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IN SILICO INSIGHTS INTO THE EFFICACY OF DARJEELING HIMALAYA'S TRADITIONAL FERMENTED BEVERAGES TO COMBAT VARIOUS HIGH-ALTITUDE SICKNESSES

– Research paper –

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Abstract: This *in silico* study has been designed to validate ethnomedicinal properties of some Himalayan fermented ethnic beverages against varied high-altitude sicknesses. Traditional beverages like *tongba*, *chhyang*, *nigar* and finger-millet *raksi* (supplemented with ethnomedicines like *khokim* and *chimphing*) were considered in this study which were sampled from villages situated in Singalila Ridge of the Himalayas. Seven major metabolites from these beverages such as ethyl alpha-D-glucopyranoside; meranzin; auraptene; cis-vaccenic acid; cyclo(pro-gly); cyclo(leu-pro); and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one were investigated *in silico* to provide insights to their efficiency in alleviating major altitude illnesses (pulmonary edema and hypoxia); high-altitude respiratory infections; pain or inflammation; cardiovascular problems; gastrointestinal diseases and disorders; and high-altitude neurological diseases and disorders. Computational tools such as Molinspiration Chemoinformatics, SwissADME, Molsoft program and vNN-ADMET were employed to evaluate physicochemical properties, pharmacokinetics and ADMET parameters. Binding affinities were computed using Auto Dock Tools. *In silico* pharmacokinetics and ADMET revealed bioactivity potentials of selected metabolites which are associated with medicinal values to treat high-altitude sickness. A total of 175 combinations of receptor-ligand docking interactions were considered out of which 141 combinations exhibited a binding energy score of ≤ -5 and 29 combinations scored ≤ -7. Outcome of this computational research affirmed the effectiveness of high-altitude's traditional drinks in healing of high-altitudinal stresses.

Keywords: Ethnic beverages, molecular docking, ADMET, high-altitude sickness

INTRODUCTION

High-altitude sickness (HAS) is a group of diseases and disorders which include major altitudinal illnesses like acute mountain sickness (AMS), peripheral edema (swelling of organs like hands, feet, and face), high-altitude cerebral edema (HACE), high-altitude pulmonary edema (HAPE) and hypoxia; infections commonly encountered at high altitude like gastrointestinal infections (*Escherichia coli*, *Salmonella*, *Shigella* etc.), respiratory infections (pneumonia, influenza, tuberculosis etc.), urological and gynaecological infections etc; inflammation or feeling of pain in the body muscle and joints; cardiovascular diseases including hypertension, high pulse rate, heart attacks etc.; gastrointestinal or digestive problems; neurological diseases and disorders including loss of appetite, sleep problems, headache, loss of coordination, cyanosis, psychiatric problems like confusion and irrational behaviour, tightness in the chest, weakness, vertigo and hallucinations etc. (Ericsson et al., 2001; Chen et al., 2018a; Majumder et al., 2021). Medical scientists from United states (Hackett et al., 1976; Johnson et al., 1984) and Switzerland (Ferrazzini et al., 1987), pioneered the discovery of medicines (acetazolamide and dexamethasone) to treat acute mountain sickness successfully. Although, till date the pathogenesis of

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this disease is poorly understood thus drug development process has been overlooked as well. Kapoor et al. (2004) reported use of some drugs for the treatment of a few categories of HAS, mainly acute mountain sickness (AMS) and high-altitude pulmonary edema. To the best of our knowledge, natural products were rarely used for the docking of proteins cumulatively associated to HAS (Liang et al., 2018; Yeerjiang et al., 2023) and with traditional foods or beverages, this study is one of a kind.

Several ethnic foods and beverages and ethnomedicines enriched with natural products found in the Himalayas are useful to inhabitants and tourists that help to endure the altitudinal stresses, recover from various high-altitude sicknesses and get acclimatized with the environmental temperature that remains frigid throughout the year (Majumder et al., 2021). Traditional knowledge of ethnic communities living at Singalila includes various plant based ethnomedicines, ethnic foods and fermented beverages like *tongba*, *chhyang nigar*, *raksi* etc. (Majumder et al., 2024). Moreover, Majumder et al. (2021) described traditional drink *raksi* as "an element of ethnopharmacology of high altitude". Earlier, people from ethnic communities living in Singalila Ridge of the Himalayas and others who healed themselves from altitude illnesses by means of traditional medicines acclaimed therapeutic properties of these traditional drinks (Majumder et al., 2021; Majumder et al., 2023). Previously, our research group analysed volatile profiles of *raksi* and *tongba* and discovered their medicinal properties that highlighted Himalayan ethnic beverages as preventatives of altitude sicknesses (Majumder et al., 2021; Majumder et al., 2022).

In this research, computational biochemistry protocols were applied to predict binding affinity of abundant metabolites detected in high-altitude's ethnic beverages towards proteins targeting six distinct categories of HAS. Prior to docking, pharmacokinetics of the molecules were evaluated by physicochemical properties, druglikeness scores and ADMET parameters. Molecular docking studies are a significant strategy within the realm of virtual screening techniques, extensively utilized to uncover and understand ligand-protein interactions. The aim of this *in silico* study was to evaluate signature volatile constituents of selected beverages as candidates to treat HAS.

MATERIALS AND METHODS

Selection of metabolites of ethnic beverages

Traditional beverages such as *tongba* (*TNB*), *chhyang* (*CHN*) and *nigar* (*NGR*) and *kodo ko raksi* or finger-millet *raksi* supplemented with *khokim* (*KKR*) and *chimphing* (*CKR*) collected from different villages of Singalila Ridge (Indo-Nepal high-altitude region) were previously analyzed within the context of biochemical analysis and metabolite profiling by GC-MS analysis (Majumder et al., 2021; Majumder et al., 2022; Majumder et al., 2024). In this follow-up *in silico* research work, seven different metabolites (major compounds with abundant peak area) of five different traditional beverages were considered. Moreover, these metabolites were from various biosynthesis pathways such as, most abundant glycoside compound from *TNB*- ethyl alpha-D-glucopyranoside or **α-EG** (53.95% in *TNB*; 0.83% in *CHN*; 3.18% in *NGR*); two coumarin derivatives from *KKR* such as meranzin or **MRN** (7.26%) and auraptene or **ARN** (14.44%); one long-chain hydrocarbon/ fatty acid metabolite from glycerol rich *CHN* i.e., cis-vaccenic acid or **CVA** (10.04%); two amino acid derivatives such as, hexahydropyrrolo[1,2-a]pyrazine-1,4-dione or cyclo(pro-gly) or **CPG** (26.47% in *CKR*) and hexahydro-3-(2-methylpropyl)-pyrrolo[1,2-a]pyrazine-1,4-dione or cyclo(leu-pro) or **CLP** (16.96% in *TNB*; 0.16% in *CHN*); and one fermented sugar catabolite as *NGR's* major compound- 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one or **DDMP** (19.4%) (Majumder et al., 2024).

Computation of molecular properties and pharmacokinetics

Evaluation of physicochemical properties and druglikeness

Molinspiration Chemoinformatics (http://www.molinspiration.com/) was used to compute the molecular attributes and druglikeness features of selected biomolecules (signature metabolites of ethnic beverages as discussed below) through parameters like partition coefficient or miLogP; topological polar surface area or TPSA; number of atoms; molecular weight; number of hydrogen bond acceptor, donor and rotatable bonds (nHBA nHBD and nRotB); molecular volume; bioactivity scores being G-protein coupled receptor or GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease and enzyme inhibitor (Lipinski, 2004). The selected compounds were subjected to further *in silico* pharmacokinetic evaluation employing two other

free web tools i.e., SwissADME (http://www.swissadme.ch/index.php#) and Molsoft program (http://www.molsoft.com/), desired to provide GI absorption, BBB score, log *Kp* (skin permeation), bioavailability score and drug likeness model score. Drug score values indicate overall potential of a compound to be a drug candidate.

Assessment of ADMET properties

The ADMET (absorption, digestion, metabolism, excretion and toxicity) features of targeted molecules corresponds to biological efficiency and metabolic output (Ghosh et al., 2022). Therefore, evaluation of ADMET properties would interpret a clear idea of the predicted side effects in addition to knowing the accompanying effects on health. ADMET analysis has been done using software vNN-ADMET (Variable Nearest Neighbour ADMET) (https://vnnadmet.bhsai.org/) in reference to the protocol of Schyman et al. (2017) which predicts the range of liver toxicity, metabolic function, membrane transport features and minimum recommended therapeutic dose of a molecules.

Retrieval of proteins and ligands

Receptors or proteins were chosen by considering diseases and disorders encountered in high-altitude places to screen altitude sicknesses for *in silico* analysis. Proteins were retrieved from Protein data bank (https://www.rcsb.org/). Selected receptors and their respective proteins (PDB id) for each disease are listed below (Table 1). To do smooth interaction during *in silico* study, water molecules were erased from protein structures and followed by addition of polar hydrogen and Kollman charges in AutoDock tools for proper optimization. The 3D structures of the signature metabolites from selected ethnic beverages were extracted from NCBI PubChem (https://pubchem.ncbi.nlm.nih.gov/).

Table 1. List of proteins and corresponding PDB Ids chosen to target various high-altitude sicknesses (HAS)

Names of targeted diseases	**Name of protein/receptor**	**PDB Id**
Major altitude illnesses (pulmonary edema and hypoxia)	Beta-2 adrenergic receptor	3KJ6
	Rhodopsin-like GPCR	2Z73
	Hypoxia-inducible factor 1-alpha	4H6J
		4ZPR
	Hypoxia-inducible factor 2-alpha	4ZPK
High-altitude respiratory infections (Tuberculosis and pneumonia)	*Mycobacterium tuberculosis* enoyl-ACP reductase	1BVR
		5OIF
	Streptococcus pneumoniae IgA1 protease	6XJA
		6XJB
Pain or inflammation in the body muscle and joints	Human interleukin-6	1ALU
	COX-2	3LN1
		5IKV
		4COX
	COX-1	6Y3C
		3N8Y
Cardiovascular problems (hypertension related diseases)	Human Angiotensin Converting Enzyme	1O8A
	Thermolysin	5DPF
Gastrointestinal diseases and disorders (Gastroesophageal reflux disease, heartburn, gastric inflammation, gastritis and gastric cancer)	Endothelial selectin	1ESL
		1G1T
	Human histamine H1	3RZE
Neurological diseases and disorders	Acetylcholinesterase (AChE)	2GYU
	Butyrylcholinesterase (BchE)	4TPK
	Adenosine A2A	3PWH
		5NM2
	Parkin E3 ligase	4I1H

Docking analysis

Molecular docking is a current approach which is implied for the identification of different protein-ligand bonds to determine the amino acids involved in the biological activity. Once the ligands and proteins were ready, molecular docking analysis was done using Auto Dock Tools (AutoDock4) and Vina (AutoDock Vina) (Trott and Olson, 2010). The three-dimensional grid was made instructed using the AUTOGRID algorithm that determines the binding energy of a ligand-receptor bond (Morris et al., 1998). The default grid size was x = 40, y = 40 and z = 40 and the distance between the grid points was considered as 0.375 Å (Morris et al., 1998). The results of docking by Auto Dock tools and Vina were visualized with PyMOL molecular visualization tool. The hydrogen bond interactions in a compound-protein binding were analysed using LigPlot^{+} software (Laskowski and Swindells, 2011).

RESULTS AND DISCUSSION

Molecular properties and pharmacokinetics of selected metabolites

Physicochemical properties and druglikeness

The results of *in silico* physicochemical and pharmacokinetic characterization of selected compounds have been provided in Table 2. These various features were evaluated according to Lipinski's rule of five which visualizes the druglikeness traits of molecules prospective to prevent HAS. The Lipinski's rule of five (LogP ≤ 5, molecular weight ≤ 500, nHBA ≤ 10 and nHBD ≤ 5) supports those potent compounds anticipated for being effective as drug molecules. If a molecule violates more than one of these parameters that may be considered as less likely as drug.

Table 2. Physicochemical properties, pharmacokinetic and druglikeness scores of selected ethnic beverage metabolites [Ethyl alpha-D-glucopyranoside (α-EG); meranzin (MRN); auraptene (ARN); cis-vaccenic acid (CVA); cyclo(pro-gly) (CPG); cyclo(leu-pro) (CLP); and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)]

Evaluating parameters	α-EG	MRN	ARN	CVA	CPG	CLP	DDMP
miLogP	-1.65	2.8	5.57	7.58	-0.98	0.66	-0.46
MW(g/mol)	208.21	260.29	298.38	282.47	154.17	210.28	144.13
nHBA	6	4	3	2	4	4	4
nHBD	4	0	0	1	1	1	2
TPSA (Å^2)	99.38	51.97	39.45	37.3	49.41	49.41	66.76
n-Atoms	14	19	22	20	11	15	10
nRotB	3	3	6	15	0	2	0
Volume (g/mol)	186.14	235.53	292.49	318.84	138.14	204.92	123.4
pK$_a$ of most Basic/Acidic group	<0./13.01	<0./21.66	<0./24.17	<0./4.26	-5.64/14.17	-4.68/13.46	<0./8.59
Number of stereo centers	5	1	0	0	1	2	1
Violation of Lipinski's rule of 5	0	0	1	1	0	0	0
GPCR ligand	-0.25	-0.38	-0.3	0.17	-0.32	0.22	-1.59
Ion channel modulator	0.1	-0.67	-0.35	0.07	-0.24	0.2	-0.96
Kinase inhibitor	-0.54	-0.7	-0.56	-0.22	-1.23	-0.56	-2.25
Nuclear receptor ligand	-0.78	0.29	0.18	0.23	-1.26	-0.3	-1.6
Protease inhibitor	-0.4	-0.36	-0.46	0.07	0.05	0.67	-1.53
Enzyme inhibitor	0.38	0.29	0.17	0.27	-0.34	0.08	-0.65
GI absorption	High	High	High	High	Low	High	High
BBB score	2.1	4.53	4.89	4.4	3.16	3.7	2.24
log Kp (skin permeation) (cm/s)	-9.19	-6.15	-4.50	-2.60	-7.67	-6.79	-7.44
Bioavailability Score (L:T ratio)	0.55	0.55	0.55	0.85	0.55	0.55	0.85
Drug-likeness model score	0.01	-0.63	-0.3	-0.3	-0.75	-0.15	-0.96

Here, physicochemical and further ADMET properties revealed that compounds abided by Lipinski's criteria, with only one violation (miLogP ≤ 5 for **ARN** and **CVA)** which indicates that selected molecules would not have oral bioavailability problems. The biophysical scores of the query molecules are as follows: partition coefficient (miLogP) value ranged between -1.65 (**α-EG**) to 7.58 (**CVA**); molecular weight (MW) lied between 154.17 (**CPG**) to 298.38 g/mol (**ARN**); topological polar surface area (TPSA) value ranged between 37.3 $Å^2$ (**CVA**) to 99.38 $Å^2$ (**α-EG**); highest numerical count of HBA (6) and HBD (4) was found in **α-EG** without violating Lipinski's rule of five (Table 2). TPSA is a frequently used metric of medicinal chemistry for the optimization of a drug's ability to permeate cells. Molecules with TPSA score greater than 140 tend to be poor at permeating cell membranes (Pajouhesh and Lenz, 2005). The nRotB is a crucial factor that helps in determination of conformational flexibility in a drug compound that affects the binding mechanism of ligand molecules to respective receptor proteins. In order to qualify the oral bioavailability standard, the count of rotatable bonds in a drug target should be ≤10 (Priya et al., 2015). Here, all query molecules except **CVA** (nRotB = 15) matched the criterion.

The pharmacological activity explains the beneficial effects of molecules in living beings. Molecules pretend to bind with biological target proteins such as enzymes, ion channels, and receptors to exhibit bioactivity. The bioactivity scores computed for the molecules of ethnic beverages revealed abilities as GPCR ligand, nuclear receptor ligand, ion channel modulator, kinase inhibitor, protease inhibitor, and enzyme activity inhibitor. All the parameters, calculated with the help of online software Molinspiration (www.molinspiration.com) predicted varying biological activity for the compounds. The bioactivity score is given in Table 2. Molecule showing bioactivity scores more than 0.00 is most likely to show significant biological activities, while molecule scoring between -0.50 and 0.00 are expected to be moderately active and values less than -0.50 is corresponding to an inactive state (Paramashivam et al., 2015). As per the bioactivity scores, the best compound was identified to be **CVA** with five high bioactivity scores and one moderate activity (Table 2). **DDMP** was found to be inactive scoring less than -0.50 in all. All compounds except **DDMP** were active as GPCR ligand and protease inhibitor while all compounds except **CVA** were inactive as kinase inhibitor. Moreover, metabolites resulted moderate (**ARN** and **CPG**) to high bioactivity (**α-EG**, **CLP** and **CVA**) scores as ion channel modulator except **MRN** and **DDMP**. Beside **DDMP**, bioactivity scores for nuclear receptor ligand by **α-EG** and **CPG** were also less than -0.50. Overall, GPCR ligand, protease inhibitor and ion channel modulator were found as moderate to high scoring among all pharmacokinetic parameters and their direct or indirect association with HAS could be a matter of concern in this study. Trzaskowski et al. (2012) stated that GPCRs, being involved in signalling pathways related to many diseases i.e., mental, metabolic including endocrinological disorders, immunological including viral infections, cardiovascular, inflammatory, sensory disorders, and cancer, are considered as targets for about most of the drugs available in the market. In this research we also targeted GPCRs (beta-2 adrenergic receptor and rhodopsin-like GPCR) specifically to target major altitude illness- high-altitude pulmonary and cardiovascular diseases (Table 1). Protease inhibitory property of query compounds may also help to prevent high altitude respiratory infection by targeting *Streptococcus pneumoniae* IgA1 protein as selected here (Table 1). Ion channels are also essential for all cardiac functions, including rhythmicity and contractility. Consequently, it is used for therapeutics aimed at cardiac pathophysiologies such as atrial fibrillation or angina (Priest and McDermott 2015). Many of the popular drugs in market exert their effect by modulation of ion channels which are also used for the treatment of diverse diseases. A significant fraction of ion channel programs targets on excitatory cells, such as muscle and nerve cells. In this regard, most studied diseases are stroke, schizophrenia, depression, memory dysfunction, anxiety, cardiac arrhythmia, and arterial hypertension (Willumsen et al., 2003) which can also be categorised as high-altitude illnesses.

