Kurdish Studies
January 2024
Volume: 12, No:1, pp. 5095-5110
ISSN: 2051-4883 (Print) | ISSN 2051-4891 (Online)
www.KurdishStudies.net

DOI: 10.53555/ks.v12i1.3267

Computational Analysis to Investigate the Anti-inflammatory and Diuretic Potential of Natural Compounds to Treat High Altitude Pulmonary Oedema by Targeting Phosphodiesterase Type 5

Asma Ahmed[1], Anam Naz[2*], Aiza Ashfaq[3], Mubashar Hassan[4], Iqra Sharif[5], Afaq Akram[6], Noman Khalique[7], Rafia Mehmood[8]

[1,2*,3,5,6,8]Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore 54000, Punjab, Pakistan
[4]Department of Chemistry, Superior University, Lahore, Punjab, Pakistan
[7]RANI omico-informatics Feed and Pharmaceuticals, Lahore, Punjab Pakistan

***Corresponding Author:** Anam Naz
E-mail; anam.naz@imbb.uol.edu.pk

Abstract: High altitude pulmonary edema (HAPE) is a syndrome of non-specific symptoms including dizziness, lack of energy, cough, cyanosis and nausea. HAPE is caused by fluid accumulation in lungs. Treatment of HAPE includes diuretics and pulmonary vasodilator medications. *Bergenia stracheyi* (Hook.f. & Thomson) Engl. and *Anagallis arvensis* L. can be potential antioxidant and anti-inflammatory components. Thus, active components of these plants could be used as ligands against targeted proteins to treat pulmonary oedema. β-eudesmol from *B. strachyei* (Hook.f. & Thomson) Engl. was utilized as a ligand and phosphodiesterase-5 as the targeted protein for molecular docking. Targeted protein and selected ligand showed fulfilled ADMET properties as well as good binding energy with targeted protein. β-eudesmolshowed-5.6 binding affinity with targeted protein and appeared to have a good binding affinity. Molecular docking analysis used in this study for identification of novel compounds from therapeutic interest and ligand-target interactions at molecular level, utilized different online tools and softwares; (PubChem, ChemSketch, PyMOL, UniProt, Chimera, Depth residue, PyRx and Discovery Studio) in order to predict binding energies and appropriate drug targets. Thus, this complex is also suitable for MD simulation for novel drug discovery. MD simulation was performed by Desmond software, and it showed stability of complex. Thus, *B. strachyei* (Hook.f. & Thomson) Engl. showed great evidence of anti-inflammatory and diuretic activities and it can be further studied for *in vitro* trial experiments for their potential treatment of high-altitude pulmonary oedema as compared to *A. arvensis* L.

Keywords: *Anagallis arvensis* L.; Anti-inflammatory; *Bergenia stracheyi* (Hook.f. & Thomson) Engl.; High altitude pulmonary oedema; Hypoxic pulmonary vasoconstriction; Molecular docking

1. Introduction

The introduction Plants have a crucial role in our life as they have been utilized in medicinal and cosmetic applications since time immemorial. In fact, pharmaceutical and cosmetic industries production depend on plants. A single plant can produce different types of bioactive compounds such as glycosides, alkaloids, flavonoids, polyphenols, phytosterols, terpenoids, etc (Christian, B. 2021). Moreover, secondary metabolites or phytochemicals that are present in fruits, vegetables, grains, stem, leaves, roots and other plant parts give the basic nutrition and health benefits. These include decreasing the risk of chronic inflammatory diseases, increased activity of enzymes, DNA metabolism and repair, reducing cell proliferation, reduction in oxidative damage by acting as enzyme inhibitors, peroxide decomposers, singlet oxygen quenchers, etc.(Lobo *et al.*, 2010). Medicinal plants, for instance, have been used for centuries in traditional medicines (Hosseinzadehet *et al.*,2015). Ayurvedic (Mangal *et al.*,2017), herbal and homeopathic industries production completely depends on medicinal plant extraction which give life-saving medicines such as anti-inflammatory, anti-oxidant, diuretics, anti-cancer and cardiac drugs with less toxic side effects than those of synthetic medicines (Tesfaye *et al.* , 2020). In fact, a number of pharmaceuticals in today's clinical use that are approved by FDA, are originated from natural products derivatives and their analogues (De Smet, P.A.G.M., 1997). Pharmaceutical industries are in search of alternative, more natural and environmentally friendly pharmaceuticals and medicinal plants are a good source of attraction by chemists, bio-chemist, and pharmaceutics because of phenolics, nitrogen compounds, vitamins, terpenoids along with other secondary metabolites and these are with more antioxidant, anti-inflammatory, antitumor, antimutagenic, anti-carcinogenic, antibacterial, or anti-viral activities (Maridassand D. B, 2008; Mollazadeh *et al.* , 2019). Herbal medicines derived from plants are being promoted to treat clinical diseases. However, considerable attention has been paid to the protective effects of natural anti-inflammatory and anti-oxidants against many life-threating diseases such as pulmonary diseases (Licciardi and Underwood, 2011). HAPE is a non-cardiogenic disease of non-specific symptoms including dizziness, headache, lassitude and nausea, chest pain, pink sputum, wheezing and swelling in lower extremities. In HAPE fluid accumulation occurs in alveolar ducts due to hypoxia and activate inflammatory mediators causing a serious illness. HAPE occurs in two forms, the first occurs when un-acclimatized lowlanders travel to altitudes greater than 2500-3000 m, while the second form, called re-entry HAPE, occurs when landers return after a journey at a lower altitude (Paralikar S.J, 2012).

A significant number of human populations has occupied high altitude environment, including the mountainous geographic regions of the Andes, Pamir, Ethiopian highlands, Tibet, and Tian-Shan. Moreover, over the past few decades, the number of people moving to high altitudes has been continuously increasing due to economic purposes. High altitude is one of the most critical extreme environments, described by great challenges. HAPE affects about 0.2 -6% in healthy mountaineers at altitude above 2500 m and rarely occur below that height (Korzeniewsket *et al.*, 2015). There is an in-crease in acute altitude sickness and major medical problems among people who travel to high altitudes for both work and pleasure. It was found that patients with HAPE experience a wide range of clinical symptoms when traveling for distance of around 11,500 feet. (Singh *et al.*, 2021). One of the main aspects of high-altitude environments is alveolar hypoxia, which has a well-known effect on the cardio-pulmonary system, including the development of pulmonary hypertension. The later, in turn, contributes to HAPE due to an exaggerated hypoxic pulmonary vasoconstriction (Sydykov *et al.*, 2021) (Figure 1). Moreover, the chronic exposure to high altitude hypoxia causes development of pulmonary hypertension, resulting in an increased pressure load on the right ventricle, leading to right heart failure and premature death (Naeije *et al.*, 2013). Therefore, it is strongly believed that the absence of an effective medical treatment, HAPE is one of the major causes of non-traumatic death at high altitude.

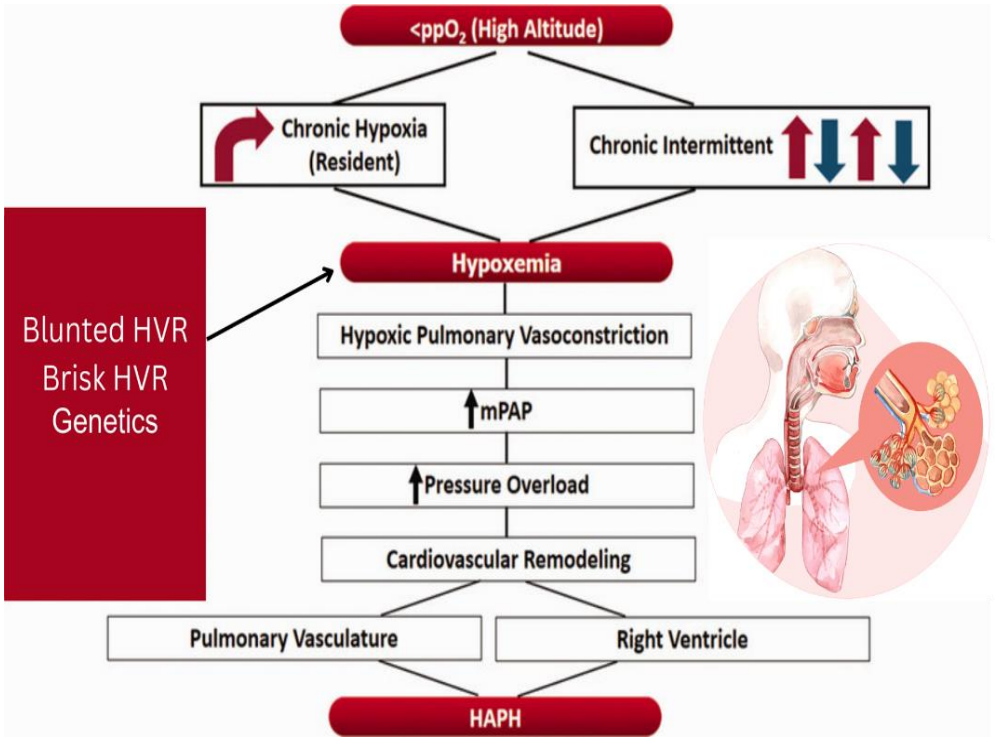


Figure 1. Flow chart of High-altitude pulmonary oedema show high altitude pulmonaryhypertension (HAPH) and capillary stress failure caused by hypoxia (Scherrer *et al.*, 1996).

