), Topological Polar Surface Area (TPSA) of 96.36 Å^2 (< 140 Å^2), and molar refractivity of 127.49 (40–130). The low logP value of Ashwagandha indicated good absorption and permeation with higher hydrophilicity. CYP2D6 and CYP3A4 are two main Cytochrome P450 enzymes that play significant roles during drug metabolism in the liver. The analysis identified Ashwagandha as a CYP2D6 substrate/non-inhibitor and CYP3A4 non-substrate/non-inhibitor, indicating that the drug may be metabolized in the liver. Bioavailability score for Ashwagandha was observed as 0.55, which implied that it had 55% probability of rat bioavailability (higher than 10%).

ADMET profile of shilajith:

Through the SwissADME analysis it was observed that Shilajit follows Lipinski's rule of five towards drug-likeness with molecular weight 308.24 (less than 500 g/mol) with four H-bond donor (not more than 5), eight H-bond acceptor (not more than 10), AlogP value of 1.39 (not more than 5), 1 rotatable bond (not more than 10), Topological Polar Surface Area (TPSA) of 137.43 Å^2 (< 140 Å^2), and molar refractivity of 73.21 (40–130). It showed good Gastrointestinal absorption . CYP2D6 and CYP3A4 are two main Cytochrome P450 enzymes that play significant roles during drug metabolism in the liver. The analysis identified Shilajit as a CYP2D6 substrate/non-inhibitor and CYP3A4 non-substrate/non-inhibitor, indicating that the drug may be metabolized in the liver. Bioavailability score for Shilajit was observed as 0.56, which implied that it had 56% probability of rat bioavailability (higher than 10%).

ADMET profile of Liquorice:

Through the SwissADME analysis it was observed that Liquorice violates Lipinski's rule of five towards drug-likeness with molecular weight 822.93 (less than 500 g/mol) with eight H-bond donor (not more than 5), 16 H-bond acceptor (not more than 10), AlogP value of 1.89 (not more than 5), 7 rotatable bonds (not more than 10), Topological Polar Surface Area (TPSA) of 267.04 Å^2 (< 140 Å^2), and molar refractivity of 202.84 (40–130).CYP2D6 and CYP3A4 are two main Cytochrome P450 enzymes that play significant roles during drug metabolism in the liver. The analysis identified Liquorice as a CYP2D6 substrate/non-inhibitor and CYP3A4 non-substrate/non-inhibitor, indicating that the drug may

be metabolized in the liver. Bioavailability score for Liquorice was observed as 0.11, which implied that it had 11% probability of rat bioavailability (higher than 10%). Though some of the parameters were violated, liquorice showed very good binding affinity as exhibited by the docking scores and hence considered.

ADMET profile of Alangi:

Through the SwissADME analysis it was observed that Shilajit follows Lipinski's rule of five towards drug-likeness with molecular weight 122.12 (less than 500 g/mol) with one H-bond donor (not more than 5), two H-bond acceptor (not more than 10), AlogP value of 0.7 (not more than 5), 1 rotatable bond (not more than 10), Topological Polar Surface Area (TPSA) of 55.98 $Å^2$ (< 140 $Å^2$), and molar refractivity of 32.33 (40–130). It showed good Gastrointestinal absorption. The low logP value of Ashwagandha indicated good absorption and permeation with higher hydrophilicity. CYP2D6 and CYP3A4 are two main Cytochrome P450 enzymes that play significant roles during drug metabolism in the liver. The analysis identified Alangi as a CYP2D6 substrate/non-inhibitor and CYP3A4 non-substrate/non-inhibitor, indicating that the drug may be metabolized in the liver. . Bioavailability score for Alangi was observed as 0.55, which implied that it had 55% probability of rat bioavailability (higher than 10%).

CONCLUSIONS

Different levels of binding affinities were shown by the compounds for the protein targets, with some giving docking scores that were higher than those of the standard ligands. Proinflammatory pathways' essential proteins may interact molecularly to modify their activities and subsequently control inflammatory responses. The enzyme caspase-1, which converts pro-interleukin (IL)-1 to its active form (IL-1), is in charge of this process. IL-1 is a crucial pro-inflammatory cytokine that is released in response to tissue damage, illnesses, or infections and frequently has negative effects. IL-1 activity can be reduced by inhibiting caspase-1, which is a promising therapeutic measure to prevent unfavorable inflammatory reactions. A G protein-coupled receptor called ADRB2 modulates inflammatory and immune responses in both directions. Adrenergic receptor activation may have pro- or anti-inflammatory (regulatory) effects, depending on a number of variables. Therefore, molecular interaction with this receptor may control its immunomodulatory activity, which in turn may control inflammatory responses, which is crucial for the treatment of IBD. Depending on a number of factors, the activation of adrenergic receptors may have pro- or anti-inflammatory (regulatory) effects. Thus, molecular interaction with this receptor may regulate its immunomodulatory activity, which may regulate inflammatory responses, which is important for the treatment of IBD. Therefore, the ability of these ancient herbs' compounds to interact with and possibly control the actions of these protein targets could be related to their capacity to reduce inflammation.

All the selected ancient herbs exhibited various binding affinities for the anti-inflammatory targets' caspase-1, ADRB2, COX-2 and TNF-α – amongst which Liquorice showed the maximum binding affinity, however further research in this area is required by *invivo* studies to demonstrate its effects on IBD. Further moving a step forward any herbal formulation which could be made with these herbs like rectal gel or rectal suppository and testing its efficacy by *invivo* studies could possibly validate the anti-inflammatory, antioxidant and wound healing actions of these herbs which make up the main requisite for treatment of IBD. Because these herbs are from natural sources, it is generally perceived as harmless, although there are only a few studies on safety. A further study with more focus on toxicology is therefore suggested.

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