The blood-brain barrier (BBB) score denotes the likelihood of the drug molecule of crossing the blood-brain barrier border and bringing about therapeutic modifications. The BBB protects human brain from any toxic side effects of drugs and exogenous molecules. However, medications particularly developed for neurological disorders should cross the brain barrier in therapeutic concentrations. Therefore, understanding the BBB can guide effective and efficient drug design (Gupta et al., 2019). Previously, research revealed that (tested with 270 CNS and 720 non-CNS drugs) the BBB Score is a typical predictor of CNS drug-likeness. Molecules with BBB scores between 0 to <4 can be classified as non-CNS drugs, while molecules with BBB scores between 4 to 6 can be classified as CNS drugs (Ghose et al., 2012; Gupta

et al., 2019). Compound such as **MRN** (4.53), **ARN** (4.89) and **CVA** (4.4) resulted such BBB scores (Table 2) that confirm their likeliness as CNS drugs. **ARN** was already classified as neuroprotective agent (Bibak et al., 2019) for preventing high altitude neurological and psychiatric illnesses like anxiety, depression, dizziness, and Alzheimer's disease (Arabi et al., 2021; Majumder et al., 2021). **MRN** has antidepressant properties too (Liu et al., 2021). CNS depressants are used to treat insomnia, anxiety, depression, panic attacks, and seizures (https://www.cancer.gov/publications/dictionaries/cancer-terms/def/cns-depressant). In molecular docking section, we have chosen receptors of various neurological diseases and disorders to target high-altitude illnesses where it remains to be seen whether the docking scores of these three BBB permeant natural products can affirm their bioactivity like any other CNS drug or support their activity as a CNS drug. Moreover, TPSA values for **MRN**, **ARN** and **CVA** were computed as 51.97 $Å^2$, 39.45 $Å^2$ and 37.3 $Å^2$ respectively (Table 2) which also suggest their capability to penetrate the blood–brain barrier to act on receptors in the central nervous system, as the suggested limit of TPSA value for a standard CNS drug should be less than 90 $Å^2$ (Hitchcock, 2012) corresponding to these three query compounds. Gastrointestinal absorption is a crucial pharmacokinetic behaviour just like brain access (BBB) for the estimation of druglikeness (Daina and Zoete, 2016). The Brain Or IntestinaL EstimateD permeation method (BOILED-Egg) was earlier proposed as an accurate predictive model for computing the lipophilicity and polarity of small molecules (Daina and Zoete, 2016). Bioavailability is multifactorial, but primarily depends on gastrointestinal absorption. The BOILED-Egg assessed by SwissADME predicted high gastrointestinal absorption for signature ethnic beverage metabolites (only except **CPG**) signifying high bioavailability favourable with druglikeness (Figure 1).

log *Kp* value of selected compounds were obtained from SwissADME. The skin permeability (*Kp*) identifies the rate of a chemical penetrating across the stratum corneum. log *Kp* indicates skin absorption which is widely used to predict the transport of molecules in the outermost epidermal layer of skin (Chen et al., 2018b). This prediction of permeability coefficient (*Kp*) for the transport of molecules through mammalian epidermis is based on the linear model proposed by Potts and Guy (1992). The greater the negative value of log Kp, the lower the molecule's permeability through the skin. Here, the highest negative log *Kp* has been found for **α-EG** among the selected ligands. The epidermis is a barrier that protects the body from microbial pathogens, oxidative stresses, and chemical compounds, and provides mechanical resistance to minor injury.

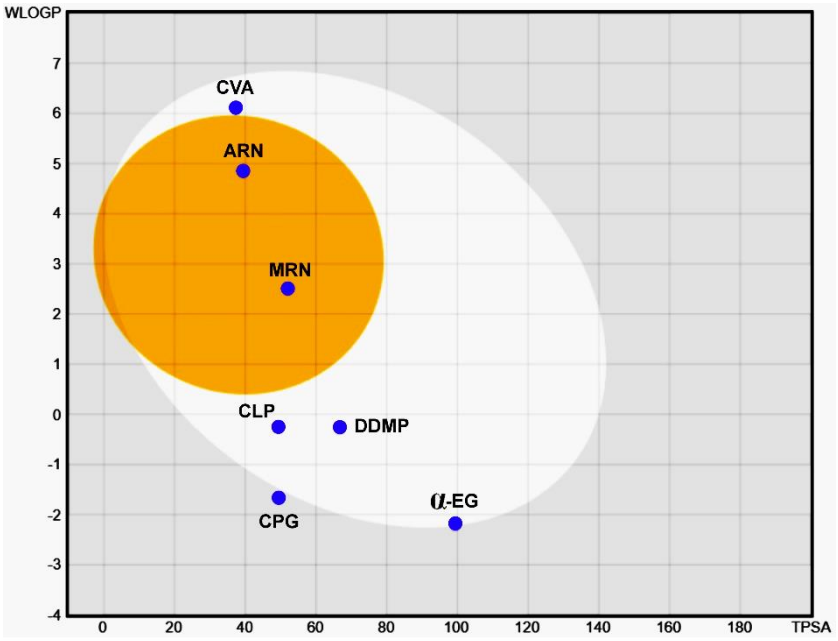


Figure 1. SwissADME generated "The Brain Or IntestinaL EstimateD permeation method" BOILED-Egg assessment. The white zone represents very probable HIA (GI) absorption, whereas the yellow zone shows very likely BBB permeability. The outer grey region is made up of compounds that are expected to have poor absorption but no brain penetration. [Ethyl alpha-D-glucopyranoside (**α-EG**); meranzin (**MRN**); auraptene (**ARN**); cis-vaccenic acid (**CVA**); cyclo(pro-gly) (**CPG**); cyclo(leu-pro) (**CLP**); and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (**DDMP**)]

Stratum corneum plays the major role of barrier. Consumption of glycoside **α-EG** through fermented beverages like Japanese *sake* or *tongba* is involved in collagen production which help in the maintenance and improvement of skin homeostasis and in the prevention of dermal dehydration (a prime high-altitude problem) with moisturizing functions in human body (Bogaki et al., 2017; Majumder et al., 2022). Collagen is the main structural protein in the extracellular matrix and is the primary building block of body's skin, muscles, bones, tendons and ligaments and other connective tissues. Previously, Bogaki et al. (2017) demonstrated role of **α-EG** in the proliferation of normal human dermal fibroblasts (NHDF). Therefore, this *in silico* study suggests the less skin-permeating nature of **α-EG** (log *Kp* = -9.19 cm/s) does not allow it to disperse elsewhere after reaching its site of function i.e., dermal layers which leads to retention of this compound in the skin, muscle, bone, tendon, ligament and other connective tissues. So, this is how *TNB*, by its signature molecule, could alleviate some major dermatoses encountered in high-altitude areas i.e., UV-related skin disorders like tanning, photomelanosis, acute and chronic sunburn, polymorphic light eruption, chronic actinic dermatitis, actinic cheilitis, etc.; cold injuries like frostbite, chilblains, acrocyanosis, erythrocyanosis, etc.; nail issues like koilonychias; airborne contact dermatitis; insect bite reaction; xerosis; and skin carcinomas (Singh, 2017).

The L:T ratio (L: local bioavailability; T: total systemic bioavailability) is considered as the meter to evaluate bioavailability of a targeted drug (Borgström, 1998). An L:T ratio of 0.15 signifies that 85% of the systemically available drug has not exerted any anticipated effects in the targeted organ (stie of bioactivity) before reaching the systemic circulation. This portion should be regarded as a "waste of drug". Likewise, if the L:T ratio is 0.55, that means 45% is wasted. A high L:T ratio defines a good targeting ability of the combination of substance and device or a low contribution from the GI tract. A high L:T ratio also reflects a more beneficial balance between wanted and unwanted effects (Borgström, 1998). With a bioavailability score of 0.85, the parameter of drug-likeness is considered high, but only after it fits the Lipinski, Verber, and Egan rule (Al Azzam et al., 2022). Results show **CVA** and **DDMP** as most bioavailable (with a score of 0.85) compared to other compounds who were moderately bioavailable.

Molecules are considered as drug-like when they show positive values of drug-likeness model score (Ghosh et al., 2022). Only **α-EG** among the query compounds possessed a positive drug-likeness model score of 0.01, which was very low though. However, in this research low or negative drug-likeness model score is not an issue as far as complementary or alternative therapy is concerned. So, biological activities of these ethnic beverages against high-altitude sicknesses can be anticipated as a non-drug treatment.

ADMET properties

ADMET properties always need to be taken into consideration when studying *in silico* bioactivity of a compound whether to be developed as a new drug or not. The pharmacokinetic characteristics and safety profile of selected metabolites through ADMET predictions were estimated employing the variable nearest neighbour (vNN) computational methodology as given in Figure 2. ADMET or 'Absorption, Distribution, Metabolism, Excretion and Toxicity' impacts the quantity of drug requirement and drug exposure kinetics of the apprehensive tissue system. **MRN** and **ARN** of *KKR* and **DDMP** of NGR were computed as responsible for probable drug induced liver injury (DILI). Hepatic-cytotoxicity was associated with *CHN* metabolite **CVA** and *CKR* metabolite **CPG**. However, **CPG**'s low bioavailability, specifically low gastrointestinal absorption, as revealed in pharmacokinetics brings no issue with its cytotoxic characteristic. ADMET prediction denotes *TNB*'s major compound **α-EG** as the most favourable to be drug molecule among all. No cellular toxicity and adverse effects are predicted for **α-EG** only except inhibition of Cytochrome P450 (Cyp) for human liver microsomal (HLM). Moreover, this undesirable trait (HLM inhibition) was the common one among all ADMET parameters as observed in other query compounds too. Further computation revealed the inhibition of Cytochrome P450 (for 3A4, 2C9 and 2C19) by **ARN** only which may affect significant drug-drug interactions to cause unanticipated adverse reactions or therapeutic failures and severe toxicity.

Being an essential cell membrane protein, P-glycoprotein (P-gp) extracts out many foreign materials from the cell (Amin, 2013). Cancer cells often overexpress P-gp and as a result chemotherapeutic agents outflow from the cell which prevents treatment by reducing the effective intracellular concentrations of such agents. This phenomenon is known as multidrug resistance. Hence, the identification of molecules that can be either transported out of the cell by P-gp (substrates) or hinder P-gp function (inhibitors) is highly intriguing (Amin, 2013). Inhibition of P-gp can result in increased bioavailability of the susceptible

drug. Here, **MRN**; **ARN**; **CPG**; and **CLP** were such compound that act as membrane transporters via P-gp inhibitory activity while **MRN**, **ARN** and **CLP** are highly bioavailable metabolites as well. Moreover, many inhibitors (verapamil, cyclosporin A, trans-flupenthixol, etc.) are also transported out of the cell by P-gp (Amin, 2013), **ARN** is one of such examples. P-gp substrate efflux can be a major obstacle in the success of CNS-targeted therapeutic delivery as well as a critical pharmacokinetic factor for causing drug-drug interactions (Chen et al., 2018b). P-gp binds to a wide range of structurally varied substrates, hence, although a drug may seem promising in the initial stages but an efflux pump like P-gp can reduce its efficacy by pumping the drug out of the cell and away from the target site, resulting in drug resistance. Therefore, the Food and Drug Administration (FDA) encourages to check for possible PGP substrates during the initial stages of drug development (Juvale et al., 2022).

The hERG (cardiotoxicity) or "ether-a-go-go related gene" encodes the potassium channel Kv11.1 and helps to generate cardiac action potentials. Hence, hERG channel inhibitors are associated with QT prolongation and may cause severe cardiac side effects including sudden cardiac death (Saxena et al., 2017). ADMET analysis anticipated both cyclic dipeptides (**CPG** and **CLP**) above all selected signature ethnic beverage compound to be human ether-a-go-go-related gene (hERG) blocker. **CLP** was also associated with mitochondrial toxicity or induction of MMP or (Matrix metalloproteinase protease). Mitochondrial dysfunction has been associated with cancer, diabetes, neurodegenerative disorders, and cardiovascular diseases. Furthermore, mutagens cause abnormal genetic mutations leading to cancer which was also taken into consideration. *In silico* prediction of the Ames *Salmonella*/microsome mutagenicity assay (Ames test) revealed **ARN** and **DDMP** as positive inducers. Ames test is a short-term bacterial reverse mutation assay specifically designed to evaluate a chemical's potential genotoxicity (Mortelmans and Zeiger 2000). Moreover, the maximum recommended therapeutic dose (MRTD) was significantly lower in case of **CVA** (104 mg/day) followed by **MRN** (152 mg/day), **ARN** (214 mg/day), **CLP** (266 mg/day) and least toxic **α-EG** (708 mg/day). Comparatively high MRTD values were computed in **DDMP** (4858 mg/day) and **CPG** (3260 mg/day).

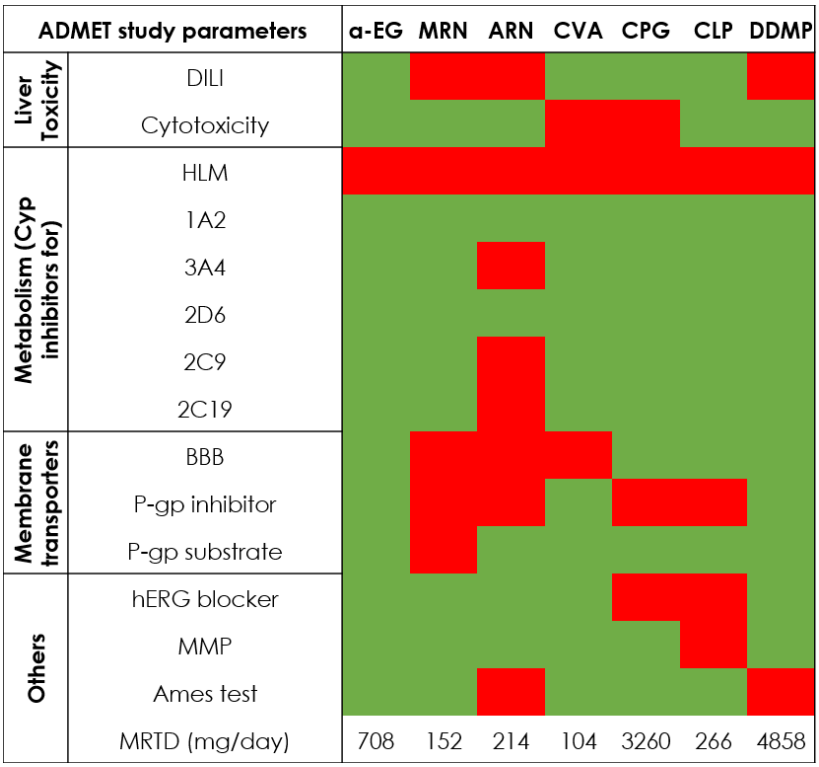


Figure 2. ADMET properties of selected Himalayan ethnic fermented beverage metabolites (green colour denotes "No" and red denotes "Yes") [Ethyl alpha-D-glucopyranoside (**α-EG**); meranzin (**MRN**); auraptene (**ARN**); cis-vaccenic acid (**CVA**); cyclo(pro-gly) (**CPG**); cyclo(leu-pro) (**CLP**); and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (**DDMP**)]

Docking scores by metabolites against high-altitude sicknesses

Docking score mimics the potential energy change when the protein and ligand are about to bind. A more negative Autodock and Autodock Vina score indicates a strong binding, while a less negative or potentially positive score indicates a weak or absent binding. Docking energy scores (Vina scores) for all the proteins computed against every selected ethnic beverage metabolite (**α-EG**, **MRN**, **ARN**, **CVA**, **CPG**, **CLP**, and **DDMP**) are included in Table 3. High altitude sicknesses (HAS) have been categorised into six diverse groups of diseases and disorders which have been mentioned in the Table 2 and discussed below.