Among many of natural plants with medicinal and bioactive properties, *A.arvensis* L. (poor man's medicine), possessing a variety of medicinal properties such as anti-inflammatory, anti-oxidant, anti-cytotoxic, anti-microbial and anti-viral activities. Various studies have also shown the presence of bioactive compounds such as glycosides, kaempferol, stigmasterol, alkaloids, anagalligen in, carbohydrates and arvenin-I and II in different parts of *A. arvensis* L. (Yasmeen *et al.*,2020). It was shown that the phytochemicals present in *A. arvensis* L. have a significant effect on the reduction of prostaglandin synthesis, hence reduces inflammation (Saleem*et al.*,2020). A. arvens is also act as bioactive antioxidant and enzyme inhibitor (Aldughaylibi*et al.*,2022). For in-stance, studies show the anti-inflammatory properties of *A. arvensis* L. which were measured using COX-1 and COX-2 assays resulting in inhibition as well as anti-oxidant potential and scavenging of superoxide free radicals (Saleem *et al.*, 2020). In-fact, free radical damage causes a variety of diseases, including pulmonary diseases, cardiac diseases, cancer, stroke, and rheumatoid arthritis. Another important natural plant is Bergenia (*B. strachyei* (Hook.f. & Thomson) Engl.), commonly known as zakhm-e-hayat, which possesses several biological activities including being diuretics, anti-inflammatory, anti-oxidant, anti-diabetic and anti-cancer activities, etc. (Bhadra*et al.*,2022). The Bergenia's plant plays an important role as an anti-oxidant by reducing free radical damage and also act as a diuretic to help kidneys release more sodium into urine and manage symptoms of oedema and hypertension (Aggarwal *et al.*, 2011). Therefore, Bergenia is used to treat pulmonary disorders due to the aforementioned properties (Usman *et al.*, 2012). Phytochemicals such as anagalligenin, anagalligenone, 1,8-Cineole, β-eudesmol and damascenone present in *A. arvensis* L. and *B. strachyei* (Hook.f. & Thomson) Engl. are the target anti-inflammatory ligands to be investigated against high altitude pulmonary edema in this study via studying the interactions of the target ligands with different proteins; phosphodiestrase-5, carbonic anhydrase-2, creatinine kinase, phosphotidyl inositol-3 kinase and phospholipase A2.

Carbonic anhydrase -2 inhibitors that act as diuretics, are FDA approved drugs for altitude sickness prophylaxis (Aslam & Gupta, 2022). The main function of these inhibitors is preventing the breakdown of carbonic acid resulting in lower blood pH (*i.e.*, more

acidic) (Jimenez *et al.*,2019). PDE-5 are present in various tissues including vascular and smooth muscle cells of vessels. The main function of PDE-5 is to break-down cGMP, which is a second messenger that induces relaxation of smooth muscle cells as well as regulates fluid and salt transport within the nephrons. cGMP mediates its function via different cellular targets: cGKs, cGMP-gated cation channels, and phosphodiesterases (PDEs) (Mergia and Stegbauer, 2016). It shows up-regulation (Significantly increased messenger RNA and protein level) of PDE-5 inhibitors which ultimately reduced hypoxia (Zhao *et al.*, 2022). Inhibition of PDE-5 enzyme prevents the breakdown of cGMP causing the activation of protein kinase G which causes the relaxation of vascular smooth muscles. Phospholipase A2 and NF-ɤB inhibitor are another class of medications with anti-inflammatory and immuno-modulating properties (He-riansyah*et al.*, 2021). Phospholipase A2 enzyme exists in many isoforms in tissues, which catalyzes the hydrolysis of the ester bond at the sn-2 position of membrane glycerophospholipids releasing free fatty acids including arachidonic acid (AA), which in turn, is transformed into a variety of bioactive lipophilic molecules known as eicosanoids, such as prostaglandins (PGs) and leukotrienes (LTs) which are involved in numerous homeostatic biological functions and inflammatory diseases (BBA, 2019). Phospholipase A2 binds to intracellular glucocorticoid receptors (GRs) which induces expression of glucocorticoid-responsive genes that act as anti-inflammatory mediators. Therefore, anti-inflammatory such as cytokines, interleukin 10, and lipocortins inhibit phospholipase A2, thereby blocking the release of arachidonic acid from membrane phospholipids and prevent the synthesis of prostaglandins and leukotrienes which both are mediators of inflammation. Phosphotidyl inositol 3-Kinase (PI3K) inhibitors are FDA approved for cancer treatments (Yang *et al.*,2019).

PI3K is an intracellular kinase mainly located on the medial side of the cell. The main role of PI3K is the regulation of the survival, migration, proliferation, differentiation, transcription and translation of cells in the context of atherosclerosis, which occurs through the activation of signaling pathways. PI3K also plays a crucial role in the progression and regression of atherosclerosis. It was reported that the inhibition of PI3K enzyme causes cells death, inhibits cell proliferation and inhibits many signaling pathways (Sowmithra *et al.,* 2020), which in turn, can reduce symptoms of hypertension (Daniela *et al.,* 2012). Creatine kinase enzyme can also be inhibited to prevent platelet aggregation (Brewster, 2018). Moreover, up-regulation of the creatine kinase pathway helps to maintain muscle creatine levels and limits cellular energy failure in skeletal muscles, hence, protect muscle tissues damage due to hypoxia (Arazi*et al.,*2021).

To the best of our knowledge, *A. arvensis* L. and *B. stracheyi* (Hook.f. & Thomson) Engl. anti-inflammatory properties have not been investigated for high-altitude pulmonary edema. Therefore, the main objective of our study is to screen phytochemicals of *A. arvensis* L. and *B. stracheyi* (Hook.f. & Thomson) Engl. against inflammation resulted from high altitude pulmonary edema via studying their interaction with different target proteins.

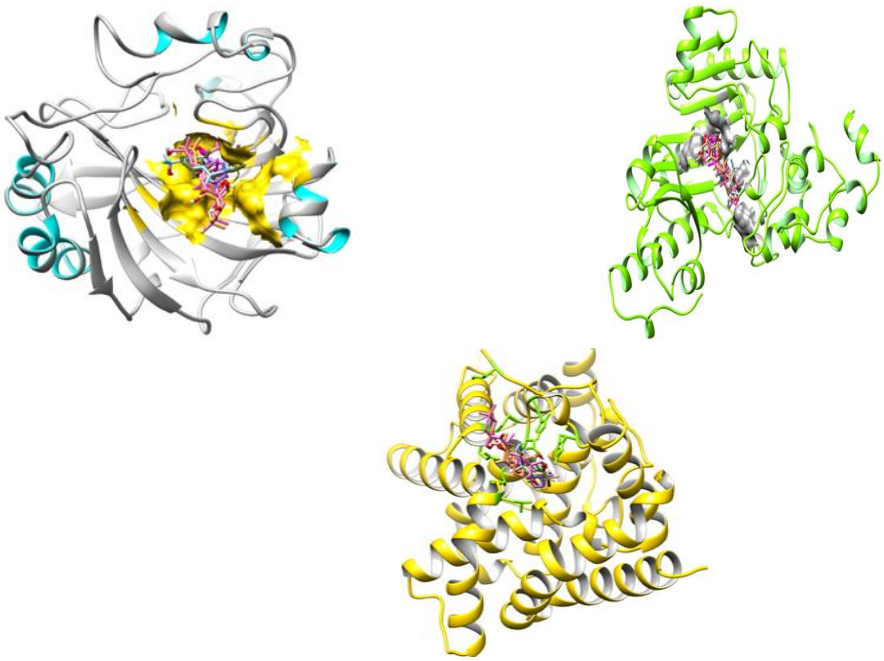
2. Results

2.1. Evaluation of selected proteins structures

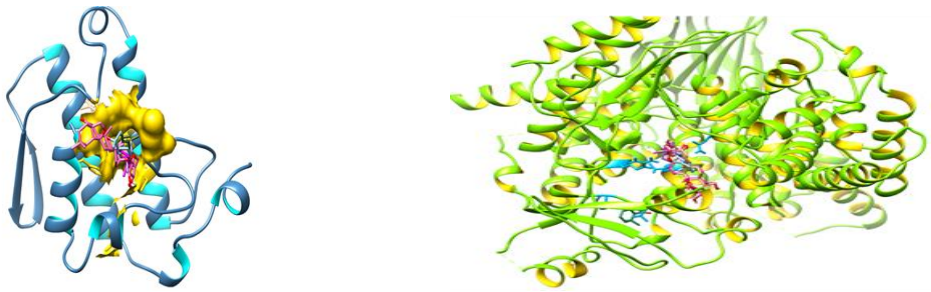
Gene behind the protein, their role, accession number and molecular weight of carbonic anhydrase-2, phosphodiestrase-5, phospholipase A_2, creatine kinase and phosphotidylinositol-3 kinase has been determined (Table 1) and structures are depicted in Figure 1.

Table 1. Structural feature of selected proteins.