Table 3. Docking energy scores or Vina scores (Kcal/mol) of signature metabolites from selected ethnic beverages exhibiting binding affinity with all twenty-five target proteins to investigate high-altitude sicknesses. [Ethyl alpha-D-glucopyranoside (α-EG); meranzin (MRN); auraptene (ARN); cis-vaccenic acid (CVA); cyclo(pro-gly) (CPG); cyclo(leu-pro) (CLP); and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)]

Name of the disease	Targeted receptor	PDB ID	α-EG	MRN	ARN	CVA	CPG	CLP	DDMP
Major altitude illnesses	HB2AR	3KJ6	-5.1	-6.3	-7.6	-4.6	-5	-5.8	-4.8
	R-GPCR	2Z73	-4.4	-5.9	-7	-4.1	-4.8	-5.5	-4.3
	HIF-1A	4H6J	-5	-6.2	-7.3	-4.9	-5.4	-5.5	-5.2
		4ZPR	-5.8	-7.3	-8.3	-5.2	-5.6	-6.7	-5.8
	HIF-2A	4ZPK	-5.6	-6	-7.6	-5.2	-6	-6.4	-5.5
High-altitude respiratory infections	TB InhA	1BVR	-5.6	-6.8	-7	-4.9	-5.1	-5.8	-5.1
		5O1F	-5.5	-7.2	-8	-4.3	-5.7	-5.6	-5.5
	SP-IgA1 protease	6XJA	-5.4	-6.3	-6.2	-5	-4.9	-5.6	-4.9
		6XJB	-5.8	-7.3	-7.4	-5.1	-5.9	-6.5	-5.4
Pain or inflammation	IL6	1ALU	-5.1	-5.7	-5.8	-3.9	-5.1	-5.5	-4.7
	COX-2	3LN1	-5.6	-7.4	-7.1	-4.9	-5.5	-6.3	-5.4
		5IKV	-6.3	-8	-8.3	-5.1	-5.9	-6.7	-6
		4COX	-6.1	-8	-7.8	-5	-6	-7	-6
	COX-1	6Y3C	-5.7	-7.9	-8.2	-5.6	-6.1	-7	-6
		3N8Y	-6.8	-7.6	-8	-6.1	-6	-6.6	-6
Cardiovascular problems	ACE	1O8A	-6	-7.1	-7.9	-5.1	-5.8	-6.4	-5.5
	Thermolysin	5DPF	-5.3	-6.5	-6.6	-4.2	-5	-5.4	-4.7
Gastrointestinal diseases and disorders	E-selectin	1ESL	-5	-5.6	-6.3	-3.7	-4.5	-5.1	-4.4
		1G1T	-4.8	-5.8	-6.4	-4.2	-4.8	-5.1	-4.5
	HH1	3RZE	-4.8	-6	-6.4	-4	-4.8	-5.1	-4.5
Neurological diseases and disorders	AChE	2GYU	-6.2	-6.9	-7.4	-5	-6.2	-6.8	-5.7
	BchE	4TPK	-6.5	-7.7	-8.7	-6.1	-5.9	-6.8	-5.6
	A2AR	3PWH	-5	-6.5	-7.6	-5.2	-5.3	-6.1	-4.8
		5NM2	-4.8	-6.3	-6.6	-4.5	-4.7	-5.8	-4.7
	PE3L	4I1H	-5.2	-6.2	-6.8	-4.5	-5.3	-6	-4.7

Major altitude illnesses

High altitude pulmonary edema and hypoxia are major medical problems associated with AMS or major altitude illnesses (Clarke, 2006). The objective behind selection of human β2 adrenergic receptor or HB2AR (PDB: 3KJ6) was to treat high altitude pulmonary edema by studying its interaction with signature metabolites of ethnic beverages. HB2AR is a G-protein-coupled receptor or GPCR having importance in pharmaceutical to target pulmonary and cardiovascular diseases. According to Chandramoorthi et al. (2008), the HB2AR plays a very important role in the pathogenesis of HAPE or high-altitude pulmonary edema. Selected metabolites such as **MRN**, **ARN** and **CPG** were previously reported to attenuate edema (Majumder et al., 2021; Ferro et al., 2015). Aiming GPCR, a rhodopsin-like GPCR protein (R-GPCR) was also chosen in this study to (PDB: 2Z73). Moreover, hypoxia was chosen as another target categorized as a major altitude illness. Hypoxemia manifests at high altitudes due to the reduced inspired oxygen partial pressure (hypoxia) resulting from the lowered barometric pressure. Wide-spectrum high-altitude illnesses can occur in the body if the hypoxic stress beats body's acclimatization ability (Imray et al., 2010).

Hypoxia represents a hazardous state arising from diminished oxygen tension in the arterial blood, brought about by the lungs' inability to adequately oxygenate the blood. Ethnic beverage *KKR* metabolite **ARN**, one of the selected metabolites in this study, was reported to exhibit hypoxia preventive activity (Majumder et al., 2021). Hypoxia-inducible factor 1-alpha or HIF-1A (PDB: 4H6J and 4ZPR) and hypoxia-inducible factor 2-alpha or HIF-2A (PDB: 4ZPK) were chosen to target hypoxia in this study. Binding energy scores by signature metabolites against major altitude illnesses has been given in Table 3. Both the *KKR* metabolites (**ARN** and **MRN**) exhibited comparatively high binding energy scores against every protein. The strongest binding affinity (-8.3 kcal/mol) was recorded between **ARN** (ligand) and 4ZPR (protein) targeting HIF-1A (Figure 3). Overall, selected ligands showed better energy score while binding with 4ZPR (HIF-1A) and 4ZPK (HIF-2A) (Table 3; Figure 3) among proteins selected to screen major altitude illnesses, establishing effectiveness of the metabolites particularly against hypoxia. Previously, one of our research findings indicated efficacy of *khokim raksi* metabolites (**ARN** and **MRN**) in the prevention of high-altitude pulmonary edema (Majumder et al., 2021). This bioactivity has been supported *in silico* through this docking as remarkable binding energies were computed for **ARN** and **MRN** (-7.6 kcal/mol and -6.3 kcal/mol respectively) against the protein 3KJ6 (targeting HB2AR).

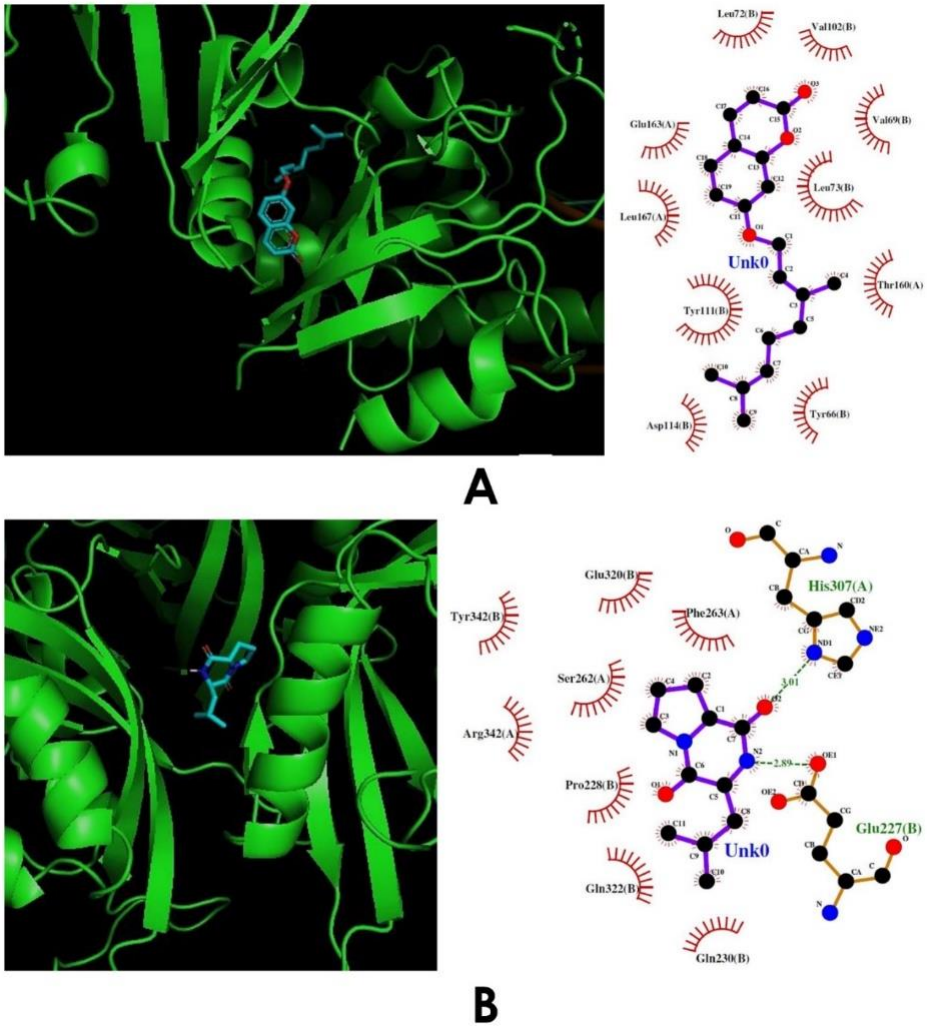


Figure 3. Docking of **ARN** (auraptene) with hypoxia-inducible factor 1-alpha (**A**) and **CLP** [cyclo(leu-pro)] with hypoxia-inducible factor 2-alpha (**B**) analysed to target major high-altitude illness- hypoxia

High-altitude respiratory infections

Zhao et al. (2021) reported potential antimicrobial activity of selected metabolite **CLP** and various other properties of pharmacological importance (Acharyya et al., 2021). Antibacterial properties of **CPG** and **CVA** were previously reported (Majumder et al., 2021; Semwal et al., 2018). However, there is no report available describing antibacterial activity of these signature metabolites against tuberculosis and pneumonia which have been considered here as particular high-altitude infections. In this docking-based study, binding affinity of ligands were investigated with important types of proteins generally taken for *in silico* analysis. To analyse *in silico* antituberculosis activity, selected ethnic beverages' metabolites were screened for the inhibition of enoyl-ACP reductase or InhA i.e., PDB: 1BVR and PDB: 5OIF two pharmacologically important proteins from *Mycobacterium tuberculosis* (TB). InhA proteins are associated in vital function i.e., synthesis of mycolic acids, essential part of mycobacterial cell wall (Rozwarski et al., 1999). Against 1BVR, **ARN** and **MRN** exhibited comparatively high binding affinities i.e., -7 kcal/mol and -6.8 kcal/mol respectively (Table 3), followed by *TNB*'s signature metabolites **CLP** (-5.8 kcal/mol) and **α-EG** (-5.6 kcal/mol). Against 5OIF, metabolites which featured strong binding energy scores were coumarins- **ARN** (-8) (Figure 4-A) and **MRN** (-7.2) followed by two dipeptide metabolites- **CPG** (-5.7) and **CLP** (-5.6). Pneumococcal IgA1 protease are a group of proteolytic enzymes, a virulence factor produced by pathogenic *Streptococcus pneumoniae* (SP) that infect and colonise mucosal surfaces and may sabotage the protective effects of IgA (Janoff et al., 2014). Therefore, SP-IgA1 proteases i.e., PDB: 6XJA and PDB: 6XJB were chosen in this study. Metabolites **ARN** (-7.4) and MRN (-7.3) (Figure 4-B) showed promising binding affinity scores against PDB: 6XJB, a targeted SP-IgA1 proteases (Table 3). Overall results of docking targeting high-altitude respiratory infections are given in Table 3.

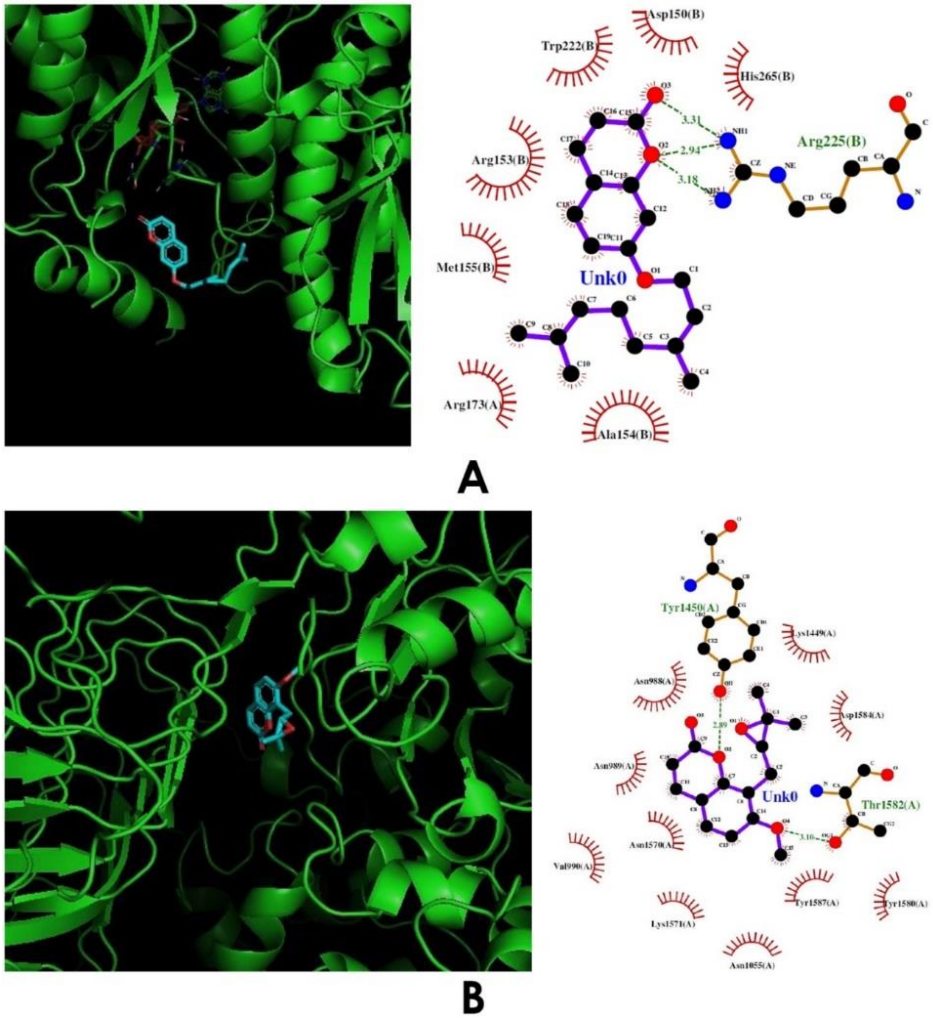


Figure 4. Docking of **ARN** (auraptene) with enoyl-ACP reductase of *Mycobacterium tuberculosis* (**A**) and **MRN** (meranzin) with IgA1 protease of *Streptococcus pneumoniae* (**B**) analysed to target high-altitude's respiratory infections

Pain or inflammation

Pain or inflammation is a common symptom of several health problems encountered due to exposure of high-altitudinal stresses (Majumder et al., 2021). Mainly tourists and sometimes native mountain people face severe pain in the body muscle and joints after various physiological activities where ethnic medicines, foods and beverages, considered as therapeutic, are consumed as remedy (Majumder et al., 2021). Addition of bioactive coumarin rich ethnomedicine *khokim* in preparation of *raksi* reportedly could play prevent altitude sicknesses (Majumder et al., 2021). In high altitudes of Nepal, Sikkim and Darjeeling, *khokim* is often used to treat fever, body ache, muscular pain etc. (Singh et al., 2002). Beverages studied in this research may exhibit pain-relieving property possibly because of containing anti-inflammatory compounds detected by GC-MS i.e., **ARN** (Bibak et al., 2019) and **MRN** (Patel 2022) from *KKR*; **CPG** (Ferro et al., 2015) from *CKR*, and **CVA** (Jacome-Sosa et al., 2016) from *CHN* etc. To validate their *in silico* activity, molecular docking of the bioactive compounds with proteins such as Human Interleukin-6 or IL6 (PDB: 1ALU), COX-2 (PDB: 3LN1; 5IKV and 4COX) and COX-1 (PDB: 6Y3C and 3N8Y) were performed in this research. IL6 encodes a cytokine that play role in inflammation and the maturation of B cells. IL6 is primarily synthesized at sites of acute and chronic inflammation. It induces a transcriptional inflammatory response through interleukin 6 receptor, alpha (https://www.ncbi.nlm.nih.gov/gene/3569) after being secreted into the serum. Cyclooxygenase (COX-1 and COX-2) enzymes converts arachidonic acid to prostaglandins and other lipid mediators (Smith et al., 1998). Famous Japanese immunologist Kishimoto (2005) reported implication of IL6 in a wide variety of inflammation-associated diseases including susceptibility to diabetes mellitus, rheumatoid arthritis and Castleman's disease. COX-2 is the most appropriate target protein to study anti-inflammatory activity (Kishimoto, 2005) for being induced by inflammatory stimuli while COX-1 might be the major player in neuro-inflammation (Choi et al., 2009). However, both enzymes produce prostaglandins that promote inflammation, pain, and fever; thus, considered useful as targets for anti-inflammatory drugs. COX inhibitors could alleviate the symptoms of AMS (Yi et al., 2020), the point of focus of this study. Distinct role of COX-1 and COX-2 on neuroinflammation (hippocampal inflammation- a high-altitude problem) and related cognitive deficits in the high-altitude hypoxia condition has also been highlighted in a recent study by Chauhan et al., 2019. COX-2 inhibitors have potential to treat mild-to-moderate pain and inflammation caused by osteoarthritis, rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, menstrual cramps or dysmenorrhea, familial adenomatous polyposis, short-term pain like sports injuries (a high-altitude issue), gout, migraine headaches or other conditions (https://my.clevelandclinic.org/health/drugs/23119-cox-2-inhibitors). Docking scores against selected COX-2 proteins were in the range of -4.9 to -8.3 and against receptor COX-1, the range was found -5.6 to -8.2 (Table 3). 1ALU showed comparatively lesser binding scores than that exhibited by COX-2 or COX-1 proteins. Binding affinities for this Human Interleukin-6 protein were found in this subsequent order, i.e., **ARN** > **MRN** > **CLP** > **CPG**/**α-EG** > **DDMP** > **CVA** (-5.8, -5.7, -5.5, -5.1, -4.7 and -3.9kcal/mol), respectively (Table 3). Among the compounds, **CVA** resulted least binding energy score in every computed interaction and highest scores were resulted by **ARN** and **MRN** alternately. Docking images of **MRN** with COX-2 and **α-EG** with COX-1 have been given in Figure 5.