Proteins	Genes	Role	Accession Numbers	Molecular weight	Figure No.
Carbonic anhydrase-2	CA2, one of sixteen forms of α carbonic anhydrases	catalyzes reversible hydration of CO_2	P00918	29.2 kDa	3 (A)
Phosphodiestrase-5	949 amino-acids were present in PDE-5	Regulate cGMP- specific signaling pathways such as smooth muscle contraction and relaxation	Q3SBD3	108,494 kDa.	3 (B)
Phospholipase A_2	PLA_2	phospholipid digestion and metabolism, signal transduction and host defense	P00630	19,058 kDa	3 (C)
Creatine kinase	CK, contain 381 amino-acids	Reversibly catalyzes the transfer of phosphate between ATP. Play crucial role in energy transduction in tissues with large, fluctuating energy demands	P06732	43,101 kDa	3 (D)
Phosphotidylinositol-3 kinase	contain 728 amino-acids	activate signaling cascades involved in cell growth, survival, proliferation, motility and morphology	O00459	81, 5 kDa	(E)



(A).Complex of Carbonic anhydrase-2 (B) Complex of Creatine kinase (C) Complex of phosphodiesterase-5



(D) Complex of phospholipase A_2 (E) Complex of phosphotidyl inositol-3 kinase

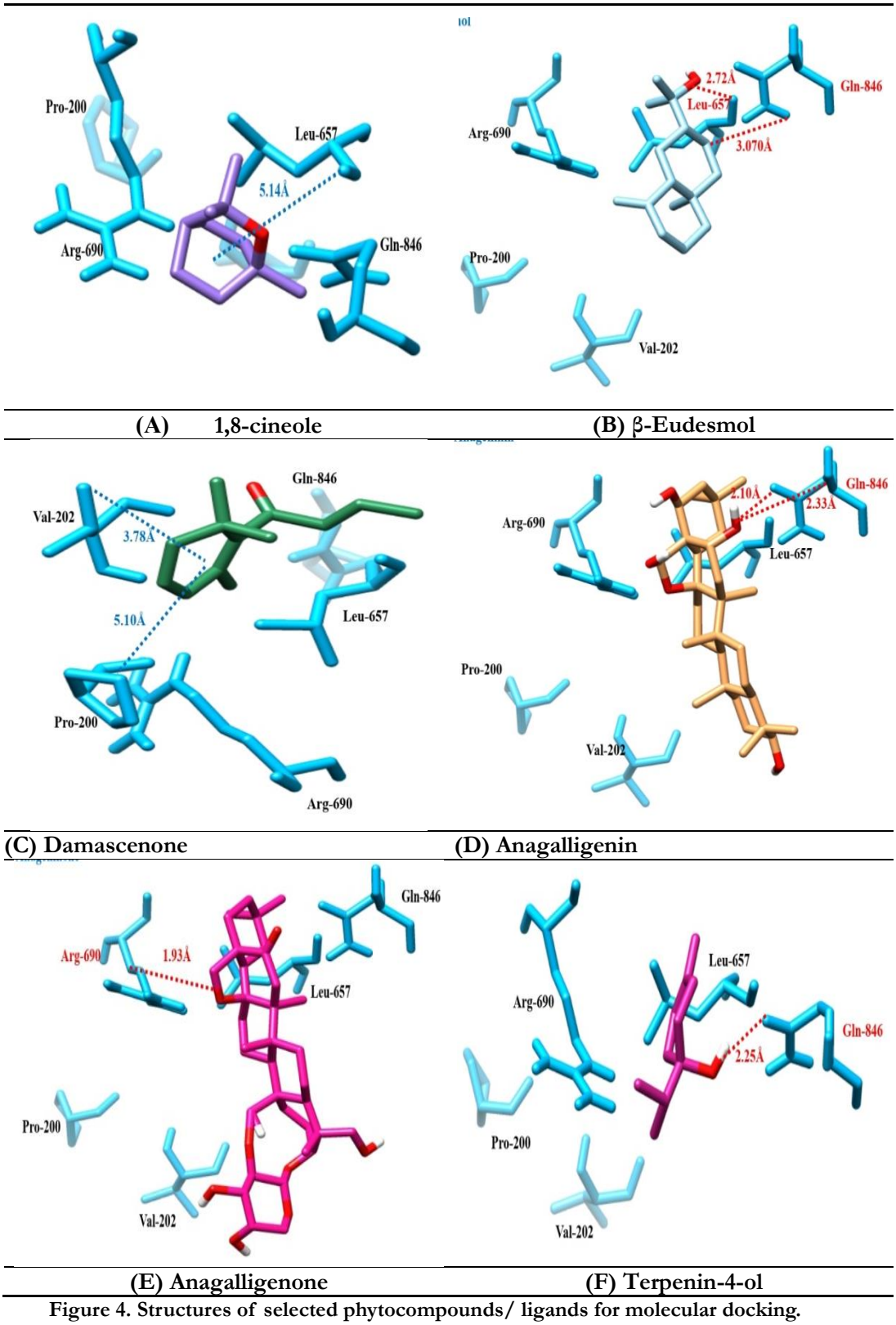
Figure 3. Structures of targeted proteins A-E.

2.2. Evaluation of selected phytocompounds/ligand Structures

Selected phytocompounds or ligands have different structures (Figure 4), pubchem ID, molecular formulas and molecular weights (Table 2).

Table 2. Selected Phytocompounds/ ligand Structures.

Selected compounds	PubChem IDs	Molecular formula	Molecular weight	Figures
1,8-cineole	2758	$C_{10}H_{18}O$	154.25	4 (A)
β-Eudesmol	91557	$C_{15}H_{26}O$	222.37	4 (B)
Damascenone	5366074	$C_{13}H_{18}O$	190.28	4 (C)
Anagalligenone	72375825	$C_{35}H_{56}O$	604.8	4 (D)
Anagalligenin	70680295	$C_{30}H_{50}O_5$	490.7	4 (E)
Terpenin-4-ol	2724161	$C_{10}H_{18}O$	154.25.	4 (F)



(A) 1,8-cineole **(B) β-Eudesmol**

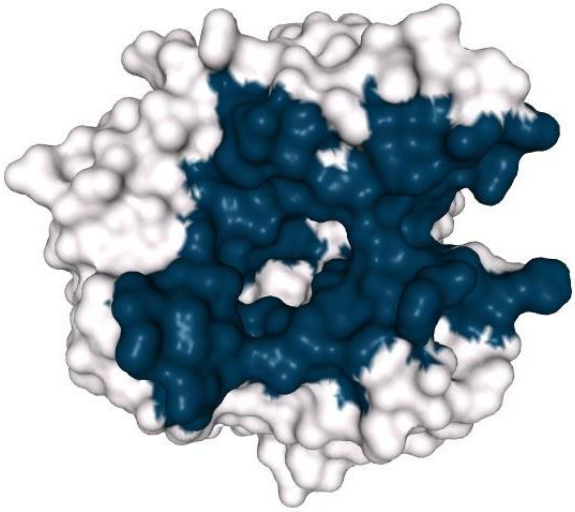
(C) Damascenone **(D) Anagalligenin**

(E) Anagalligenone **(F) Terpenin-4-ol**

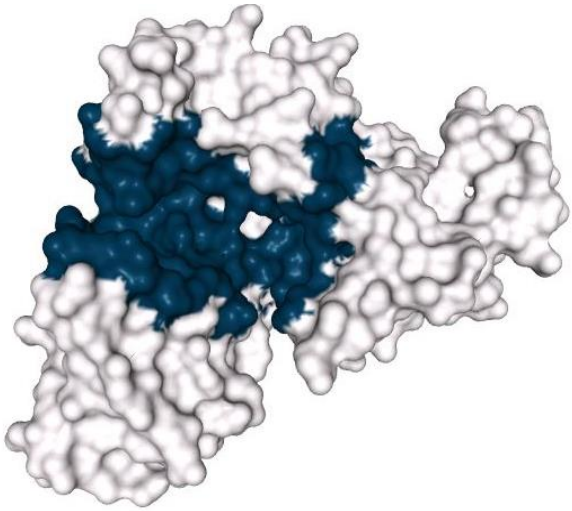
Figure 4. Structures of selected phytocompounds/ ligands for molecular docking.

2.3. Active Site of proteins

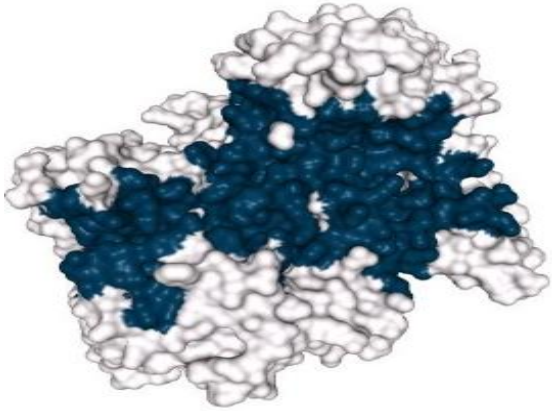
Active sites of carbonic anhydrase-2, creatine kinase, phosphotidyl inositol-3 kinase, phosphodiestrase-5, phospholipase A_2 showed binding pockets and number amino acid residues where probability of binding ligands was more accurate (Figure 5). Peaks elevation in graphs showed high binding possibility of ligands at specific residue number (Figure 6).



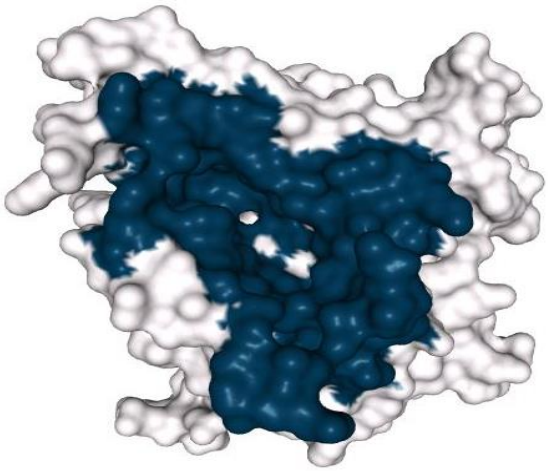
(A) Carbonic anhydrase-2



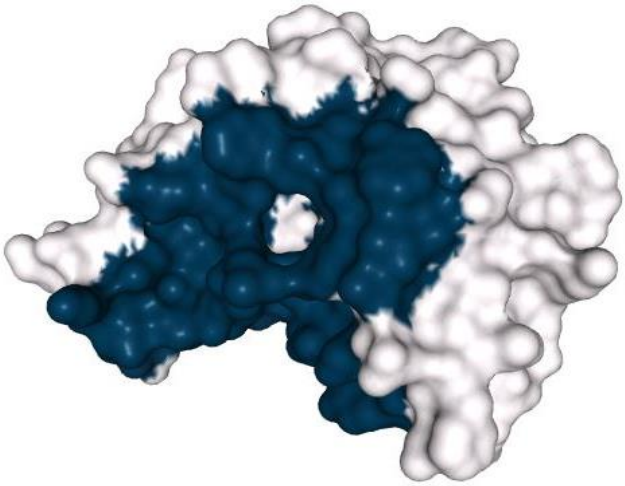
(B) Creatine kinase



(C) Phosphatidyl inositol-3 kinase



(D) Phosphodiesterase-5



(E) Phospholipase A_2

Figure 5. Cloud model of proteins

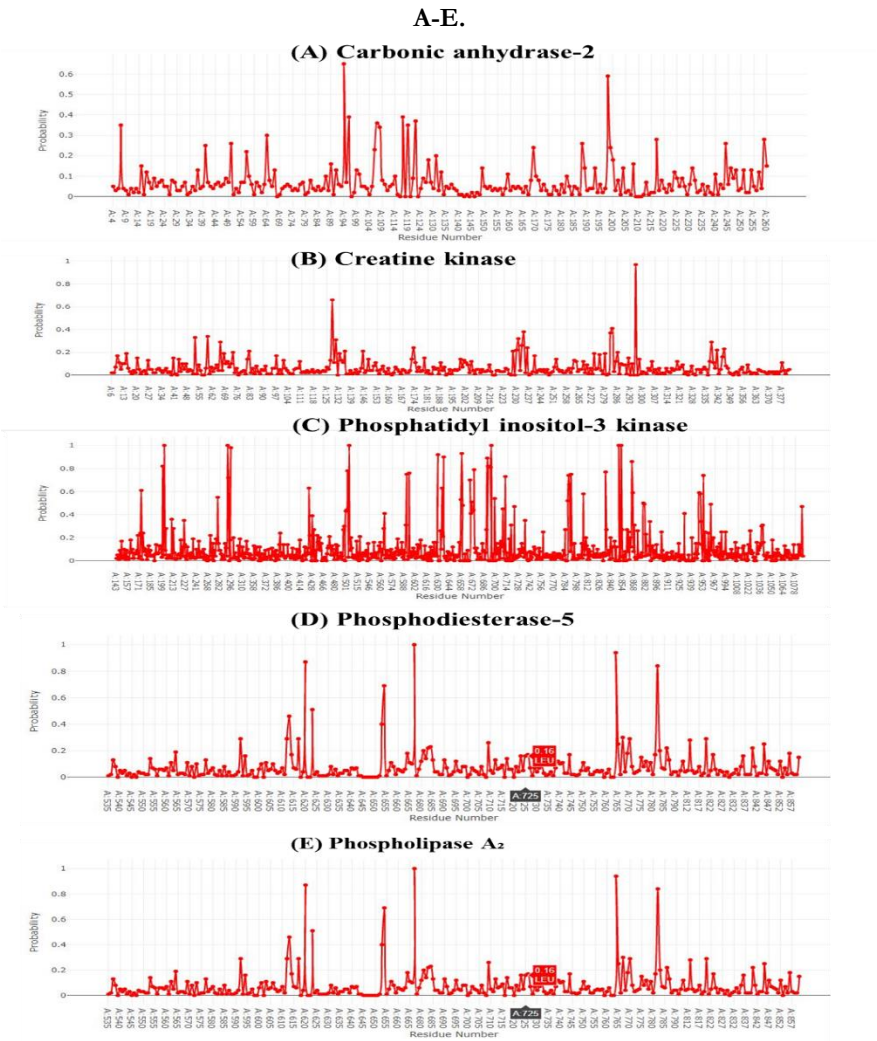


Figure 6. Residue number and probability of protein with selected compounds A-E.

2.4. ADMET screening of selected drugs

Chemical distribution, absorption, metabolism, toxicity and distribution play avital role in drug development and discovery. High-quality drugs show efficacy against therapeutic targets and show appropriate ADMET properties at therapeutic dose. Development of *in silico* models have predicted ADMET properties and gave ADMET scores on the basis of 18 ADMET properties by SwissADME (Table 3 and Figure 7).

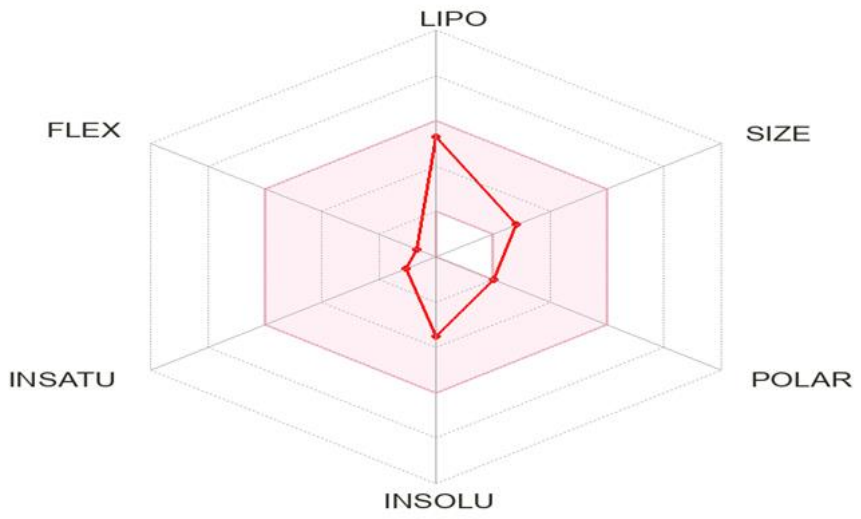


Figure 7. ADMET screening of β-Eudesmol.

Table 3. Physiochemical properties and lipophilicity.

Physicochemical Properties		**Lipophilicity**	
Formula	**$C_{15}H_{26}O$**	Log $P_{o/w}$ (iLOGP)	3.06
Molecular weight	222.37 g/mol	Log $P_{o/w}$ (XLOGP3)	3.74
Num. heavy atoms	16	Log $P_{o/w}$ (WLOGP)	3.92
Num. arom. heavy atoms	0	Log $P_{o/w}$ (MLOGP)	3.67
Fraction Csp3	0.87	Log $P_{o/w}$ (SILICOS-IT)	3.64
Num. rotatable bonds	1	Consensus Log $P_{o/w}$	3.60
Num. H-bond acceptors	1	**Water Solubility**	
Num. H-bond donors	1	Log S (ESOL)	-3.51
Molar Refractivity	70.46	Solubility	6.89^{e-02} mg/ml ; 3.10^{e-04} mol/l
TPSA	20.23 Å²	Class	Soluble
Pharmacokinetics		Log S (Ali)	-3.86
GI absorption	High	Solubility	3.09^{e-02} mg/ml ; 1.39^{e-04} mol/l
BBB permeant	Yes	Class	Soluble
P-gp substrate	No	Log S (SILICOS-IT)	-3.21
CYP1A2 inhibitor	No	Solubility	1.36^{e-01} mg/ml ; 6.12^{e-04} mol/l
CYP2C19 inhibitor	No	Class	Soluble
CYP2C9 inhibitor	Yes	**C**	
CYP2D6 inhibitor	No	Lipinski	Yes; 0 violation
CYP3A4 inhibitor	No	Ghose	Yes
Log K_p (skin permeation)	-5.00 cm/s	Veber	Yes
Medicinal Chemistry		Egan	Yes
PAINS	0 alert	Muegge	No; 1 violation: Heteroatoms<2
Brenk	1 alert: isolated_alkene	Bioavailability Score	0.55
Leadlikeness	No; 2 violations: MW<250, XLOGP3>3.5		
Synthetic accessibility	3.38		

2.5. Complex of targeted proteins with selected ligands

Hydrogen bond shows bond distance and bond length between specific amino-acid residues and interactions of selected ligands with targeted proteins.

With carbonic anhydrase-2, anagalligenin showed hydrophobic interactions with bond distance of 4.76Å, 4.88 Å and 5.38Å between His-94, Leu-198 and Phe-131 respectively, while hydrogen interaction with a bond distance of 2.78Å between Thr-199. Anagalligenone showed hydrogen interaction with a bond distance of 2.30Å, 2.72Å and 2.80Å with Thr-199, Thr-209 and His-199, respectively. β-eudesmol and 1,8-Cineole showed both hydrophobic (bond distance 4.9Å with Leu-198 and 4.86Å with Leu-198 respectively) and hydrogen interactions (bond distance of 2.17Å and 2.25Å between Asn-67 and His-94 respectively by β-eudesmol and 2.46Å with Gln-92 by 1,8-Cineole). Damascenone showed hydrophobic interactions (at 4.35Å, 4.80Å and 3.95Å with val-135, Phe-131 and His-94 respectively) and H- interactions of bond distance 2.24Å with Gln-92. Terpinen-4-ol only showed hydrophobic interactions with bond distances of 4.99Å, 4.22Å, 4.96Å and 4.10Å with His-94, His-119, Thr-209 and Leu-198 (Figure 8 A).