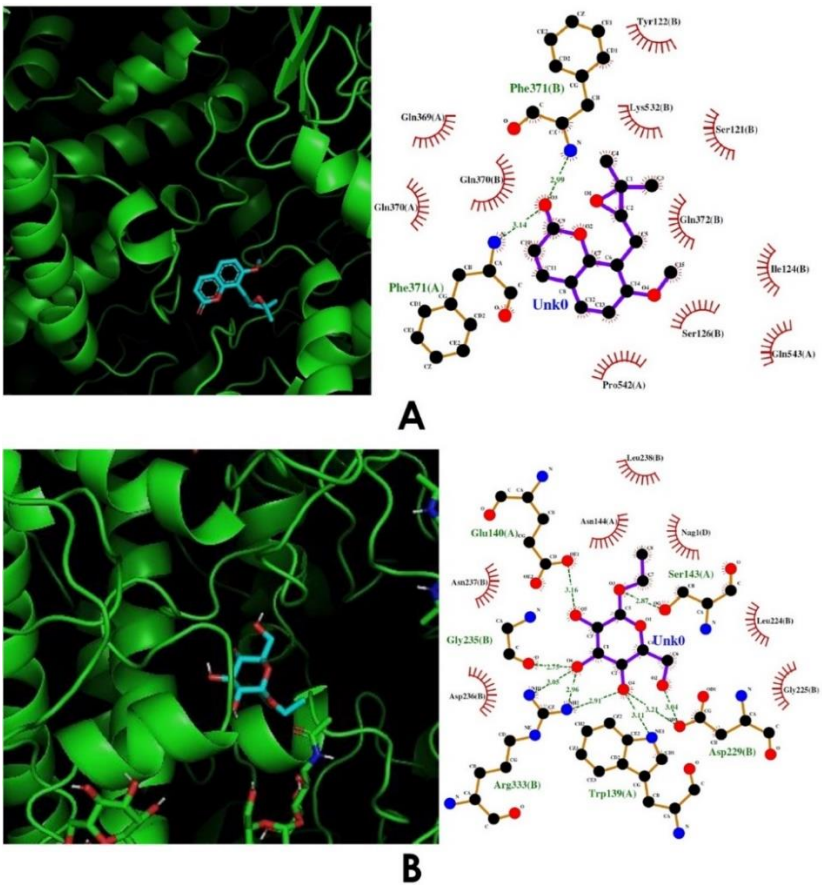


Figure 5. Docking of **MRN** (meranzin) with COX-2 receptor (**A**) and **α-EG** (ethyl alpha-glucopyranoside) with COX-1 receptor (**B**) analysed to target pain or inflammation commonly encountered in high-altitude

Cardiovascular problems

Regarding selected ligands, monoterpene-coumarin **ARN** is a potential vasodilator and prevents hypertension to exhibit cardio-protectivity; coumarin **MRN** improves atherosclerosis; and **CPG** prevents cardio-metabolic disorders by normalizing systolic blood pressure as reported (Arabi et al., 2021; Li et al., 2019; Li et al., 2020). Human angiotensin-converting enzyme (ACE) (PDB: 1O8A) plays critical role in cardiovascular function by cleaving the carboxy terminal His-Leu dipeptide from angiotensin I that produces a potent vasopressor octapeptide, angiotensin II. Angiotensin II is a vasopressor and aldosterone-stimulating peptide that actually controls blood pressure and fluid-electrolyte balance (Natesh et al., 2003; MacLeod-Carey et al., 2020). ACE encodes an enzyme that functions in blood pressure regulation and electrolyte balance. Inhibitors of ACE are a first line of therapy to treat diseases like hypertension, heart failure, myocardial infarction and diabetic nephropathy. Accordingly, the encoded enzyme increases blood pressure and is a drug target of ACE inhibitors, which are often prescribed to reduce blood pressure (https://www.ncbi.nlm.nih.gov/gene/1636). the renin-angiotensin-aldosterone system (RAAS) is the principal target of arterial hypertension (Te Riet et al., 2015). In this regard, several researchers focused on designing RAAS inhibitors using thermolysin as target model (MacLeod-Carey et al., 2020). Thermolysin inhibitors may also inhibit ACE and NEP and be potential antihypertensives as thermolysin has structural and functional similarities to NEP and ACE. Previously MacLeod-Carey et al. (2020) designed antihypertensive drugs *in silico* using thermolysin (PDB: 5DPF) as a target. So, both ACE and thermolysin were selected as proteins to evaluate cardiovascular protective (antihypertensive) properties of signature compounds from studied high-altitude beverages. The docking results (Table 3) showed that the bioactive compounds showed better affinity towards the human angiotensin-converting enzyme (ACE) as it returned better docking scores that were in the range of -5.1 to -7.9 (Table 3; Figure 6) compared to that for Thermolysin (a range of -4.2 to -6.6), indicating better binding or interacting with the protein involved in overall cardiopathy including electrolyte disbalance, hypertension, heart failure, myocardial infarction, diabetic nephropathy etc.

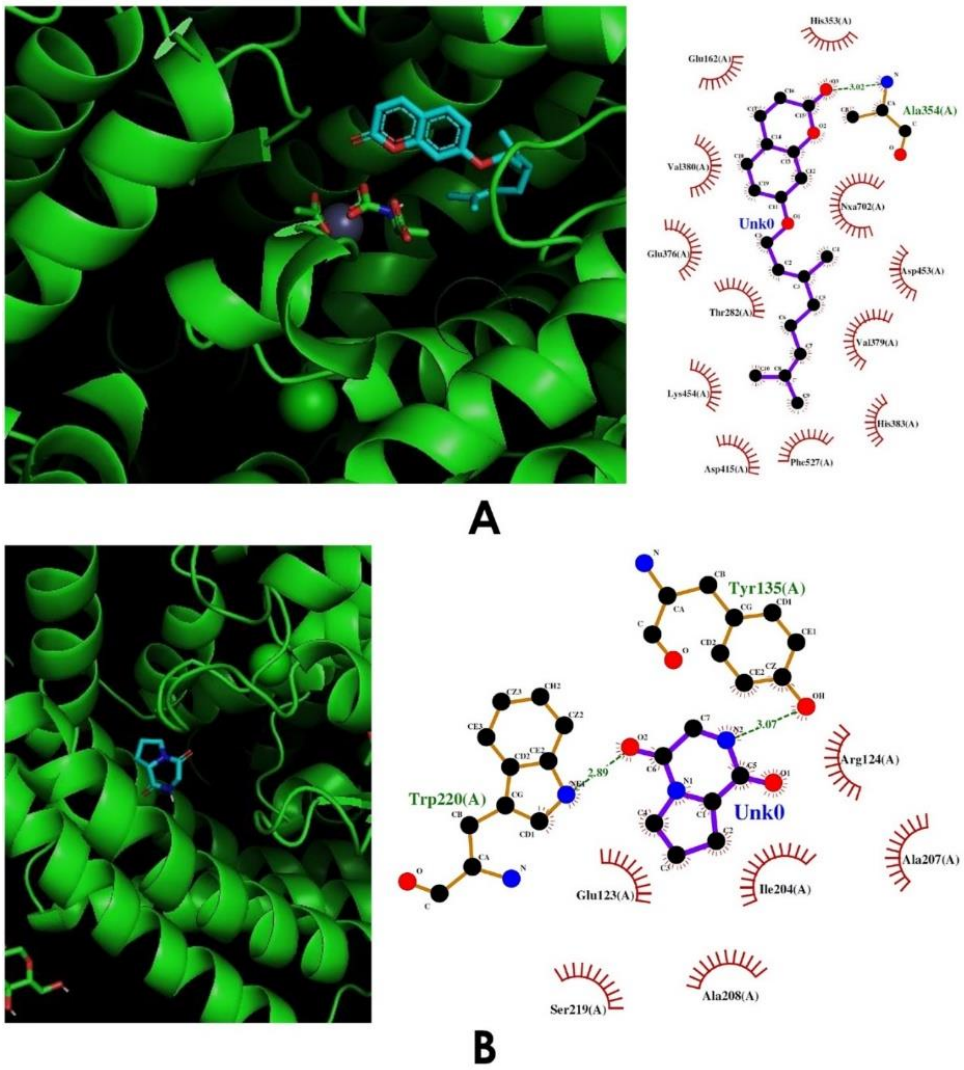


Figure 6. Docking of auraptene or **ARN** (**A**) and cyclo(pro-gly) or **CPG** (**B**) with human Angiotensin Converting Enzyme (ACE) as chosen to target hypertension related diseases

Gastrointestinal diseases and disorders

Sharma et al. (2014) reported atrophic gastritis as a prevalent feature in patients experiencing dyspepsia in a high-altitude area. Epigastric pain, heartburn, regurgitation, bloating, belching, nocturnal pain, discomfort after meals, early satiety, nausea, postprandial fullness usually happen with people who have dyspepsia (Sharma et al., 2014). Dyspepsia or indigestion is a common symptom in high altitude that may lead to heartburn, gastric inflammation, gastritis and gastric cancer (Sharma et al., 2014). Studied compound- **ARN** can attenuate chronic gastritis and gastric cancer (Sekiguchi et al., 2012). However, studies to date have not demonstrated other selected signature metabolites' relationship with gastrointestinal diseases and disorders linked to HAS. Endothelial selectin (E-selectin) proteins (PDB: 1ESL and 1G1T) were chosen because the aim of this study was to target gastrointestinal diseases including gastritis (Graczyk et al., 2013) and gastric cancer (Jung et al., 2012). Another targeted protein histamine is a major mediator in allergic diseases. Histamine regulates various pathophysiological and physiological processes including secretion of gastric acid and pepsin (Riaz et al., 2019). Histaminergic H1 (PDB: 3RZE) has been targeted for computational evaluation of gastrointestinal disorders to study anti-diarrheal, anti-secretory, anti-spasmodic, anti-motility and anti-ulcer activities as demonstrated by Riaz et al. (2019). Binding affinities for receptors aiming gastrointestinal diseases and disorders, arranged as high to low, were found in this order (docking score ranges are included inside round brackets): **ARN** (-6.3 to -6.4) > **MRN** (-5.6 to -6) > **CLP** (-5.1) > **α-EG** (-4.8 to -5) > **CPG** (-4.5 to -4.8) > **DDMP** (-4.4 to -4.5) > **CVA** (-3.7 to -4.2). Results are given in Table 3 and images of **α-EG** and **ARN** docking with endothelial selectin protein are added in Figure 7.

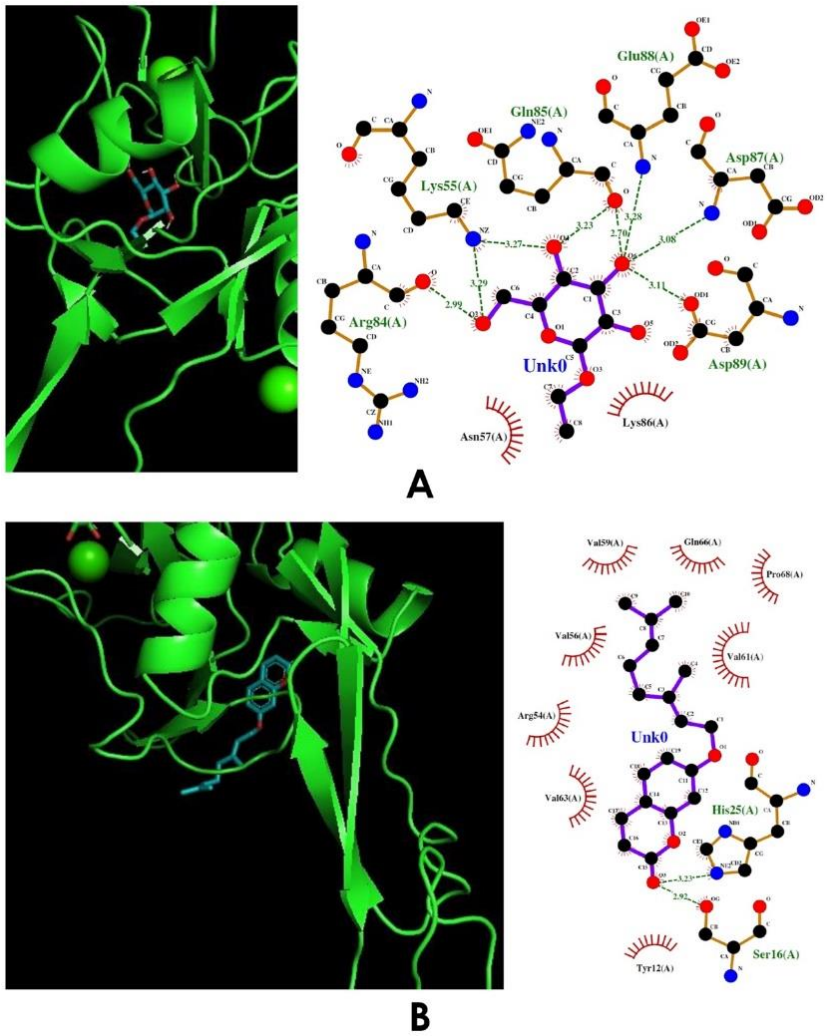


Figure 7. Docking of alpha-ethyl glucopyranoside or **α-EG** (**A**) and auraptene or **ARN** (**B**) with endothelial selectin protein selected to target gastrointestinal diseases and disorders

Neurological diseases and disorders

Raksi compounds **ARN** and **CPG** are reported as potential neuroprotective components (Bibak et al., 2019; Acharyya et al., 2021). Selected coumarin **ARN** exhibits a variety of bioactivities to counter anxiety, depression, dizziness, and Alzheimer's disease (Arabi et al., 2021; Majumder et al., 2021) and **MRN** has antidepressant property too (Liu et al., 2021). Therefore, they were reported to treat such neurological diseases considered as high altitude neurological and psychiatric illnesses in this research. Recently, Savioli et al. (2022) have pointed out acetylcholine depletion as the reason behind fatigue, which is a common disorder at high-altitude. Chronic exposure to high altitude (CEHA) is associated to deficits of cognitive functions as reported by Sharma et al., 2019. In the same study, it was stated that synaptic changes in the olfactory cortex of CEHA animals can cause alterations in the release of acetylcholine. According to Gibson and Duffy (1981), the cholinergic system is particularly vulnerable to hypoxia condition (major high-altitude disease) where neurotransmitter acetylcholine (ACh) is primarily affected. In the lungs, acetylcholine causes bronchoconstriction via smooth muscle contraction responsible for wheezing and shortness of breath, major high-altitude problems (Buels and Fryer 2012). Moreover, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are commonly targeted as proteins to study anti-neurodegenerative property (Alzheimer's disease and myasthenia gravis) and their inhibitors are considered as potential neuroprotective agents. So, receptor proteins such as AChE (PDB: 2GYU) and BChE (PDB: 4TPK) were targeted to evaluate possible neuroprotective potential of selected beverage metabolites. The adenosine A2A receptor (A2AR), another selected protein, is a G-protein-coupled receptor that is a major target of stimulants like caffeine and have a crucial role in the modulation of inflammation, myocardial oxygen consumption, coronary blood flow, and regulation of CNS neurotransmitters, thus a receptor of interest for new

neuroprotective drug development (Chen et al., 2013). A2AR antagonists indicate their potential medicinal value in the treatment of Parkinson's disease, a neurodegenerative disorder. Inhibition of A2AR not only decreases mobility disorders, but also reveals the neuroprotective effect (Cieślak et al., 2008). Cognitive impairments are quite common in hypobaric hypoxia (HH) conditions (Chen et al., 2018a). Broad-spectrum cognitive processes such as, attention; short-term memory; decision-making; simple and complex reaction time; and mood are altered in HH conditions, and the severity of these impairments is closely related to the altitude (Chen et al., 2018a). At 3000–4000 m (altitude range similar to our study area- Singalila Ridge of the Himalayas) psychomotor abilities can be reduced, while above 6000 m mountaineers reportedly experienced hallucinations (Chen et al., 2018a). At high altitudes, imbalanced oxygen supply affects haemostasis of the central nerve system (CNS) and causes pathophysiological responses. It has been discussed earlier that inflammation is a key response in hypoxia signalling, causes high-altitude diseases such as AMS or acute mountain sickness and HAPE or high-altitude pulmonary edema. Furthermore, neuroinflammation, featuring as the activation of glia cells and the release of cytokines within the CNS, can occur and contribute to cognitive impairments in high-altitude conditions (Chen et al., 2018a). Later, Chen et al. (2018a) investigated the effect of adenosine A2A receptor on spatial memory and mood after acute HH exposure. They explored activity of A2A receptor in accumulation of microglia and production of proinflammatory cytokine TNF-α in the hippocampus of acute HH mice model. Therefore, neuroinflammation caused by A2AR activation involves in acute HH-induced spatial memory impairment; so, this protein is important in the study of pharmacotherapy regarding cognitive dysfunction at high altitudes (Chen et al., 2018a). Pulmonary disorders- asthma and chronic obstructive pulmonary disease (COPD); and shortness of breath induced sleeping problem (altitude-induced insomnia) can also be treated by adenosine A2A agonists (Polosa and Blackburn, 2009; Scammell et al., 2001). For docking neurological diseases and disorders connected with HAS, A2AR proteins (PDB: 3PWH and 5NM2) were targeted in this *in silico* study. Parkin is a RING-between-RING E3 ligase (PE3L) enzyme which functions in the covalent attachment of ubiquitin to specific substrates, and mutations in Parkin causes Parkinson's disease, cancer and mycobacterial infection (Riley et al., 2013). PE3L (PDB: 4I1H) has also been targeted to study neuroprotectivity of selected compounds not only because Parkinson's disease is one of the common targeted neurological disorders in such studies but also parkinsonism (slowness, stiffness and walking/balance difficulties) is reported as a major neurologic symptom linked to severe altitude sickness (https://www.apdaparkinson.org/article/high-altitude-cold-weather-effects-parkinsons). Highest docking scores were resulted by **ARN** and **MRN** alternately. The obtained results show that the query compounds have binding affinity values within a range of -6.6 to -8.7 kcal/mol for **ARN**; -6.2 to -7.7 kcal/mol for **MRN**; -5.8 to -6.8 kcal/mol for **CLP**; -4.8 to -6.5 kcal/mol for **α-EG**; -4.7 to -6.3 kcal/mol for **CPG**; -4.7 to -5.7 for **DDMP**; and -4.5 to -6.1 for **CVA** (Table 3). Docking score between **ARN** and butyrylcholinesterase (BChE) was recorded as the highest (-8.7) (Figure 8-B) among all the combinations tested in this research. **MRN** and **ARN** not only showed better docking scores among others while targeting proteins selected for neurological diseases and disorders but also they ranked as top two compounds with high BBB scores during pharmacokinetics analysis justifying their likeliness as well as bio-activeness as CNS drugs.