Anagalligenone, β-eudesmol, anagalligenin, and 1,8-cineole showed only hydrogen interaction with creatine kinase at bond distance of 2.56Å with Arg-130, 2.47Å and 2.49Å with Asn-286 and Arg-132, 1.85Å and 2.15Å with Arg-96 and 2.86Å with Arg-236 respectively. Damascenone and terpenin-4-ol showed both type of interactions. Damascenone had hydrophobic interactions with bond distance 2.88Å with Trp-228 and 4.31Å with Ile-238, while its H- interactions was with bond distance 1.81Å and 2.57Å with Arg-130. Terpenin-4-ol showed hydrophobic interactions at 3.91Å with Trp-228 and 5.28Å with Arg-132, while its H-interaction was with bond distance of 2.08Å with Arg-236 (Figure 8 B).

Anagalligenin, anagalligenone, β-eudesmol, 1, 8-cineole, damascenone and terpenin-4-ol showed hydrogen interaction with protein phosphodiesterase-5 at bond distance of 1.93Å, 2.31Å, 2.57Å and 2.58Å with Glu-682, His-657, Leu-725 and Thr-723 by anagalligenin, 1.88Å and 2.84Å with Asp-654 and 2.92Å with His-613 by anagalligenone 1.93Å and 2.10Å with His-613 by β-eudesmol and cineole respectively, 2.00Å with His-657 by dmascenone and 1.97Å with Thr-723 and 3.97Å with Met-681 by tepenin-4-ol (Figure 8 C).

Phospholipase A2 showed hydrophobic interactions with anagalligenin, β-eudesmol, damascenone, terpenin-4-ol and 1,8-cineole at bond distance of 1.63Å and 2.39Å with Thr-47 and Cys-51 by anagalligenin respectively and 3.81Å with Tyr-69 and 5.41Å with His-48 by β-eudesmol, 4.57Å with Tyr-52 and 4.84Å with Leu-31 by damascenone, 4.20Å with Tyr-69 and 4.49Å with Val-2 by terpenin-4-ol and 4.37Å with Phe-5, 4.79Å with Val-2, 4.88Å and 5.19Å with Tyr-52 by 1,8-cineole. Anagalligenone showed only H-interaction at bond distance of 2.28Å with Tyr-22, 2.38Å with Cys-45 and 2.73Å with Tyr-52 (Figure 8 D).

Phosphotidyl inositol-3 kinase showed H-interactions of bond distance 2.33Å and 2.10Å with Gln-846 and hydrophobic interaction 1.63Å with Thr-47 and 2.39Å with Cys-51 with anagalligenin. Anagalligenone (at bond distance of 1.93Å with Arg-690), β-eudesmol (at bond distance of 2.72Å with Leu-657 and 3.070Å with Gln-846) and terpenin-4 (at bond distance of 2.25Å with Gln-846) showed only H-interaction with phosphotidyl inositol-3 kinase. 1,8-cineole and damascenone showed hydrophobic interactions with bond distance of 5.14Å with Leu-657 by 1,8-cineole and 3.78Å with Val-202 and 5.10Å (Figure 8 E).

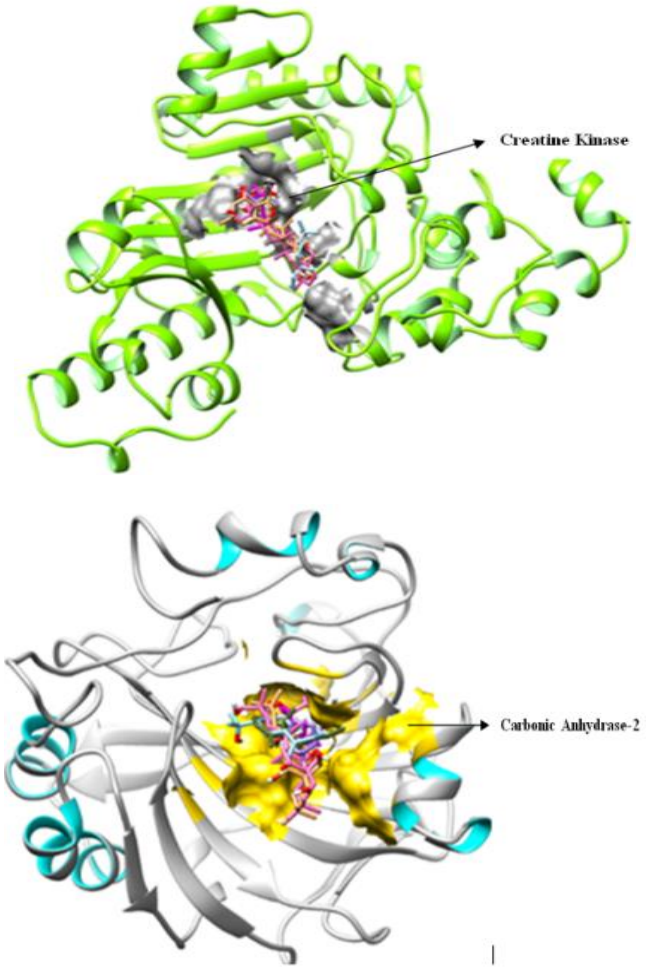


Figure 8. Complex of targeted proteins with selected ligands A-E.

2.6.RMSD and RMSF via MD Simulation

Complex of all selected ligands with PDE-5 (Figure 9 C) was selected for MD simulation, whose results showed that Gln-817 possess a strong H-interaction and thus, protein-ligand RMSD showed stability at 10ns which had been increased with the passage of time and it's changes within range (Figure 9 A, B, C). RMSD showed global changes in whole structure of complex. RMSF showed individual residues fluctuation as its longer peak showed loop and coils and small peaks showed rigid structure of α helix (54.86%) and β sheets (0.02% and secondary structure elements 54.88%) as compared to secondary structure (coils and loops) of protein and part of protein which was in contact with ligandalso showed low fluctuations and more stability. RMSF depends on secondary structures of protein, out of which 55% residues were helixes and 45% were coils and loops and these caused fluctuations and high RMSD (Figure 9 D, E).

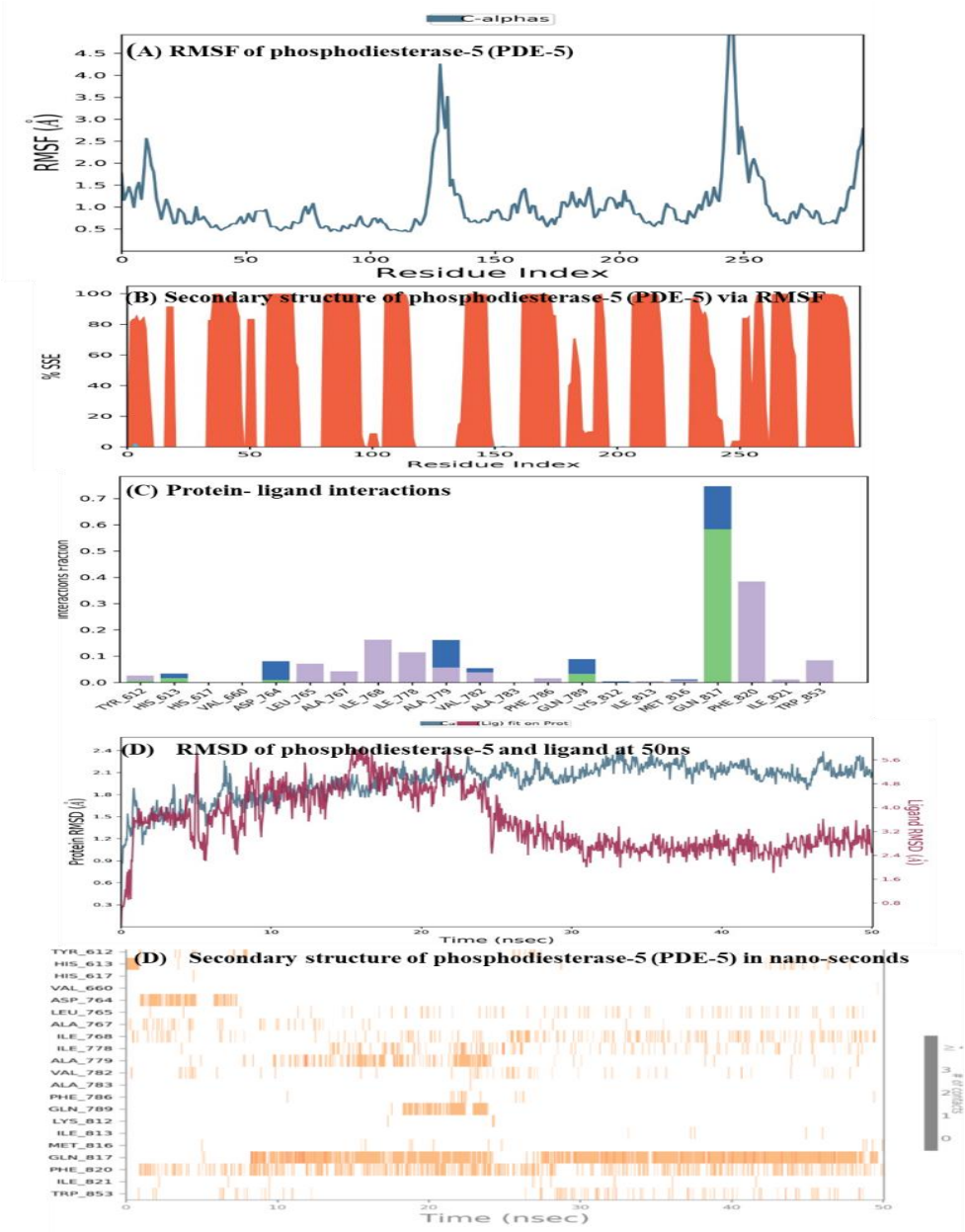


Figure 9. MD simulation of phosphodiesterase-5 (PDE-5) by Desmon.