Total 175 combinations of protein-ligand docking interactions were examined out of which 141 combinations (80.6%) had a binding energy score of ≤ -5 and 29 (16.6%) had an affinity ≤ -7. In order to have a better understanding, docking scores given in Table 3 have been graphically represented via a radar chart (Figure 9-A) prepared in Microsoft Excel Worksheet. The overall outcome depicts that among the query compounds, **CVA** resulted least binding energy score in every computed interaction while strong bindings were observed in the interactions performed by **ARN** followed by **MRN** (Figure 9-B). Among the groups of diseases, average docking score for proteins (Figure 9-C) targeting pain or inflammation were the highest (average score: -6.3) which is an evident of strong anti-inflammatory activities or pain-relieving properties linked to these ethnic beverages as acclaimed by people living in high altitudes (Majumder et al., 2021).

Neurological diseases and disorders ranked second among selected categories with an average docking score of -5.9. Receptor proteins chosen to target respiratory infection (*Mycobacterium tuberculosis* and *Streptococcus pneumoniae*) showed quite good interactions with ethnic beverage metabolites resulting an average score of -5.85 affirming antibacterial properties.

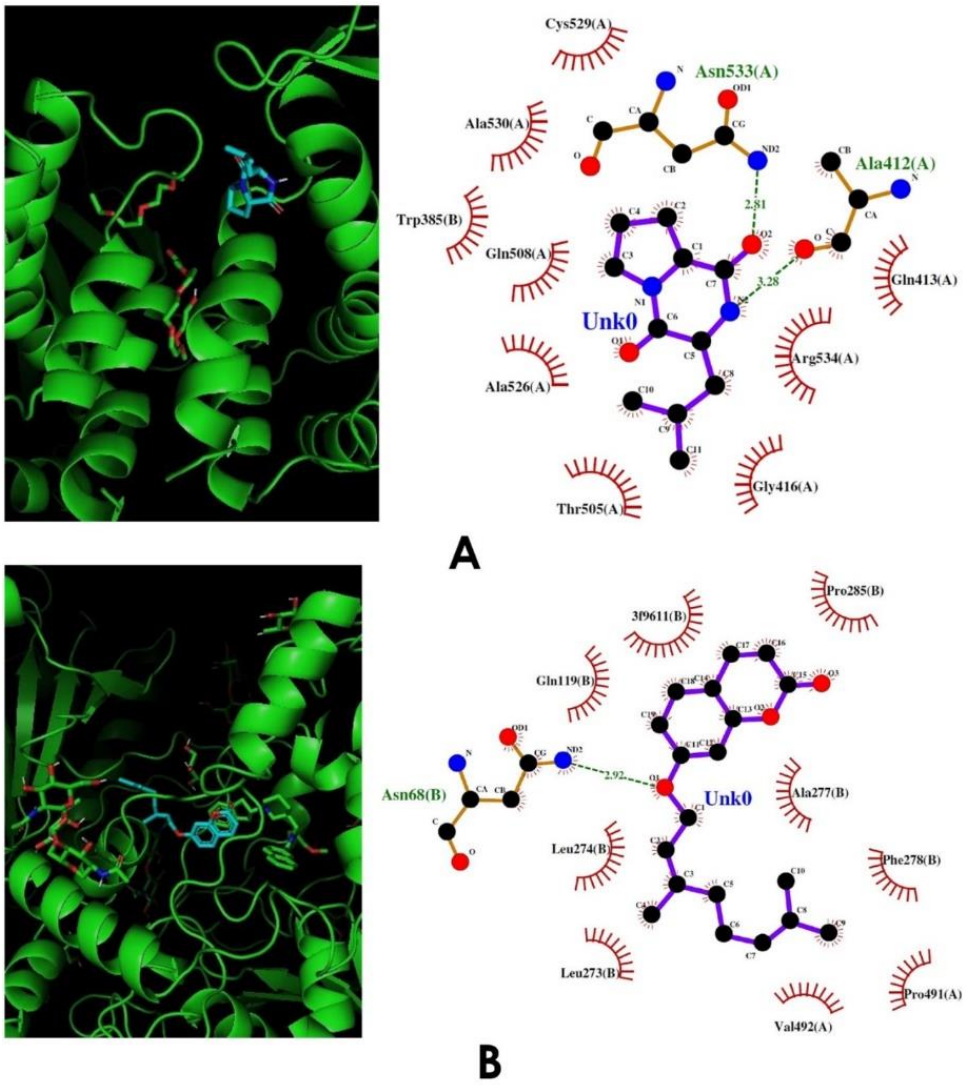


Figure 8. Docking of cyclo(leu-pro) or **CLP** with acetylcholinesterase (AChE) (**A**) and auraptene or **ARN** with butyrylcholinesterase (BchE) (**B**) analysed to study anti-neurodegenerative property to evaluate possible neuroprotective potential of ethnic fermented beverages

Average docking scores for major altitude illnesses (AMS) and cardiovascular problems were -5.8 for each category which validated promising curative activities by the beverages against AMS, pulmonary edema, hypoxia, hypertension and other cardiovascular problems. Binding strengths were minimum with an average score of -5 as exhibited by proteins selected for gastrointestinal diseases and disorders.

During molecular docking, the amino acid residues involved in hydrogen-binding and hydrophobic interactions were recorded from 2-D representations of protein-ligand complexes obtained from LigPlot^{+} software as given in Supplementary Table S1. Hydrophobic interactions are always stronger than other weak intermolecular forces (i.e., Van der Waals interactions or Hydrogen bonds). Compounds- **ARN**, **MRN**, **CVA** and **CPG** showed significant binding scores with a greater number of hydrophobic interactions compared to hydrogen bonds. Moreover, these strong protein-ligand binding by hydrophobic bonds have many benefits as hydrophobic nature of interaction is energetically favourable (exothermic), it keeps protein stable and biologically active with the interaction and decreases surface area to stop undesirable interactions during the time of molecule's function. Certainly, it's evident that hydrophobic interactions significantly contribute to the stabilization of ligands at the binding interface. A heatmap (Figure 10) designed for comparative analysis is given representing number of hydrogen bonds and hydrophobic interactions formed during protein-ligand binding. Some docking images (visualized with PyMOL molecular visualization tool) including of different categories of HAS have been represented in Figure 3, Figure 4, Figure 5, Figure 6, Figure 7, and Figure 8.

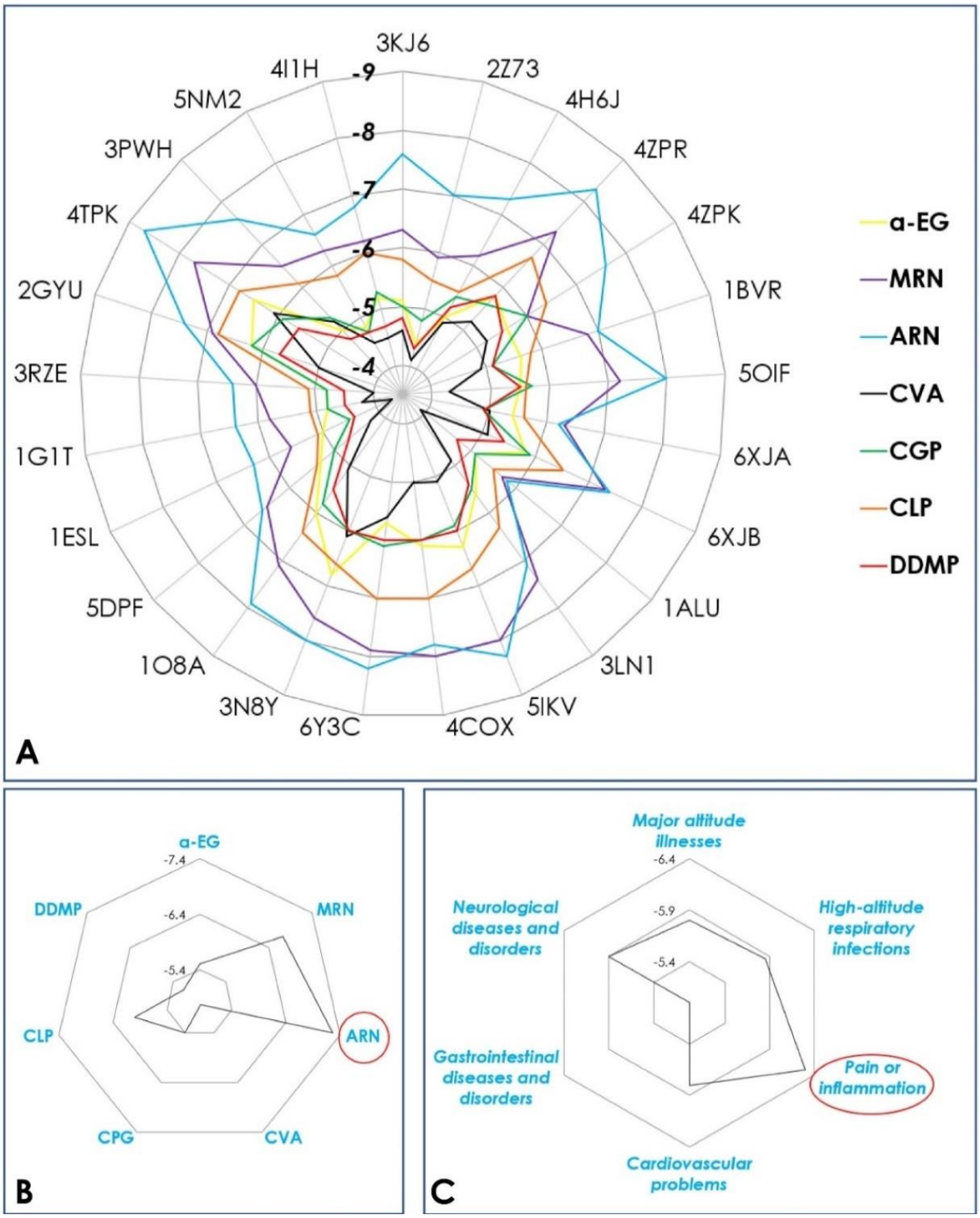


Figure 9. Comparative docking scores: **A**- Radar chart showing binding scores of different metabolites against different receptor proteins; **B**- Radar chart prepared with mean docking scores exhibited by different metabolites swowing auraptene or **ARN** as the most promising scorer; **C**- Radar chart prepared with mean docking scores computed for each category of HAS or high-altitude sicknesses shwowing receptor proteins chosen for pain or inflammation as involved in stronger binding compared to other categories of HAS [Ethyl alpha-D-glucopyranoside (**α-EG**); meranzin (**MRN**); auraptene (**ARN**); cis-vaccenic acid (**CVA**); cyclo(pro-gly) (**CPG**); cyclo(leu-pro) (**CLP**); and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (**DDMP**)]

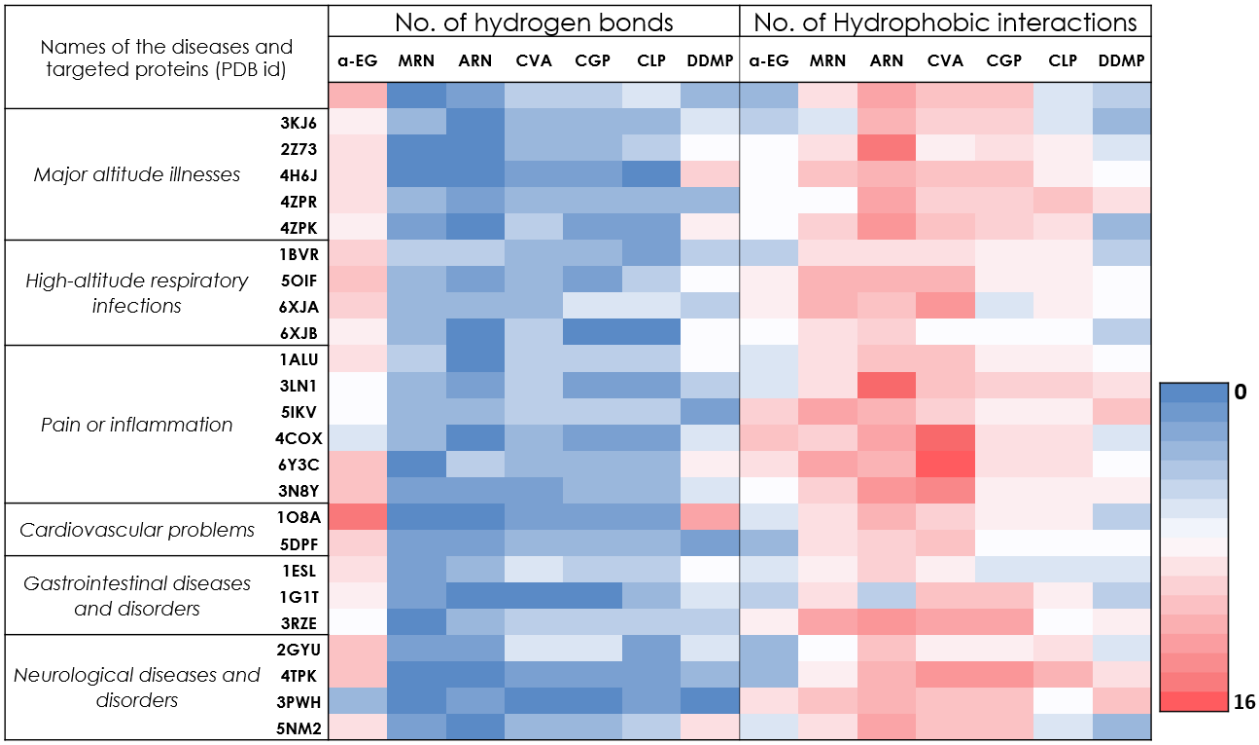


Figure 10. Heatmap prepared using Blue-White-Red (0 to 15) colour scale showing number of hydrogen bonds and hydrophobic interactions formed during protein-ligand binding. [Ethyl alpha-D-glucopyranoside (**α-EG**); meranzin (**MRN**); auraptene (**ARN**); cis-vaccenic acid (**CVA**); cyclo(pro-gly) (**CPG**); cyclo(leu-pro) (**CLP**); and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (**DDMP**)]

CONCLUSIONS

The current study was designed as a novel approach combining physicochemical properties, *in silico* pharmacokinetics, ADMET analysis and molecular docking to validate that HAS can be alleviated by Himalayan ethnic beverages. The concept of this research i.e., prevention of HAS through consumption of traditional fermented drinks was raised after a series of research on metabolomics conducted by our research group to validate ethnomedicinal claims. Here, outcome of this computational research has demonstrated HAS healing properties of Himalayan traditional fermented drinks through bioinformatics which led the hypothesis, high-altitude sickness versus high altitude's ethnic food, to a new high. These beverages are categorised under non-drug traditional therapy, so medicinal activities are associated to consumption. But excess consumption of alcoholic beverages is a matter of concern, therefore intaking limited quantity of these traditional beverages in high altitudinal areas can be an effective regimen towards coping against high altitude sicknesses owing to cumulative effect of the constituent metabolites. Results further suggest that exploration of traditional knowledge shared by Indigenous people could be essential in designing and implementing solutions for the healthcare challenges or drawbacks in remote ecosystems, in this case- prevention of HAS by these beverages collected from high-altitude remote locations. The current results may advise future in-depth studies on pharmacology of ethnic beverages, *in vivo* experiments and cellular assays.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

SM conceptualized the research. SM and MB designed the study. SC and SM performed the experiments. AG and MB assisted in the experiments. SM wrote the manuscript. MB supervised and administered the research. All authors have revised and approved the manuscript.

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SUPPLEMENTARY INFORMATION

Supplementary Table S1: List of important amino acid residues of selected proteins involved in hydrogen- binding and hydrophobic (*) interactions with ligands. [Ethyl alpha-D-glucopyranoside (**α-EG**); meranzin (MRN); auraptene (**ARN**); cis-vaccenic acid (**CVA**); cyclo(pro-gly) (**CPG**); cyclo(leu-pro) (**CLP**); and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (**DDMP**)]

REFERENCES

1. Acharyya, S., Saha, S., Majumder, S., & Bhattacharya, M. (2021). Characterization of a mercury tolerant strain of Staphylococcus arlettae from Darjeeling hills with an account of its antibiotic resistance pattern and metabolome. *Archives of Microbiology*, *203*, 5745-5754.
2. Al Azzam, K. M., Negim, E. S., & Aboul-Enein, H. Y. (2022). ADME studies of TUG-770 (a GPR-40 inhibitor agonist) for the treatment of type 2 diabetes using SwissADME predictor: In silico study. *Journal of Applied Pharmaceutical Science*, *12*(4), 159-169.
3. Amin, M. L. (2013). P-glycoprotein inhibition for optimal drug delivery. *Drug Target Insights*, *7*, DTI-S12519.
4. Arabi, M., Nasab, S. H., Lorigooini, Z., Boroujeni, S. N., Mortazavi, S. M., Anjomshoa, M., & Amini-Khoei, H. (2021). Auraptene exerts protective effects on maternal separation stress-induced changes in behavior, hippocampus, heart and serum of mice. *International Immunopharmacology*, *93*, 107436.
5. Bibak, B., Shakeri, F., Barreto, G. E., Keshavarzi, Z., Sathyapalan, T., & Sahebkar, A. (2019). A review of the pharmacological and therapeutic effects of auraptene. *BioFactors*, *45*(6), 867-879.
6. Bogaki, T., Mitani, K., Oura, Y., & Ozeki, K. (2017). Effects of ethyl-α-d-glucoside on human dermal fibroblasts. *Bioscience, Biotechnology, and Biochemistry*, *81*(9), 1706-1711.
7. Borgström, L. (1998). Local versus total systemic bioavailability as a means to compare different inhaled formulations of the same substance. *Journal of Aerosol Medicine*, *11*(1), 55-63.
8. Buels, K. S., & Fryer, A. D. (2012). Muscarinic receptor antagonists: effects on pulmonary function. *Muscarinic Receptors*, 317-341.
9. Chandramoorthi, G. D., Piramanayagam, S., & Marimuthu, P. (2008). An insilico approach to high altitude pulmonary edema-molecular modeling of human β2 adrenergic receptor and its interaction with salmeterol and nifedipine. *Journal of Molecular Modeling*, *14*, 849-856.
10. Chauhan, G., Roy, K., Kumar, G., Kumari, P., Alam, S., Kishore, K., Panjwani, U., & Ray, K. (2019). Distinct influence of COX-1 and COX-2 on neuroinflammatory response and associated cognitive deficits during high altitude hypoxia. *Neuropharmacology*, *146*, 138-148.
11. Chen, J. F., Eltzschig, H. K., & Fredholm, B. B. (2013). Adenosine receptors as drug targets—what are the challenges?. *Nature reviews Drug discovery*, *12*(4), 265-286.
12. Chen, P. Z., He, W. J., Zhu, Z. R., Guo-Ji, E., Xu, G., Chen, D. W., & Gao, Y. Q. (2018a). Adenosine A2A receptor involves in neuroinflammation-mediated cognitive decline through activating microglia under acute hypobaric hypoxia. *Behavioural Brain Research*, *347*, 99-107.
13. Chen, C. P., Chen, C. C., Huang, C. W., & Chang, Y. C. (2018b). Evaluating molecular properties involved in transport of small molecules in stratum corneum: A quantitative structure-activity relationship for skin permeability. *Molecules*, *23*(4), 911.
14. Choi, S. H., Aid, S., & Bosetti, F. (2009). The distinct roles of cyclooxygenase-1 and-2 in neuroinflammation: implications for translational research. *Trends in Pharmacological Sciences*, *30*(4), 174-181.
15. Cieślak, M., Komoszyński, M., & Wojtczak, A. (2008). Adenosine A 2A receptors in Parkinson's disease treatment. *Purinergic Signalling*, *4*, 305-312.
16. Clarke C. (2006). Acute mountain sickness: medical problems associated with acute and subacute exposure to hypobaric hypoxia. *Postgraduate Medical Journal, 82*(973), 748–753. https://doi.org/10.1136/pgmj.2006.047662
17. Daina, A., & Zoete, V. (2016). A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. *ChemMedChem*, *11*(11), 1117-1121.

18. Ericsson, C. D., Steffen, R., Basnyat, B., Cumbo, T. A., & Edelman, R. (2001). Infections at high altitude. *Clinical Infectious Diseases*, *33*(11), 1887-1891.
19. Ferrazzini, G., Maggiorini, M., Kriemler, S., Bärtsch, P., & Oelz, O. (1987). Successful treatment of acute mountain sickness with dexamethasone. *British Medical Journal (Clinical Research Ed..)*, *294*(6584), 1380-1382.
20. Ferro, J. N. D. S., de Aquino, F. L. T., de Brito, R. G., dos Santos, P. L., Quintans, J. D. S. S., de Souza, L. C., de Araújo, A. F., Diaz, B. L., Lucca-Júnior, W., Quintans-Júnior, L. J., & Barreto, E. (2015). Cyclo-Gly-Pro, a cyclic dipeptide, attenuates nociceptive behaviour and inflammatory response in mice. *Clinical and Experimental Pharmacology and Physiology*, *42*(12), 1287-1295.
21. Ghose, A. K., Herbertz, T., Hudkins, R. L., Dorsey, B. D., & Mallamo, J. P. (2012). Knowledge-based, central nervous system (CNS) lead selection and lead optimization for CNS drug discovery. *ACS Chemical Neuroscience*, *3*(1), 50-68.
22. Ghosh, A., Chakraborty, S., Majumder, S., & Bhattacharya, M. (2022). Comprehensive In silico Investigation Validates Two Flavonoid Compounds of Derris robusta (Roxb. ex DC.) Benth. to Supplant Remdesivir as Natural Therapeutic Remedy Against a Range of Coronaviruses. *Letters in Applied NanoBioscience, 12*, 4, 108
23. Gibson, G. E., & Duffy, T. E. (1981). Impaired synthesis of acetylcholine by mild hypoxic hypoxia or nitrous oxide. *Journal of Neurochemistry*, *36*(1), 28-33.
24. Graczyk, M., Przybyszewski, M., Kuźmiński, A., Tlappa, J., Mućka, J., Napiórkowska, K., Szynkiewicz, E., Żbikowska-Gotz, M., & Bartuzi, Z. (2013). Role of E-selectin and platelet endothelial cell adhesion molecule 1 in gastritis in food allergy patients. *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii*, *30*(5), 271-276.
25. Gupta, M., Lee, H. J., Barden, C. J., & Weaver, D. F. (2019). The blood–brain barrier (BBB) score. *Journal of Medicinal Chemistry*, *62*(21), 9824-9836.
26. Hackett, P., Rennie, D., & Levine, H. (1976). The incidence, importance, and prophylaxis of acute mountain sickness. *The Lancet*, *308*(7996), 1149-1155.
27. Hitchcock, S. A. (2012). Structural modifications that alter the P-glycoprotein efflux properties of compounds. *Journal of Medicinal Chemistry*, *55*(11), 4877-4895.
28. Imray, C., Wright, A., Subudhi, A., & Roach, R. (2010). Acute mountain sickness: pathophysiology, prevention, and treatment. *Progress in Cardiovascular Diseases*, *52*(6), 467-484.
29. Jacome-Sosa, M., Vacca, C., Mangat, R., Diane, A., Nelson, R.C., Reaney, M. J., Shen, J., Curtis, J. M., Vine, D. F., Field, C. J., & Igarashi, M. (2016). Vaccenic acid suppresses intestinal inflammation by increasing anandamide and related N-acylethanolamines in the JCR: LA-cp rat [S]. *Journal of Lipid Research*, *57*(4), 638-649.
30. Janoff, E. N., Rubins, J. B., Fasching, C., Charboneau, D., Rahkola, J. T., Plaut, A. G., & Weiser, J. N. (2014). Pneumococcal IgA1 protease subverts specific protection by human IgA1. *Mucosal Immunology*, *7*(2), 249-256.
31. Johnson, T. S., Rock, P. B., Fulco, C. S., Trad, L. A., Spark, R. F., & Maher, J. T. (1984). Prevention of acute mountain sickness by dexamethasone. *New England Journal of Medicine*, *310*(11), 683-686.
32. Jung, W. C., Jang, Y. J., Kim, J. H., Park, S. S., Park, S. H., Kim, S. J., Mok, Y. J., & Kim, C.S. (2012). Expression of intercellular adhesion molecule-1 and e-selectin in gastric cancer and their clinical significance. *Journal of Gastric Cancer*, *12*(3), 140-148.
33. Juvale, I. I. A., Hamid, A. A. A., Abd Halim, K. B., & Has, A. T. C. (2022). P-glycoprotein: new insights into structure, physiological function, regulation and alterations in disease. *Heliyon*, e09777.
34. Kapoor, R., Narula, A. S., & Anand, A. C. (2004). Treatment of acute mountain sickness and high altitude pulmonary oedema. *Medical Journal Armed Forces India*, *60*(4), 384-387.
35. Kishimoto, T. (2005). Interleukin-6: from basic science to medicine—40 years in immunology. *Annual Review of Immunology*, *23*, 1-21.
36. Laskowski, R. A., & Swindells, M. B. (2011). LigPlot+: multiple ligand–protein interaction diagrams for drug discovery. *Journal of Chemical Information and Modeling, 51* (10), 2778-2786
37. Li, L., Yu, A. L., Wang, Z. L., Chen, K., Zheng, W., Zhou, J. J., Xie, Q., Yan, H. B., Ren, P., & Huang, X. (2019). Chaihu-Shugan-San and absorbed meranzin hydrate induce anti-atherosclerosis and behavioral improvements in high-fat diet ApoE-/-mice via anti-inflammatory and BDNF-TrkB pathway. *Biomedicine & Pharmacotherapy*, *115*, p.108893.

38. Li, F., Liu, K., Gray, C., Harris, P., Reynolds, C. M., Vickers, M. H., & Guan, J. (2020). Cyclic glycine-proline normalizes systolic blood pressure in high-fat diet-induced obese male rats. *Nutrition, Metabolism and Cardiovascular Diseases*, *30*(2), 339-346.
39. Liang, Z. L., Zhang, X. Y., Wang, F., Zhang, K., Liu, H. F., & Liu, H. L. (2018). Understanding molecular mechanisms of Rhodiola rosea for the treatment of acute mountain sickness through computational approaches (a STROBE-compliant article). *Medicine*, *97*(39).
40. Lipinski, C. A. (2004). Lead-and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today: Technologies*, *1*(4), 337-341.
41. Liu, X., Zhou, J., Zhang, T., Chen, K., Xu, M., Wu, L., Liu, J., Huang, Y., Nie, B., Shen, X., Ren, P., & Huang, X. (2021). Meranzin hydrate elicits antidepressant effects and restores reward circuitry. *Behavioural Brain Research*, 398, 112898. https://doi.org/10.1016/j.bbr.2020.112898
42. MacLeod-Carey, D., Solis-Céspedes, E., Lamazares, E., & Mena-Ulecia, K. (2020). Evaluation of new antihypertensive drugs designed in silico using Thermolysin as a target. *Saudi Pharmaceutical Journal*, *28*(5), 582-592.
43. Majumder, S., Ghosh, A., Chakraborty, S., Saha, S., & Bhattacharya, M. (2021). Metabolomics affirms traditional alcoholic beverage raksi as a remedy for high-altitude sickness. *Journal of Ethnic Foods*, *8*(1), 1-10.
44. Majumder, S., Chakraborty, S., Ghosh, A., & Bhattacharya, M. (2022). The Himalayan ethnic beverage tongba with therapeutic properties in high-altitude illnesses and metabolomic similarities to Japanese sake. *Acta Universitatis Sapientiae, Alimentaria*, *15*(1), 67-83.
45. Majumder, S., Ghosh, A., & Bhattacharya, M. (2023). In vitro bioactivities and simulated gastrointestinal digestion studies on Guras-based traditional fermented drinks collected from Singalila ridge of the Himalayas. *Canrea Journal: Food Technology, Nutritions, and Culinary Journal, 6*(2), 182–198.
46. Majumder, S., Ghosh, A., Saha, S., Acharyya, S., Chakraborty, S., Subba, P., Nandi, S., Sarkar, S., & Bhattacharya, M. (2024). In vitro bioactivities and gastrointestinal simulation validate ethnomedicinal efficacy of five fermented kodo-based Himalayan traditional drinks and bioaccessibility of bioactive components. *Food Production, Process and Nutrition* (in press)
47. Morris, G. M., Goodsell, D. S., Halliday, R. S., Huey, R., Hart, W. E., Belew, R. K., & Olson, A. J. (1998). Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *Journal of Computational Chemistry*, *19*(14), 1639-1662.
48. Mortelmans, K., & Zeiger, E. (2000). The Ames Salmonella/microsome mutagenicity assay. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *455*(1-2), 29-60.
49. Natesh, R., Schwager, S. L., Sturrock, E. D., & Acharya, K. R. (2003). Crystal structure of the human angiotensin-converting enzyme–lisinopril complex. *Nature*, *421*(6922), 551-554.
50. Pajouhesh, H., and George R. Lenz. (2005). Medicinal chemical properties of successful central nervous system drugs. *NeuroRx, 2,* 541-553.
51. Paramashivam, S. K., Elayaperumal, K., bhagavan Natarajan, B., devi Ramamoorthy, M., Balasubramanian, S., & Dhiraviam, K. N. (2015). In silico pharmacokinetic and molecular docking studies of small molecules derived from Indigofera aspalathoides Vahl targeting receptor tyrosine kinases. *Bioinformation*, *11*(2), 73.
52. Patel, D.K. (2022). Biological potential and therapeutic effectiveness of meranzin hydrate on intestinal motility. *International Journal of Surgery*, *100*, p.106289.
53. Polosa, R., & Blackburn, M. R. (2009). Adenosine receptors as targets for therapeutic intervention in asthma and chronic obstructive pulmonary disease. *Trends in Pharmacological Sciences*, *30*(10), 528-535.
54. Potts, R. O., & Guy, R. H. (1992). Predicting skin permeability. *Pharmaceutical Research*, *9*, 663-669.
55. Priest, B.T., & McDermott, J.S. (2015). Cardiac ion channels. *Channels*, *9*(6), 352-359.
56. Priya, R., Sumitha, R., Doss, C. G. P., Rajasekaran, C., Babu, S., Seenivasan, R., & Siva, R. (2015). Molecular docking and molecular dynamics to identify a novel human immunodeficiency virus inhibitor from alkaloids of Toddalia asiatica. *Pharmacognosy Magazine*, *11*(Suppl 3), S414.
57. Riaz, M. B., Khan, A. U., & Qazi, N. G. (2019). Pharmacological and computational evaluation of fig for therapeutic potential in hyperactive gastrointestinal disorders. *BMC Complementary and Alternative Medicine*, *19*, 1-22.
58. Riley, B. E., Lougheed, J. C., Callaway, K., Velasquez, M., Brecht, E., Nguyen, L., Shaler, T., Walker, D., Yang, Y., Regnstrom, K., & Diep, L. (2013). Structure and function of Parkin E3 ubiquitin ligase reveals aspects of RING and HECT ligases. *Nature Communications*, *4*(1), p.1982.

59. Rozwarski, D. A., Vilcheze, C., Sugantino, M., Bittman, R., & Sacchettini, J. C. (1999). Crystal structure of the Mycobacterium tuberculosis enoyl-ACP reductase, InhA, in complex with NAD+ and a C16 fatty acyl substrate. *Journal of Biological Chemistry*, *274*(22), 15582-15589.
60. Savioli, G., Ceresa, I. F., Gori, G., Fumoso, F., Gri, N., Floris, V., Varesi, A., Martuscelli, E., Marchisio, S., Longhitano, Y., & Ricevuti, G. (2022). Pathophysiology and therapy of high-altitude sickness: Practical approach in emergency and critical care. *Journal of Clinical Medicine*, *11*(14), p.3937.
61. Saxena, P., Hortigon-Vinagre, M. P., Beyl, S., Baburin, I., Andranovits, S., Iqbal, S. M., Costa, A., IJzerman, A. P., Kügler, P., Timin, E., & Smith, G. L. (2017). Correlation between human ether-a-go-go-related gene channel inhibition and action potential prolongation. *British Journal of Pharmacology, 174*(18), 3081-3093.
62. Scammell, T. E., Gerashchenko, D. Y., Mochizuki, T., McCarthy, M. T., Estabrooke, I. V., Sears, C. A., Saper, C. B., Urade, Y., & Hayaishi, O. (2001). An adenosine A2a agonist increases sleep and induces Fos in ventrolateral preoptic neurons. *Neuroscience*, *107*(4), 653-663.
63. Schyman, P., Liu, R., Desai, V., & Wallqvist, A. (2017). vNN web server for ADMET predictions. *Frontiers in Pharmacology*, *8*, p.889.
64. Sekiguchi, H., Takabayashi, F., Irie, K., & Murakami, A. (2012). Auraptene attenuates gastritis via reduction of Helicobacter pylori colonization and pro-inflammatory mediator production in C57BL/6 mice. *Journal of Medicinal Food*, *15*(7), 658-663.
65. Semwal, P., Painuli, S., Badoni, H., & Bacheti, R. K. (2018). Screening of phytoconstituents and antibacterial activity of leaves and bark of Quercus leucotrichophora A. Camus from Uttarakhand Himalaya. *Clinical Phytoscience*, *4*, 1-6.
66. Sharma, P. K., Suri, T. M., Venigalla, P. M., Garg, S. K., Mohammad, G., Das, P., Sood, S., Saraya, A., & Ahuja, V. (2014). Atrophic gastritis with high prevalence of Helicobacter pylori is a predominant feature in patients with dyspepsia in a high altitude area. *Tropical gastroenterology : official journal of the Digestive Diseases Foundation, 35*(4), 246–251. https://doi.org/10.7869/tg.224
67. Sharma, R., Cramer, N. P., Perry, B., Adahman, Z., Murphy, E. K., Xu, X., Dardzinski, B. J., Galdzicki, Z., Perl, D. P., Dickstein, D. L., & Iacono, D. (2019). Chronic exposure to high altitude: synaptic, astroglial and memory changes. *Scientific Reports*, *9*(1), p.16406.
68. Singh, H. B., Prasad, P., & Rai, L.K. (2002). Folk medicinal plants in the Sikkim Himalayas of India. *Asian Folklore Studies*, *61*, 295–310.
69. Singh, L. C. G. (2017). High altitude dermatology. *Indian Journal of Dermatology*, *62*(1), 59.
70. Smith, C. J., Zhang, Y., Koboldt, C. M., Muhammad, J., Zweifel, B. S., Shaffer, A., Talley, J. J., Masferrer, J. L., Seibert, K., & Isakson, P. C. (1998). Pharmacological analysis of cyclooxygenase-1 in inflammation. *Proceedings of the National Academy of Sciences*, *95*(22), 13313-13318.
71. Te Riet, L., van Esch, J. H., Roks, A. J., van den Meiracker, A. H., & Danser, A. J. (2015). Hypertension: renin–angiotensin–aldosterone system alterations. *Circulation research*, *116*(6), 960-975.
72. Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31*(2), 455-461.
73. Trzaskowski, B., Latek, D., Yuan, S., Ghoshdastider, U., Debinski, A., & Filipek, S. (2012). Action of molecular switches in GPCRs-theoretical and experimental studies. *Current Medicinal Chemistry*, *19*(8), 1090-1109.
74. Willumsen, N. J., Bech, M., Olesen, S. P., Jensen, B. S., Korsgaard, M. P., & Christophersen, P. (2003). High throughput electrophysiology: new perspectives for ion channel drug discovery. *Receptors and Channels*, *9*(1), 3-12.
75. Yeerjiang, A., Maimaitiyiming, D., Yang, T., Fang, L., Aihemaitituoheti, A., Aimaiti, M., Zhang, X., & Nijiati, Y. (2023). Pomegranate Peel in the Amelioration of High-Altitude Disease: A Network Pharmacology and Molecular Docking Study of Underlying Mechanisms. *Journal of Food Biochemistry*, *2023*, 7186747.
76. Yi, H., Wang, K., Gan, X., Li, L., Zhang, Q., Xiang, J., Yuan, X., Zhang, Y., & Wang, Y. (2020). Prophylaxis of ibuprofen in acute mountain sickness: A protocol for systematic review and meta-analysis. *Medicine*, *99*(46).
77. Zhao, K., Xing, R., & Yan, X. (2021). Cyclic dipeptides: Biological activities and self-assembled materials. *Peptide Science*, *113*(2), e24202.