3. Discussion

Alveolar hypoxia is important feature of pulmonary hypertension, caused pulmonary vasoconstriction. HAPE is a syndrome of non-specific symptoms that cause serious complication by increasing oxidative stress and inflammation in lungs. Genetic factors and mitochondrial redox play important role in high altitude pulmonary oedema pathophysiology (Luo *et al.*,2012). Medicinal plants have been used for centuries in traditional medicines (Hosseinzadeh*et al.*,2015). Phytochemicals in plants reduce the risk of oxidative damage act as enzyme inhibitors, peroxide decomposers, singlet oxygen quencher etc. (Lobo *et al.*,2010). *A.arvensis* L. and *B. stracyei* (Hook.f. & Thomson) Engl. are important medicinal plant which have diuretics, anti-inflammatory and anti-oxidant properties. Phytochemicals of *A. arvensis* L. and *B. strachyei* (Hook.f. & Thomson) Engl. included anagalligenin, anagalligenone, 1,8-Cineole, β-eudesmol, damascenone are used as anti-inflammatory ligand. Phosphodiestrase-5, carbonic anhydrase-2, creatinine kinase, phosphotidyl inositol-3 kinase and phospholipase A2 are proteins used for novel drug discovery by interaction of anti-inflammatory ligands and proteins against high altitude pulmonary oedema.

Molecular docking of selected ligands with targeted proteins showed binding affinities. All selected ligands showed negative binding energies with targeted proteins (Afroz *et al.* , 2019; Poornima, G. and Sunitha, K. 2023). However, current results showed that anagalligenone and anagalligenin have a high binding energy with targeted protein and due to high molecular weight of both ligands, ADMET properties are not fulfilled. Therefore, for MD simulation, those ligands were selected which fulfil Lipinksin's rule of 5 and ADMET properties which describe GI absorption, solubility of ligand, number of H- bond donar and acceptor and whether it crosses blood brain barrier or not (Anish *et al.* , 2023; Tao *et al.* , 2020). MD simulation is used in computational

chemistry, and it describesthe interaction between selected ligand with targeted protein. For MD simulation β- eudesmol of *B. strachyei* (Hook.f. & Thomson) Engl. was selected as ligand. Bergenia plays animportant role as anti-oxidant by reducing free radical damage and also act as diuretics and help kidneys release more sodium into urine and manage symptoms of oedema and hypertension (Aggarwal *et al.,*2011) and protein Phosphodiestrase-5. It 's up-regulated pathway (Significantly increased messenger RNA and protein level) reduced hypoxia (Zhao *et al.,*2022). Inhibition of this enzyme, prevent from degradation of cGMP cyclic (Guanosine-mono phosphate) by PDE-5. cGMP causes activation of protein kinase G which causes the relaxation of vascular smooth muscles. Phosphodiestrase-5 show interaction with all selected ligand but according to other rules of MD simulation we would select β-eudesmol due to fulfill ADMET properties. It showed -5.6 binding affinity with phosphodiesterase-5. β-eudesmol molecular weight was 222.37 g/mol, number of H-bond acceptor and donor was 1. This ligand showed molecular weight < 500, < 5H- bond donors, <10H-bond acceptors

Complex of targeted proteins with selected ligands show hydrophobic and hydrogen interactions. MD simulation of 50ns by Desmond show result of ligand and protein complex. For running simulation number of ions concertation of Na and Cl 50.246mM and total charge for ions +28 for Na and -28 for Cl. Protein-ligand RMSD show protein and ligand complex stability at 10ns. Ligand show stability by the increase of time and it' s changes within range. Therefore, result shows that this selected complex is a stable complex. RMSD show global changes in whole structure of complex. RMSF show individual residues fluctuation. RMSF longer peak shows loop and coils and small peaks show rigid structure of α helix and β sheets as compared to protein secondary structure coils and loops, that part of protein which in contact with ligand also show low fluctuations and more stability. RMSF depend on protein secondary structure element composition. It show helix 54.86% and β-strands 0.02% and secondary structure elements 54.88%. 55% residues makes helixes and 45% residues make coils and loops which cause fluctuations and it 's RMSD high. Another simulation graph also show fluctuations with respect to time. MD simulation also describe hydrophobic and hydrogen interactions. Results of protein ligand contacts of simulation show that Gln-817 show strong H-interaction. Thus, selected complex show stability and could play a beneficial effect in future novel drug discovery for treatment of high-altitude pulmonary edema.

4. Materials and Methods

The whole scheme of work has been presented in Figure 9 flowchart.

4.1. Selection and preparation of ligand/ Compound

1,8-Cineole, β-eudesmol, damascenone, terpinen-4-ol, anagalligenin and anagalligenone from *A. arvensis* L. and *B. stracheyi* (Hook.f. & Thomson) Engl. were selected as ligand due to their potential anti-inflammatory, anti-oxidant and diuretic role (Yasmeen *et al.,*2020) to predict protein-ligand interaction and for drug development before it advances to the more costly stages of clinical trial. Selected ligand's structure had H-bonds Ball and sticks. Moreover, ADMET properties were checked via Lipinski 's rule of 5 for safety and efficacy of drug candidate for regulatory approval. Lipinski 's rule of 5 give molecular weight<500, logP<5, <5H-bond donors, <10H-bond acceptors. SDF files of 3D structure of ligand were obtained by PubChem database (Granata*et al.,*2021) while PDB file format of ligand structures were drawn by using ChemSketch to get 2-d structure, followed by PyMolsoftwares (Bohnuud*et al.,*2017).

4.2. Target/Protein identification

Structures of targeted proteins (phosphodiestrase-5, carbonic anhydrase-2, phosphotidyl inositol 3-kinase, phospholipase A_2, creatine kinase, used as anti-inflammatory mediators to relax vascular smooth muscles and were used to decrease pulmonary hypertension and capillary stress failure in high altitude pulmonary oedema) of pulmonary oedema pathways (pulmonary vasoconstriction, pulmonary hypertension and capillary stress failure) were obtained through PDB and UniProt, while their 3d structure were refined using UCSF chimera to remove all water molecules to obtain only a single chain of protein on basis of homology pattern (Goddard *et al.,*2007).

4.3. Prediction of active sites

Active sites were predicted to provide required microenvironment for catalysis and to allow substrate to form enough contact points for strong binding as if the binding site is identified, then docking calculates the binding affinity and stability. To predict the active sites of targeted proteins, an online tool depth residue was used (Myung *et al.,*2022).

4.4. Receptor grid generation

As each ligand has a specific binding site with proteins, so a computed grid consisting of selected amino acids has been made, by keeping all the parameters default which produced a cubic box surrounding the active site of receptor for docking experiment. For docking analysis, grid box was set at the retained coordinates of 1,8-Cineole, β-eudesmol, damascenone, terpinen-4-ol, anagalligenin and anagalligenone and it was set for carbonic-anhydrase 2 (centre x = -11.176 Å, centre y = -1.5639 Å, centre z = 18.8511 Å), creatine kinase (centre x = -20.44 Å, centre y = 3.8494 Å and centre z = -13. 121 Å), phosphatidylinositol-3 kinase (centre x =30.73 Å, centre y = -8.8488 Å, centre z = 25.822 Å), phosphodiesterase 5 (centre x =40.479 Å, centre y = 4.132 Å, centre z = 52.934 Å) and phospholipase-2 (centre x = 1.9152 Å, centre y = 67.439 Å, centre z = 136.440 Å), while exhaustiveness values (8) of all proteins were configured to obtain the finest binding conformational pose of selected compounds.

4.5. Molecular docking

Molecular docking analysis was performed by PyRx (working on principle of Auto Dock Vina 4) considering the protein as macromolecule and the ligand as phyto-compounds (sekar and Habib, 2022). All ligands were given as an input parameter with all proteins separately to obtain different protein-ligand binding patterns and binding energies for the formation of their 2- and 3-dimensional graphics.

2.6. Interaction analysis

Interaction of protein and ligand was analyzed for interacting residues, bond type, bond length and bond distance by Discovery Studio (Holcomb and Harris, 2022).

2.7. MD Simulation

To determine the stability, confirmation, and intermolecular interaction of the ligand with the targeted proteins, a molecular dynamics (MD) simulation was run using the Desmond package, while time-dependent alteration of the complexes was computed over 50 ns in a thermodynamic environment (applied volume, density, pressure, and temperature) and by using ensembles, the entire system was annealed until reached equilibrium. Additionally, the last manufacturing stage looked into the complex's structural adjustment and to assess the degree of structural changes, the trajectories of each complex were also subjected to a number of specific parameters, including RMSD, RMSF, protein secondary structure element (SSE), conformational modification of ligands, and intermolecular interactions.