Supplementary Table S1**.** List of important amino acid residues of selected proteins involved in hydrogen- bonds and hydrophobic (*) interactions with ligands. [Ethyl alpha-D-glucopyranoside (α-EG); meranzin (MRN); auraptene (ARN); cis-vaccenic acid (CVA); cyclo(pro-gly) (CPG); cyclo(leu-pro) (CLP); and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)]

PDB-ID	α-EG	MRN	ARN	CVA	CGP	CLP	DDMP
3KJ6	Gly101(H)	Glu148(H)*	Thr26(L)	Ala168(H)	Arg63(H)	Arg98(H)	Glu237(A)
	Thr46(L)	Pro149(H)*	Pro44(L)*	Val150(H)	Met4(L)	Phe240(A)*	Tyr33(H)
	Trp103(H)	Gly106(H)*	Trp103(H)*	Tyr145(H)	Phe98(L)	Val242(A)*	Leu266(A)*
	Pro44(L)	Phe95(H)*	Ser43(L)*	Leu177(H)*	Lys3(L)*	Val55(L)*	Tyr52(H)*
	Ser43(L)	Val93(H)*	Lys45(L)*	Glu148(H)*	Asp1(L)*	Tyr102(H)*	Cys265(A)*
	Tyr102(H)*	Gln105(H)*	Tyr102(H)*	Val93(H)*	Ile2(L)*	Gly101(H)*	
	Lys45(L)*	Thr108(H)*	Val55(L)*	Thr108(H)*	Thr97(L)*	Asp56(L)*	
			Gly101(H)*	Gly106(H)*			
			Arg239(A)*	Gln105(H)*			
			Arg98(H)*	Phe95(H)*			
			Asp56(L)*	Pro149(H)*			
			Phe240(A)*				
2Z73	Tyr13(B)	Thr10(A)	Thr10(A)*	Tyr177(B)	Gln28(A)	Phe26(B)*	Thr178(B)
	Asn14(B)	Glu9(A)	Glu25(B)*	Ala176(B)*	Gln28(B)	Glu290(B)*	Val18(B)
	Ile17(B)	Glu25(B)*	Trp12(A)*	Phe105(B)*	Asp27(B)*	Glu25(B)*	Ser192(B)
	Val19(B)	Phe26(B)*	Arg24(A)*	Thr178(B)*	Trp11(A)*	Arg24(A)*	Asp189(B)
	Arg24(B)	Glu290(B)*	Val18(A)*	Val18(B)*	Asp27(A)*	Trp12(A)*	His20(B)*
	Trp12(B)*	Tyr13(A)*	Ile17(A)*	Asp189(B)*	Trp11(B)*	Thr10(A)*	Ala176(B)*
	Glu290(A)*		Asn14(A)*	Ser192(B)*		Tyr13(A)*	
	Val18(B)*		Pro15(A)*	Pro170(B)*		Glu9(A)*	
	Pro15(B)*		Tyr13(A)*	Lys109(B)*			
			Glu9(A)*				
4H6J	Val376(B)	Arg440(B)*	Ile324(A)*	Asp238(A)	Arg362(B)	His378(B)	Val376(B)
	Asp256(A)	Thr462(B)*	Thr460(B)*	Ser451(B)	Arg440(B)	Asp377(B)*	His378(B)
	Glu257(A)	Gln333(A)*	Thr462(B)*	Asn448(B)*	Thr462(B)	Pro388(B)*	Tyr254(A)
	Tyr254(A)	Val464(B)*	Arg362(B)*	Arg258(A)*	Asn326(A)*	Val376(B)*	Glu257(A)
	His378(B)	Thr327(A)*	Arg440(B)*	Arg366(B)*	Val464(B)*	Glu257(A)*	Asp377(B)*
	Pro388(B)*	Asn326(A)*	Gln333(A)*	Thr374(B)*	Tyr325(A)*	Asp256(A)*	Pro388(B)*
	Leu392(B)*	Pro360(B)*	Pro360(B)*	Tyr456(B)*	Ile324(A)*	Phe375(B)*	Asp256(A)*
	Phe375(B)*		Cys358(B)*	Ile454(B)*	Gln333(A)*	Tyr245(A)*	Phe375(B)*
	Arg258(A)*		Asn463(B)*	Glu453(B)*	Pro360(B)*		
	Asp377(B)*		Lys465(B)*				
			Val464(B)*				
			Tyr325(A)*				
			Thr327(A)*				
			Asn326(A)*				

4ZPR	Glu318(B)	Leu72(B)*	Leu72(B)*	Leu73(B)	Leu72(B)*	Val69(B)	Glu225(B)
	Phe263(A)	Val69(B)*	Glu163(A)*	Glu163(A)*	Val102(B)*	Leu164(A)*	Arg342(A)
	Cys308(A)	Val102(B)*	Leu167(A)*	Val102(B)*	Val69(B)*	Leu73(B)*	Cys308(A)
	Ser262(A)	Tyr111(B)*	Tyr111(B)*	Tyr111(B)*	Tyr111(B)*	Tyr111(B)*	Phe263(A)
	Glu225(B)	Leu167(A)*	Asp114(B)*	Val69(B)*	Leu167(A)*	Glu163(A)*	Ser262(A)
	Arg342(A)	Glu163(A)*	Tyr66(B)*	Leu167(A)*	Leu73(B)*	Leu167(A)*	Glu225(B)
	Gln320(B)	Leu73(B)*	Thr160(A)*	Leu72(B)*		Glu170(A)*	Tyr340(B)*
	Tyr340(B)*	His199(B)*	Leu73(B)*	His199(B)*		Leu72(B)*	Thr309(A)*
	Ile264(A)*	Glu170(A)*	Val69(B)*	His166(A)*			His307(A)*
	Thr309(A)*		Val102(B)*	Glu170(A)*			Gln320(B)*
	His193(B)*						Glu318(B)*
	His307(A)*						
4ZPK	Glu227(B)	Arg260(A)	His307(A)	Gln322(B)	Asp173(A)	His307(A)	Ala171(A)
	Arg342(A)	Lys117(B)*	Phe118(B)*	Pro228(B)	Leu343(A)	Glu227(B)	Arg342(A)
	Phe263(A)	Phe118(B)*	Asp236(B)*	Gln230(B)*	Arg342(A)*	Phe267(A)*	Gly197(B)*
	His307(A)	Asn114(B)*	Glu96(B)*	Ile237(B)*	Thr196(B)*	Glu320(B)*	Val345(A)*
	Glu320(B)	Lys93(B)*	Gln322(B)*	Asp236(B)*	Asp167(B)*	Tyr342(B)*	Gln344(A)*
	Ile273(B)*	Tyr311(A)*	Tyr342(B)*	Lys117(B)*	Gln344(A)*	Ser262(A)*	Thr196(B)*
	Tyr342(B)*		Phe263(A)*	Lys191(B)*	Arg166(B)*	Arg342(A)*	Ala172(A)*
	Gln322(B)*		Ser262(A)*	Val176(B)*	Gly197(B)*	Pro228(B)*	Asp167(B)*
	Ser262(A)*		Ile237(B)*	Phe118(B)*	Val345(A)*	Gln322(B)*	Leu343(A)*
	Ile264(A)*		Pro228(B)*	Glu96(B)*	Ala172(A)*	Gln230(B)*	
			Lys117(B)*		Glu165(B)*		
			Lys191(B)*				
1BVR	Glu178(C)	Asn231(F)	Arg49(A)*	Arg49(A)	Phe108(D)	Leu268(D)	Arg173(C)
	Lys181(C)	Asp223(F)*	Asp52(A)*	Arg45(A)	Glu178(C)*	Arg225(F)*	Ser166(D)
	Asn159(D)	Ile82(A)*	Pro107(D)*	Leu71(C)*	Arg177(C)*	Leu268(F)*	Glu169(D)
	Gly104(D)	Gly229(F)*	Glu178(C)*	Ala133(C)*	Pro156(D)*	Leu269(F)*	Ser166(C)
	Asn106(D)	Ile228(F)*	Lys132(C)*	Asp110(D)*	Asn159(D)*	Gly221(D)*	Arg173(D)
	Ile105(D)	Ala252(C)*	Phe109(D)*	Pro136(C)*	Ala157(D)*	Arg225(D)*	Glu169(C)*
	Phe108(D)*	Pro251(C)*	Leu135(D)*	Tyr182(C)*	Ile105(D)*	Gly221(F)*	Ser152(C)*
	Phe174(C)*	Trp230(F)*	Pro136(C)*	Asp52(A)*	Asn106(D)*	Leu269(D)*	
	Arg177(C)*	Pro237(F)*	Tyr182(C)*	Pro107(D)*	Pro107(D)*		
	Ala157(D)*		Gln48(A)*	Phe109(D)*			
	Pro107(D)*		Arg45(A)*	Lys132(C)*			

5OIF	Ala264(A)	Arg225(B)	Arg225(B)	Gln267(A)	Glu169(A)	Ala154(B)	Arg225(B)
	Arg153(A)	Trp222(B)*	His265(B)*	Arg225(A)	Arg173(A)	Asp150(B)*	Asp150(B)
	Asp150(A)	Asp150(B)*	Asp150(B)*	Ala264(A)*	Ser166(B)*	Arg153(B)*	Met155(B)*
	Arg225(A)	Arg153(B)*	Trp222(B)*	Arg153(A)*	Glu169(B)*	Trp222(B)*	Arg153(B)*
	Trp222(A)*	Met155(B)*	Arg153(B)*	Leu218(A)*	Pro151(A)*	Leu218(B)*	Trp222(B)*
	His265(A)*	Ala154(B)*	Met155(B)*	Trp222(A)*	Arg173(B)*	Arg225(B)*	
	Met155(A)*	Leu218(B)*	Arg173(A)*	Met155(A)*	Ser152(A)*	Met155(B)*	
		His265(B)*	Ala154(B)*	Asp150(A)*	Ser166(A)*		
				His265(A)*			
6XJA	Lys1066(P)	Ser1068(P)	Lys995(P)	Pro224(H)	Pro224(H)	Tyr1450(P)	Val996(P)
	His992(P)	Asn1030(P)	Lys1066(P)*	Thr225(H)	Ala1581(P)*	Tyr1587(P)	Asn1030(P)
	Asn1028(P)	Gly1029(P)*	Ser1068(P)*	Gly1578(P)*	Gly1583(P)*	Asn1570(P)	His992(P)
	Gly994(P)	Val1996(P)*	Asn1030(P)*	His1577(P)*	Pro1627(P)*	Asp1584(P)*	Lys995(P)
	Val996(P)	Asn1028(P)*	His992(P)*	Thr1582(P)*	Arg1621(P)*	Lys1449(P)*	Asn1028(P)*
	Asn1030(P)	Gly994(P)*	Gln195(H)*	Pro226(H)*	Thr1582(P)*	Thr1582(P)*	Lys1066(P)*
	Lys995(P)	Lys995(P)*	Phe993(P)*	Thr228(H)*	His1608(P)*	Tyr1580(P)*	Gly994(P)*
	Leu197(H)*	Leu197(H)*	Lys200(H)*	Val1573(P)*		Lys1571(P)*	Gly1029(P)*
	Gln195(H)*	Gln195(H)*	Glu162(H)*	Val223(H)*		Val1572(P)*	Gly1034(P)*
	Lys200(H)*	Thr194(H)*	Leu197(H)*	Tyr1580(P)*			
	Glu162(H)*	Gly1034(P)*	Thr194(H)*	Met1589(P)*			
	Gly1034(P)*	His1036(P)*		Asn1575(P)*			
	Gly1029(P)*						
6XJB	Tyr1587(A)	Tyr1450(A)	Tyr1580(A)	Asn1028(A)	Tyr1450(A)	Tyr1450(A)	Asn1055(A)
	Tyr1450(A)	Thr1582(A)	Tyr1587(A)	Asn1055(A)	Tyr1587(A)	Lys1571(A)	Val990(A)
	Asn1570(A)	Asn988(A)*	Asn1055(A)*	Asn1570(A)*	Thr1582(A)	Asn1028(A)	Asn1028(A)
	Asn1055(A)	Asn989(A)*	Thr1582(A)*	Asp1584(A)*	Asn1055(A)	Asn1055(A)	Val1053(A)*
	Val990(A)	Asn1570(A)*	Lys1449(A)*	Thr1582(A)*	Val1990(A)*	Asn988(A)*	Thr1582(A)*
	Asn989(A)	Val990(A)*	Ser955(A)*	Asn988(A)*	Asn1570(A)*	Asn1570(A)*	Thr1027(A)*
	Asn1028(A)	Lys1571(A)*	Thr1027(A)*	Lys1449(A)*	Asn989(A)*	Thr1582(A)*	Tyr1587(A)*
	Lys1449(A)*	Asn1055(A)*	Asn988(A)*	Tyr1587(A)*	Lys1571(A)*	Thr1027(A)*	Lys1571(A)*
	Thr1582(A)*	Tyr1587(A)*	Val990(A)*	Asn989(A)*		Asn989(A)*	
	Asn988(A)*	Tyr1580(A)*	Lys1571(A)*	Tyr1450(A)*		Val990(A)*	
	Asp1584(A)*	Asp1584(A)*	Tyr1450(A)*	Thr1027(A)*			
	Thr1027(A)*	Lys1449(A)*		Tyr1580(A)*			
	Val1572(A)*			Val990(A)*			
				Lys1571(A)*			

1ALU	Glu106(A) Arg104(A) Asp160(A) Ser107(A)* Glu42(A)* Lys46(A)* Thr43(A)* Phe105(A)*	Ser107(A) Glu106(A) Phe105(A)* Thr43(A)* Lys46(A)* Arg104(A)* Asp160(A)* Thr163(A)* Gln156(A)*	Lys46(A)* Thr43(A)* Phe105(A)* Glu42(A)* Glu106(A)* Arg104(A)* Gln156(A)* Asp160(A)*	Asp160(A) Gln156(A) Arg104(A) Thr43(A)* Lys46(A)* Phe105(A)* Glu42(A)* Glu106(A)*	Arg104(A)* Lys46(A)* Asp160(A)* Thr43(A)* Phe105(A)*	Glu106(A) Ser107(A) Arg104(A) Thr43(A)* Thr163(A)* Asp160(A)* Phe105(A)*	Asp160(A) Arg104(A) Glu106(A) Lys46(A)* Glu42(A)* Ser107(A)*
3LN1	Asn19(D) Ser34(D) Trp309(C) Gln313(C) Gly121(D)* Tyr122(D)* Cys32(D)* Met33(D)*	Trp309(C) Gln313(C) Ser34(D) Gly121(D)* Ala142(D)* Asn19(D)* Glu308(C)* Pro20(D)* Met33(D)* Cys32(D)*	Gln529(C)* Arg29(D)* Tyr(108D)* Pro528(C)* Gln356(D)* Tyr359(C)* Gln358(D)* Ser112(D)* Arg46(D)*	Gln355(C) Leu352(C) Phe353(C) Tyr359(C)* Gln358(D)* Gln356(D)* Phe357(C)* Pro528(C)* Ser112(D)* Ile110(D)* Gln529(C)* Tyr108(D)*	Trp309(C) Gln313(C) Cys32(D) Cys21(D)* Gly121(D)* Tyr122(D)* Asn19(D)* Ser34(D)* Met33(D)*	Gln529(C) Ser112(D) Ile110(D)* Tyr108(D)* Ser107(D)* Pro528(C)* Gln358(D)* Gln356(D)* Phe353(C)* Tyr359(C)*	Ser34(D) Trp309(C) Cys32(D)* Gly121(D)* Asn19(D)* Gln313(C)* Met33(D)*
5IKV	Glu140(A) Ser143(A) Asp229(B) Trp139(A) Arg333(B)* Gly225(B)* Glu236(B)* Nag1(C)*	His122(A) Phe371(A) Ser121(A)* Thr118(A)* Pro542(B)* Lys369(B)* Gln370(A)* Phe371(B)* Gln372(A)*	Gln241(A) Gly536(A)* Gln374(A)* Gly225(A)* Leu224(A)* Leu145(B)* Nag1(D)* Glu140(B)* Leu238(A)* Arg333(A)* Thr237(A)* Trp139(B)* Glu236(A)* Gly235(A)* Ser143(B)* Phe142(B)*	Arg376(B) Asn375(B) Pro128(B)* Gln374(B)* Gln374(A)* Phe124(A)* Gly225(B)* Leu224(B)* Leu145(A)* Leu145(B)* Gly225(A)*	Trp139(B) Arg333(A)* Ser143(B)* Nag1(D)* Glu236(A)* Asn144(B)* Gln241(A)* Glu140(B)* Leu238(A)*	Ser143(A) Arg333(B)* Trp139(A)* Glu236(A)* Asn144(A)* Nag1(C)* Glu140(A)* Leu224(B)*	Gly235(A) Gln241(A) Nag1(D) Thr237(A)* Arg333(A)* Glu236(A)* Ser143(B)* Asn144(B)* Glu140(B)* Leu238(A)*