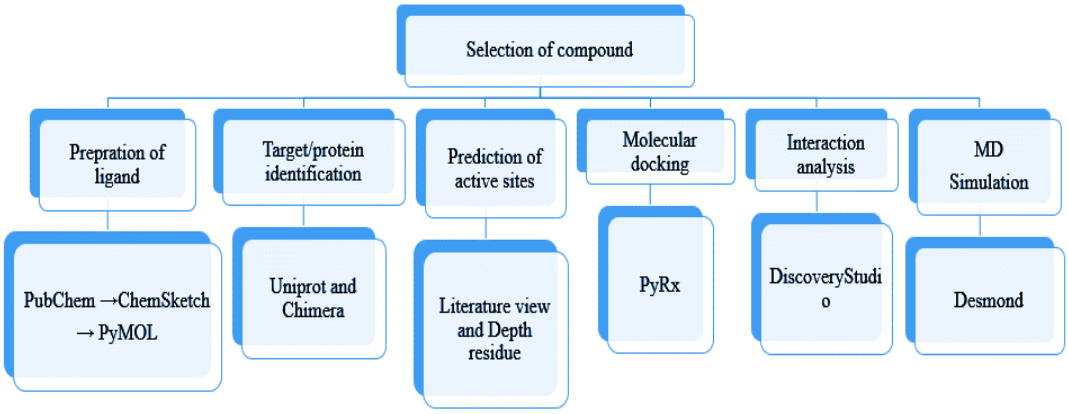


Figure 9. Flow sheet of whole experiment.

5. Conclusions

In conclusion, this study demonstrated that medicinal plant *A. arvensis* L. and *B. strachyei* (Hook.f. & Thomson) Engl. had aremarkable potential to protect the chronic symptoms of altitude sickness. Ligand β-eudesmol with protein phosphodiesterase-5 had the highest binding affinity as compared to other ligands. It shows good ADMET properties and it' s MD simulation 50ns by Desmond shows stability with protein phosphodiesterase-5 at 10ns. In traditional system of medicine natural compounds had been commonly used and played avital role in the treatment of various disorders. Various countries used the herbal components as a medicine for their health care. Therefore, it is suggested that anti-inflammatory, anti-oxidant and diuretics containing properties of herbal plants and their selected ligands had a positive impact with targeted proteins and shows protection without exerting any adverse effect by inhibitory or stimulatory mechanisms. Thus, compound of Plant B β-eudesmol can be used for the treatment of high-altitude pulmonary oedema and for further *in vitro* experiment of pulmonary oedema.

Supplementary Materials: Not Applicable

Author Contributions: "Conceptualization and validation: A.A.; methodology, A.N.; formal analysis, R.B. investigation, A.A. and A.N; resources, A.A and I.S; writing—A.A. and R.B writing—review and editing, A. N; R.B.; supervision, A.A.; project administration, A.A, A.N. and R.B.; funding acquisition, A.A. and I.S. All authors reviewed and commented on the manuscript.

Funding: This research is supported by the Researchers Supporting Project Number (RSP2023R457), King Saud University, Riyadh, Saudi Arabia.

Institutional Review Board Statement: Not Applicable

Informed Consent Statement: Not Applicable

Data Availability Statement: All the data is contained in the manuscript.

Acknowledgments: The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (NRPU-8715).The authors are very thankful to the Institute of Molecular Biology and Biotechnology for the facilitation of this research work.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Afroz, M., Zihad, S.M.N.K., Uddin, S.J., Rouf, R., Rahman, M.S., Islam, M.T., Khan, I.N., Ali, E.S., Aziz, S., Shilpi, J.A., Nahar, L. and Sarker, S.D. (2019). A systematic review on antioxidant and antiinflammatory activity of Sesame (*Sesamum indicum* L.) oil and further confirmation of antiinflammatory activity by chemical profiling and molecular docking. Phytotherapy Research, 33(10):2585-2608.
2. Akbari, B., Baghaei-Yazdi, N., Bahmaie, M and MahdaviAbhari, F. (2022). The role of plant-derived natural antioxidants in reduction of oxidative stress. BioFactors, 48(3):611-633.
3. Aldughaylibi, F. S., Raza, M. A., Naeem, S., Rafi, H., Alam, M. W., Souayeh, B and Mir, T. A. (2022). Extraction of Bioactive Compounds for Antioxidant, Antimicrobial, and Antidiabetic Applications. Molecules, 27(18), 5935.
4. Anish, R., Rumaisa, F., Aswathy, T., Kalpana, S. and Rauf, A. (2023). Molecular docking, anti-inflammatory, antimicrobial and antioxidant evaluation of *Pterospermum rubiginosum* B. Heyne. Trends in Phytochemical Research, 7(2), 95-109.
5. Arazi, H., Eghbali, E and Suzuki, K. (2021). Creatine supplementation, physical exercise and oxidative stress markers: A review of the mechanisms and effectiveness. Nutrients, 13(3), 869.
6. Arunkumar, S and Muthuselvam, M. (2009). Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. World journal of agricultural sciences, 5(5), 572-576.
7. Aggarwal, B., Prasad, S., Reuter, S., Kannappan, R., R Yadav, V., Park, B and Sung, B. (2011). Identification of novel anti-inflammatory agents from Ayurvedic medicine for prevention of chronic diseases:"reverse pharmacology" and "bedside to bench" approach. Current drug targets, 12(11), 1595-1653.
8. Bhadra, S., Pundir, C., Das, J., Kar, A., Chanda, J., Das, B and Mukherjee, P. K. (2022). Evaluation of bioactive compounds as AChE inhibitors from medicinal plants. In Evidence-Based Validation of Herbal Medicine, Elsevier,349-388.
9. Bohnuud, T., Jones, G., Schueler-Furman, O and Kozakov, D. (2017). Detection of peptide-binding sites on protein surfaces using the PeptiMap server. In Modeling Peptide-Protein Interactions , Humana Press, New York, NY ,11-20.
10. BBA - Molecular and Cell Biology of Lipids, 1864. (2019). 941–956
11. Brewster, L. M. (2018). Creatine kinase, energy reserve, and hypertension: from bench to bedside. Annals of Translational Medicine, 6(15),1-16.
12. Chen, A. Y and Chen, Y. C. (2013). A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention. Food chemistry, 138(4), 2099-2107.
13. Christian, B. (2021). Bioactive biflavonoids from *Wikstroemia indica* (L.) C.A. Mey. (Thymelaeaceae): A review. Trends in Phytochemical Research (TPR),5(4),190-198.
14. Daniela, C., Carmine, V., Giada, M., Giovanni, E., Giuseppe, C., Katiuscia, M., Alessandro, L., Giulio, S., Paolo, G., Antonio, D., Elio, F., Hannelore, H., Angelo, M., Elisa, C., Sergio, F., Giacomo, F., Orazio, M., Emilio, H. and Giuseppe, L. (2012). PI3Kγ inhibition reduces blood pressure by a vasorelaxantAkt/L-type calcium channel mechanism, Cardiovascular Research, 93(1), 200–209.
15. De Smet, P.A.G.M. (1997). The Role of plant-derived drugs and herbal medicines in healthcare. Drugs, 54, 801-840.
16. Dinan, L., Harmatha, J and Lafont, R. (2001). Chromatographic procedures for the isolation of plant steroids. Journal of chromatography A, 935(1-2), 105-123.
17. Gio-Batta, M., Sjoberg, F., Jonsson, K., Barman, M., Lundell, A. C., Adlerberth, I and Wold, A. E. (2020). Fecal short chain fatty acids in children living on farms and a link between valeric acid and protection from eczema. Scientific Reports, 10(1), 1-11.
18. Goddard, T. D., Huang, C. C and Ferrin, T. E. (2007). Visualizing density maps with UCSF Chimera. Journal of structural biology, 157(1), 281-287.
19. Granata, I., Manzo, M., Kusumastuti, A and Guarracino, M. R. (2021). Learning from metabolic networks: current trends and future directions for precision medicine. Current Medicinal Chemistry, 28(32), 6619-6653.
20. Heriansyah, T., Chomsy, I. N., Santoso, A., Ridwan, M., Aini, F and Wihastuti, T. A. (2021). Lp-PLA2 Selective Inhibitors (Darapladib) effect in lowering serum and aortic lysophosphatidylcholine (LysoPC), NF-κB and Lp-PLA2 levels on dyslipidemic rats model. Indian Journal of Forensic Medicine & Toxicology, 15(4).
21. Holcomb, A and Harris, J. (2022, August). Launching a new discipline-specific first-year discovery studio: vision, purpose, and adaptation A mid pandemic-related turbulence. In 2022 ASEE Annual Conference & Exposition.