4COX	Trp139(C)	Phe371(A)	Phe371(A)	Gln369(A)	Gln372(B)	Gln372(B)	Phe371(A)
	Asp229(D)	Phe371(B)	Lys532(B)	Gln370(B)	Ser126(B)	Ser126(B)	Tyr373(A)*
	Lys333(D)	Tyr122(B)*	Phe371(B)*	Leu366(A)	Tyr122(B)*	Tyr122(B)*	Phe367(A)*
	Gly235(D)	Lys532(B)*	Gln370(A)*	Gln543(A)*	Lys532(B)*	Gln543(A)*	Gln370(A)*
	Gly225(D)*	Ser121(B)*	Gln372(B)*	Ile124(B)*	Ile124(B)*	Phe371(B)*	Gln370(B)*
	Glu140(C)*	Gln372(B)*	Ser126(B)*	Tyr122(B)*	Ser121(B)*	Gln370(B)*	Phe371(B)*
	Asn144(C)*	Ile124(B)*	Ser121(B)*	Pro542(A)*	Gln370(B)*	Pro542(A)*	Gln372(B)*
	Nag671(C)*	Gln543(A)*	Tyr122(B)*	Phe371(B)*	Phe371(B)*	Tyr373(A)*	Pro542(A)*
	Ser143(C)*	Ser126(B)*	Pro542(A)*	Ser126(B)*		Gln369(A)*	Leu366(A)*
	Leu238(D)*	Pro542(A)*	Gln543(A)*	Gln372(B)*		Phe367(A)*	Gln369(A)*
	Thr237(D)*	Gln370(A)*	Tyr373(A)*	Tyr373(A)*		Leu366(A)*	
	Glu236(D)*	Gln370(B)*	Gln370(B)*			Phe371(A)*	
		Gln369(A)*					
6Y3C	Met522(A)	Thr206(A)	Phe210(A)*	Ser530(A)	Thr206(A)	Thr206(A)	His388(A)
	Ser530(A)	His207(A)	Tyr385(A)*	Gly526(A)*	His307(A)*	Gln207(A)	His386(A)
	Tyr385(A)	Tyr385(A)*	Ala202(A)*	Trp387(A)*	Gln203(A)*	Phe210(A)*	Thr206(A)
	Val349(A)*	His386(A)*	Thr206(A)*	Ala527(A)*	Met391(A)*	His386(A)*	Trp387(A)
	Ser353(A)*	Val447(A)*	Gln203(A)*	Phe518(A)*	His388(A)*	Leu390(A)*	Phe210(A)*
	Leu352(A)*	His388(A)*	Ala199(A)*	Ile523(A)*	Tyr385(A)*	His207(A)*	Ala202(A)*
	Tyr348(A)*	Met391(A)*	Met391(A)*	Ser353(A)*	Trp387(A)*	His388(A)*	Gln203(A)*
	Trp387(A)*	Gln203(A)*	His388(A)*	Met522(A)*	Ala202(A)*	Ala202(A)*	Tyr385(A)*
	Gly526(A)*	Trp387(A)*	His207(A)*	Leu352(A)*		Trp387(A)*	
	Phe518(A)*	Phe210(A)*	Asn382(A)*	Arg120(A)*		Tyr385(A)*	
	Ile523(A)*		His386(A)*	Leu93(A)*		Ala199(A)*	
	Ala527(A)*			Flc710(A)*			
				Leu357(A)*			
				Val116(A)*			
				Tyr355(A)*			
				Leu359(A)*			
				Val349(A)*			

3N8Y	Glu140(A)	Gln461(A)*	Arg376(A)	Gln44(B)	Trp139(A)	Cys41(B)*	Nag1(D)
	Ser143(A)	Glu465(A)*	Arg374(A)	Gly45(B)	Arg332(B)	Gly45(B)*	Glu140(A)
	Gly235(B)	Gly45(A)*	Asn375(A)*	Glu465(B)*	Asp229(B)*	Ser154(B)*	Gln241(B)
	Arg333(B)	Leu152(A)*	Arg374(B)*	Ile46(B)*	Leu238(B)*	Pro156(B)*	Gly235(B)
	Trp139(A)	Gln44(A)*	Asn375(B)*	Pro153(B)*	Asp236(B)*	Tyr39(B)*	Arg333(B)
	Asp229(B)	Ile46(A)*	Phe142(A)*	Tyr130(B)*	Asn237(B)*	Cys36(B)*	Asp236(B)*
	Leu238(B)*	Tyr130(A)*	Gly225(B)*	Asp135(B)*	Ser143(A)*	Gln461(B)*	Asp229(B)*
	Asn144(A)*	Ile151(A)*	Leu224(B)*	Pro156(B)*	Gly225(B)*	Cys47(B)*	Ser143(A)*
	Asn237(B)*	Cys47(A)*	Ser143(A)*	Cys36(B)*	Leu224(B)*	Pro153(B)*	Asn237(B)*
	Asp236(B)*	Pro153(A)*	Val145(B)*	Cys47(B)*		Leu152(B)*	Leu238(B)*
	Nag1(D)*	Arg469(A)*	Phe142(B)*	Ser154(B)*			
	Leu224(B)*		Val145(A)*	Gln461(B)*			
	Gly225(B)*			Tyr39(B)*			
				Leu152(B)*			
				Cys41(B)*			
				Gln42(B)*			
				His43(B)*			
1O8A	Lys454(A)	Arg522(A)	Ala354(A)	Tyr62(A)	Trp220(A)	Glu411(A)	Trp220(A)
	Asp415(A)	Gly404(A)*	Nxa702(A)*	Val518(A)*	Tyr135(A)	Arg522(A)*	Ser219(A)
	His383(A)	His410(A)*	Asp453(A)*	Phe512(A)*	Glu123(A)*	Pro407(A)*	Ile204(A)*
	Nxa702(A)	Pro407(A)*	Val379(A)*	Glu143(A)*	Ile204(A)*	Glu403(A)*	Tyr135(A)*
	Val379(A)*	Phe391(A)*	His383(A)*	Arg124(A)*	Arg124(A)*	His410(A)*	Arg124(A)*
	Phe527(A)*	Ala356(A)*	Phe527(A)*	Ser516(A)*	Ala207(A)*	Phe391(A)*	Glu123(A)*
	Asp4553(A)*	Act700(A)*	Asp415(A)*	Asn136(A)*	Ser219(A)*	His387(A)*	Ala207(A)*
	Glu376(A)*	Val518(A)*	Lys454(A)*	Tyr69(A)*	Ala208(A)*	Act700(A)*	Ala208(A)*
	Val380(A)*	Glu411(A)*	Thr282(A)*	Leu81(A)*			
			Glu376(A)*	Leu82(A)*			
			Val380(A)*	Asn66(A)*			
			Glu162(A)*	Asn85(A)*			
			His353(A)*	Leu120(A)*			
				Leu139(A)*			
5DPF	Tyr193(E)	Phe178(E)*	Asp16(E)*	Ser65(E)	Arg260(E)	Arg260(E)	Asp200(E)
	Glu190(E)	Tyr179(E)*	Tyr76(E)*	Val13(E)*	Phe178(E)*	Phe178(E)*	Tyr193(E)
	Asp200(E)	Tyr83(E)*	Ala180(E)*	Pro132(E)*	Val87(E)*	Tyr83(E)*	Thr194(E)
	Ile197(E)	Arg260(E)*	Tyr179(E)*	Ile131(E)*	Tyr179(E)*	Tyr179(E)*	Ile197(E)
	Thr194(E)	Val187(E)*	Asp82(E)*	Ala68(E)*	Tyr83(E)*	Val87(E)*	Ser198(E)*
	ca405(E)	Leu175(E)*	Arg35(E)*	Asp124(E)*	Asp261(E)*	Asp261(E)*	Gly199(E)*
	Leu14(E)*	Asp261(E)*	Val179(E)*	Tyr46(E)*	Leu75(E)*	Leu175(E)*	Glu190(E)*
	Asp191(E)*		Tyr75(E)*	Pro69(E)*			
	Pro195(E)*		Ile137(E)*	His105(E)*			
	Gly199(E)*		Lys182(E)*				

1ESL	Glu88(A) Gln85(A) Lys55(A) Arg84(A) Asp89(A) Asp87(A) Asn57(A)* Lys86(A)*	Lys67(A) Gln30(A)* Thr123(A)* Cys133(A)* Cys122(A)* Trp1(A)* Glu135(A)* Thr65(A)*	Lys55(A) Gln85(A)* Arg84(A)* Lys86(A)* Arg54(A)* Val63(A)* Val61(A)* Val56(A)* Asp89(A)*	Cys133(A) Gly131(A) Thr123(A)* Ser126(A)* Glu132(A)* Thr65(A)* Gly64(A)* Tyr118(A)* Trp1(A)* Cys122(A)* Glu135(A)*	Val101(A) Asn75(A) Gly102(A)* Asn39(A)* Glu36(A)* Lys32(A)* Ile35(A)*	Lys32(A) Asn75(A) Glu36(A)* Val101(A)* Asn39(A)* Ile35(A)* Gly102(A)*	Thr70(A) Gln30(A)* Lys67(A)* Ile29(A)* Trp62(A)* Ala73(A)*
1G1T	Arg84(A) Lys55(A) Gln85(A) Asn58(A)* Asn57(A)* Lys86(A)* Asp89(A)*	Asn75(A) Val101(A)* Ile35(A)* Asn39(A)* Glu36(A)* Lys32(A)* Met103(A)*	His25(A) Ser16(A) Tyr12(A)* Val63(A)* Arg54(A)* Val56(A)* Val59(A)* Gln66(A)* Pro68(A)* Val61(A)*	Tyr12(A) Ser16(A) His25(A) Trp62(A)* Val63(A)* Gln66(A)* Val61(A)* Val56(A)* Arg54(A)*	Glu80(A) Pro78(A) Arg97(A) Ala77(A)* Asp100(A)* Fuc4(B)* Tyr94(A)*	Lys32(A) Asn75(A) Glu36(A)* Asn39(A)* Gly102(A)* Val101(A)* Ile35(A)*	Lys113(A) Lys111(A) Sia3(B) Pro46(A) Ser47(A)* Tyr48(A)* Gal2(B)* Mag1(B)*
3RZE	Asn472(A) Arg139(A) Arg125(A) Asn63(A) Thr60(A) Arg409(A)* Ala413(A)* Lys412(A)*	Tyr210(A) Gly418(A)* Phe419(A)* Lys415(A)* His220(A)* Val217(A)* Tyr214(A)* Ala414(A)*	Gly418(A)* Phe219(A)* Ala422(A)* Lys415(A)* Tyr210(A)* Ala414(A)* Val217(A)* Tyr214(A)* His220(A)*	Ala211(A)* Tyr210(A)* Gly418(A)* Met421(A)* Ala422(A)* Leu426(A)* Leu207(A)* Ile429(A)* Ile425(A)*	Tyr210(A) Lys415(A)* His220(A)* Arg411(A)* Val217(A)* Ala414(A)* Tyr214(A)*	Ser128(A) Arg139(A) Glu410(A)* Arg409(A)* Pro132(A)*	Arg125(A) Ser128(A) Arg139(A) Glu410(A)* Arg409(A)* Ala413(A)*

2GYU	Tyr503(B)	Pro388(B)*	Tyr503(A)	Glu313(A)	Tyr503(B)	Asn533(A)	Arg534(A)
	Gln508(B)	Thr505(A)*	Leu524(A)	Pro232(A)	Gln508(B)	Ala412(A)	Gln413(A)
	Leu524(B)	Arg417(A)*	Ala420(A)*	Thr238(A)	Arg534(B)	Cys529(A)*	Ala526(A)
	Ala526(B)	Gly416(A)*	Arg417(A)*	Asn233(A)*	Ala412(B)*	Ala530(A)*	Ala530(A)*
	Arg534(B)	Gln413(A)*	Arg534(A)*	Gln413(A)*	Gln413(B)*	Trp385(B)*	Cys529(A)*
	Thr505(B)*	Ala412(A)*	Gln413(A)*	Pro235(A)*	Asn533(B)*	Gln508(A)*	Asn533(A)*
	Arg525(B)*	Asn533(A)*	Ala412(A)*	Asn533(A)*	Trp385(A)*	Ala526(A)*	Ala412(A)*
	Cys529(B)*	Leu524(A)*	Asn533(A)*	Pro537(A)*	Ala530(B)*	Thr505(A)*	Leu524(A)*
	Ala530(B)*	Ala526(A)*	Ala526(A)*	His405(A)*		Gly416(A)*	Gln508(A)*
	Asn533(B)*	Gln508(A)*	Cys529(A)*	Leu536(A)*		Arg534(A)*	
	Ala412(B)*	Arg534(A)*	Gln508(A)*	Leu540(A)*		Gln413(A)*	
			Trp385(B)*	Trp532(A)*			
			Gly416(A)*	Gly234(A)*			
			Thr505(A)*	Pro312(A)*			
4TPK	Asn481(A)	Asn68(B)	Asn68(B)	Ile69(B)	Gln71(B)	Gln270(B)	Gln71(B)
	Gln(270B)	3F9611(B)*	Gln119(B)*	Ser489(A)	Ile69(B)*	Gln71(B)	Trp490(A)
	Asn68(B)	Pro285(B)*	3F9611(B)*	Gln71(B)	Leu274(B)*	Asn479(A)*	Asn481(A)*
	Ile69(B)	Gly283(B)*	Pro285(B)*	Gol614(B)	Gln270(B)*	Nag1(G)*	Asn479(A)*
	Gol614(B)	Gln119(B)*	Ala277(B)*	Pro285(B)*	Asn479(A)*	Leu88(B)*	Ser489(A)*
	Gln71(B)		Phe278(B)*	Tyr332(B)*	Trp490(A)*	Asn481(A)*	Leu274(B)*
	Asn479(A)		Pro491(A)*	3f9611(B)*	Ser489(A)*	Ser489(A)*	
	Nag1(G)		Val492(A)*	Gln119(B)*	Asn481(A)*	Leu274(B)*	
	Leu88(B)*		Leu273(B)*	Asn68(B)*		Gol614(B)*	
	Trp490(A)*		Leu274(B)*	Leu274(B)*		Ile69(B)*	
						Asn68(B)*	
3PWH	Glu228(A)	Ile237(A)*	Leu194(A)*	Phe93(A)	Arg102(A)	Asn39(A)	Asn42(A)
	Thr41(A)	Ala236(A)*	Met193(A)*	Cys128(A)*	Thr41(A)*	Val45(A)*	Ala231(A)
	Asn39(A)	Phe201(A)*	Tyr197(A)*	Ile100(A)*	Ala231(A)*	Asn42(A)*	Asn39(A)*
	Asn42(A)	Tyr197(A)*	Leu198(A)*	Asp101(A)*	Ile98(A)*	Leu37(A)*	Phe295(A)*
	Arg102(A)	Leu198(A)*	Arg205(A)*	Phe44(A)*	Ala235(A)*	Glu294(A)*	Ile292(A)*
	Ala231(A)*	Leu194(A)*	Phe201(A)*	Ile104(A)*	Ile238(A)*	Asn36(A)*	Arg102(A)*
	Ile292(A)*		Ala236(A)*	Thr117(A)*	Val45(A)*	Ala231(A)*	Ser234(A)*
			Gly240(A)*	Arg120(A)*	Phe295(A)*	Ile292(A)*	Ala235(A)*
			Ala239(A)*	Val116(A)*	Tyr288(A)*	Phe295(A)*	Thr41(A)*
			Leu190(A)*	Leu115(A)*	Asn42(A)*	Tyr288(A)*	
				Ile124(A)*	Ile292(A)*		
				Ala97(A)*			
				Leu96(A)*			

5NM2	Ala231(A)	Asn36(A)*	Asn42(A)	Glu294(A)*	Asn42(A)	Asn42(A)	Ala231(A)*
	Asn39(A)	Leu37(A)*	Asn36(A)*	Leu37(A)*	Asn39(A)*	Leu37(A)*	Ile292(A)*
	Asn42(A)*	Phe295(A)*	Arg102(A)*	Ile292(A)*	Ile292(A)*	Phe295(A)*	Ser234(A)*
	Ile292(A)*	Thr41(A)*	Thr41(A)*	Ala231(A)*	Thr41(A)*	Ile298(A)*	Tyr288(A)*
	Ser234(A)*	Asn39(A)*	Ala235(A)*	Arg102(A)*	Phe295(A)*	Arg102(A)*	Ile238(A)*
	Ala235(A)*	Arg102(A)*	Ser234(A)*	Ala235(A)*	Arg102(A)*	Thr41(A)*	Ala235(A)*
	Val45(A)*	Ala235(A)*	Ala231(A)*	His230(A)*		Ala231(A)*	Val45(A)*
	Tyr288(A)*	Ile292(A)*	Ile292(A)*	Lys227(A)*		Ile98(A)*	Thr41(A)*
	Thr41(A)*	Asn42(A)*	Glu294(A)*	Arg293(A)*		Ala235(A)*	Ile98(A)*
			Leu37(A)*				
			Asn39(A)*				
4I1H	Trp447(A)	Arg314(A)	Ala206(A)*	Ser233(A)	Lys427(A)	Lys427(A)	Tyr315(A)
	Lys416(A)	Glu321(A)*	Val186(A)*	Thr410(A)	Asp464(A)	Asp464(A)	Gln316(A)
	Asn448(A)	Arg275(A)*	Phe209(A)*	Thr414(A)*	Ser233(A)	Arg234(A)	Gly319(A)
	Asp464(A)	Leu331(A)*	Val224(A)*	Lys427(A)*	Lys412(A)*	Trp447(A)*	Tyr285(A)
	Ser233(A)	Cys332(A)*	Phe210(A)*	Trp447(A)*	Tyr143(A)*	Lys416(A)*	Leu283(A)
	Lys427(A)*	Pro333(A)*	Ala225(A)*	Met432(A)*	Trp447(A)*	Val465(A)*	Leu301(A)*
	Thr414(A)*	Gln317(A)*	Leu187(A)*	Tyr143(A)*	Met432(A)*	Asn448(A)*	Gly284(A)*
	Val465(A)*	Cys337(A)*	Phe208(A)*	Asn448(A)*		Ser233(A)*	
	Met432(A)*		Pro189(A)*	Lys412(A)*		Lys412(A)*	
			Asn190(A)*	Arg234(A)*		Met432(A)*	
			Glu207(A)*	Asp464(A)*		Tyr143(A)*	

Note: Amino acid residues involved in hydrophobic interaction have been denoted with [*]