22. Hollingsworth, S. A and Dror, R. O. (2018). Molecular dynamics simulation for all. Neuron, 99(6), 1129-1143.
23. Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A and Armand, R. (2015). The application of medicinal plants in traditional and modern medicine: a review of *Thymus vulgaris*. International Journal of Clinical Medicine, 6(09), 635.
24. Jan, H. A., Samin, J., Rainer, W. B., Sher, W., Francesca, S. and Latif, A. (2020). Complementary and alternative medicine research, prospects and limitations in Pakistan: a literature review. Acta Ecologica Sinica, 40(6), 451-463.
25. Jiménez, D. L., Huelgas, R. G., Romero, A. F., Chozas, J. L., de Isla, L. P and González, J. M. (2019). Diuretic treatment of the patient with diabetes and heart failure. Role of SGLT2 inhibitors and similarities with carbonic anhydrase inhibitors. Revista Clinica Espanola (English Edition), 219(4), 208-217.
26. Komanduri, R., Chandrasekaran, N and Raff, L. M. (2000). MD simulation of indentation and scratching of single crystal aluminum. Wear, 240(1-2), 113-143.
27. Komanduri, R., Chandrasekaran, N and Raff, L. M. (2001). Molecular dynamics (MD) simulation of uniaxial tension of some single-crystal cubic metals at nanolevel. International Journal of Mechanical Sciences, 43(10), 2237-2260.
28. Korzeniewski, K., Nitsch-Osuch, A., Guzek, A and Juszczak, D. (2015). High altitude pulmonary edema in mountain climbers. Respiratory Physiology & Neurobiology, 209, 33-38.
29. Levett, P. N. (1984). Detection of Clostridium difficile in faeces by direct gas liquid chromatography. Journal of clinical pathology, 37(2), 117-119.
30. Licciardi, P.V. and Underwood, J.R. (2011). Plant-derived medicines: A novel class of immunological adjuvants. International Immunopharmacology, 11(3),390-398.
31. Lobo, V., Patil, A., Phatak, A and Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy reviews, 4(8), 118.
32. Mailafiya, M. M., Yusuf, A. J., Abdullahi, M. I., Aleku, G. A., Ibrahim, I. A., Yahaya, M. and Alebiosu, C. O. (2018). Antimicrobial activity of stigmasterol from the stem bark of *Neocarya macrophylla*. Journal of Medicinal Plants for Economic Development, 2(1), 1-5.
33. Malik, E. M and Müller, C. E. (2016). Anthraquinones as pharmacological tools and drugs. Medicinal research reviews, 36(4), 705-748.
34. Maridass, M and Britto, A.J.D. (2008). Origins of plant derived medicines. Ethnobotanical leaflets, 12, 373-387.
35. Mangal, P., Khare, P., Jagtap, S., Bishnoi, M., Kondepudi, K. K and Bhutani, K. K. (2017). Screening of six Ayurvedic medicinal plants for anti-obesity potential: An investigation on bioactive constituents from *Oroxylum indicum* (L.) Kurz bark. Journal of ethnopharmacology, 197, 138-146.
36. Mergia, E., Stegbauer, J. (2016). Role of Phosphodiesterase 5 and Cyclic GMP in Hypertension. Current Hypertension Reports,18, 39.
37. Mohammadhosseini, M., Frezza, C., Venditti, A., Sarker, S. D. (2021). Chemistry and Biodiversity, 18(11),e2100317.
38. Mollazadeh, H., Mahdian, D. and Hosseinzadeh, H. (2019). Medicinal plants in treatment of hypertriglyceridemia: A review based on their mechanisms and effectiveness. Phytomedicine, 53,43-52.
39. Muhammad, H. and Fiqih, S. S. (2022). RMSD dan binding affnity docking molecular senyawa aktif genistein dari tumbuhan Bunga Telang (*Clitoria ternatea*) dengan protease SARS-CoV-2 omicron varian 7k0e menggunakan aplikasi PyMOL dan PyRx. BIO-SMART, 1(1),1-5.
40. Myung, Y., Pires, D. E and Ascher, D. B. (2022). CSM-AB: graph-based antibody–antigen binding affinity prediction and docking scoring function. Bioinformatics, 38(4), 1141-1143.
41. Naeije, R., &Dedobbeleer, C. (2013). Pulmonary hypertension and the right ventricle in hypoxia. Experimental physiology, 98(8), 1247-1256.
42. Nayeem, N., Asdaq, S. M. B., Salem, H and AHEl-Alfqy, S. (2016). Gallic acid: a promising lead molecule for drug development. Journal of Applied Pharmacy, 8(2), 1-4.
43. Nycz, J. E., Malecki, G., Morag, M., Nowak, G., Ponikiewski, L., Kusz, J andSwitlicka, A. (2010). Arbutin: Isolation, X-ray structure and computional studies. Journal of Molecular Structure, 980(1-3), 13-17.
44. Paralikar, S.J. (2012). High altitude pulmonary edema-clinical features, pathophysiology, prevention and treatment. Indian Journal of Occupational and Environmental Medicine, 16(2):59-62.
45. Pinzi, L. and Rastelli, G. (2019). Molecular docking: shifting paradigms in drug discovery. International journal of molecular sciences, 20(18), 4331.
46. Poornima, G. and Sunitha, K. (2023). Unlocking the anthelmintic potential of *Grewia bilamellata* Gagnep.: *In-vitro* and molecular docking studies on adult Indian earthworms.Trends in Phytochemical Research, 7(2),127-140.

47. Ramawat, K., Dass, S., Mathur, M. (2009). The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. In: Ramawat, K. (eds) Herbal Drugs: Ethnomedicine to Modern Medicine. Springer, Berlin, Heidelberg.
48. Rho, T., Kil, H. W., Seo, Y. J., Shin, K. J., Wang, D and Yoon, K. D. (2020). Isolation of six anthraquinonediglucosides from *Cascara sagrada* bark by high-performance countercurrent chromatography. Journal of Separation Science, 43(21), 4036-4046.
49. Saeedi, M., Khezri, K., SeyedZakaryaei, A and Mohammadamini, H. (2021). A comprehensive review of the therapeutic potential of α-arbutin. Phytotherapy Research, 35(8), 4136-4154.
50. Saleem, H., Khurshid, U., Tousif, M. I., Anwar, S., Ali, N. A. A., Mahomoodally, M. F and Ahemad, N. (2022). A comprehensive review on the botany, traditional uses, phytochemistry, pharmacology and toxicity of *Anagallis arvensis* (L) A wild edible medicinal food plant. Food Bioscience,52,1-6.
51. Saleem, H., Zengin, G., Ahmad, I., Htar, T. T., Naidu, R., Mahomoodally, M. F and Ahemad, N. (2020). Therapeutic propensities, phytochemical composition, and toxicological evaluation of *Anagallis arvensis* (L.): a wild edible medicinal food plant. Food Research International, 137,1-4.
52. Santos, F. A., Frota, J. T., Arruda, B. R., De Melo, T. S., Da Silva, A. A. D. C. A., Brito, G. A. D. C and Rao, V. S. (2012). Antihyperglycemic and hypolipidemic effects of α, β-amyrin, a triterpenoid mixture from Protiumheptaphyllum in mice. Lipids in health and disease, 11(1), 1-8.
53. Scherrer, U., Vollenweider, L., Delabays, A., Savcic, M., Eichenberger, U., Kleger, G. R and Bartsch, P. (1996). Inhaled nitric oxide for high-altitude pulmonary edema. New England Journal of Medicine, 334(10), 624-630.
54. Shyu, Y. S and Hwang, L. S. (2002). Antioxidative activity of the crude extract of lignan glycosides from unroasted Burma black sesame meal. Food Research International, 35(4), 357-365.
55. Simirgiotis, M. J., Schmeda-Hirschmann, G., Borquez, J and Kennelly, E. J. (2013). The *Passiflora tripartita* (Banana Passion) fruit: A source of bioactive flavonoid C-glycosides isolated by HSCCC and characterized by HPLC–DAD–ESI/MS/MS. Molecules, 18(2), 1672-1692.
56. Singh, K., Singh, S. K., Singh, V., Tentu, A. K., Naik, A. K., Singh, N. and Joshii, G. (2021). Clinical, biochemical, therapeutic, and complication strategy of high-altitude pulmonary edema: Update from Western Himalayas. Indian Journal of Respiratory Care, 10(1),1-14.
57. Sowmithra, S., Jain, N. K., Bhonde, R and Datta, I. (2022). Recovery of human embryonic stem cells-derived neural progenitors exposed to hypoxic-ischemic-reperfusion injury by indirect exposure to Wharton's jelly mesenchymal stem cells through phosphatidyl-inositol-3-kinase pathway. Cellular and Molecular Neurobiology, 42, 1167-1188.
58. Sydykov, A., Mamazhakypov, A., Maripov, A., Kosanovic, D., Weissmann, N., Ghofrani, H.A., Sarybaev, A.S. and Schermuly, R.T. (2021). Pulmonary hypertension in acute and chronic high altitude maladaptation disorders. International Journal of Environmental Research and Public Health, 18(4):1-33.
59. Tao, X., Huang, Y., Wang, C., Chen, F., Yang, L., Ling, L., Che, Z. and Chen, X. (2020), Recent developments in molecular docking technology applied in food science: a review. Interational Journal of Food Science and Technology, 55(1)n, 33-45.
60. Teffo, L. S., Aderogba, M. A and Eloff, J. N. (2010). Antibacterial and antioxidant activities of four kaempferol methyl ethers isolated from *Dodonaea viscosa* Jacq. var. angustifolia leaf extracts. South African Journal of Botany, 76(1), 25-29.
61. Tesfaye, S.; Asres, K.; Lulekal, E.; Alebachew, Y.; Tewelde, E.; Kumarihamy, M.; Muhammad, I. Ethiopian Medicinal Plants Traditionally Used for the Treatment of Cancer, Part 2: A Review on Cytotoxic, Antiproliferative, and Antitumor Phytochemicals, and Future Perspective. Molecules 2020, 25, 4032.
62. Usman, R., Khan, A., Gul, S., Rauf, A. and Muhammad, N. (2012). Evaluation of *in vitro* anti-oxidant properties of selected medicinal plants. Middle-East Journal of Medicinal Plants Research, 1(2), 28-31.
63. Viklund, L., Baranchikov, Y., Schroeder, M., Efremenko, A., Demidko, D and Hedenström, E. (2022). Identification of sex-specific compounds in the invasive four-eyed fir bark beetle Polygraphusproximus. Chemoecology, 32(6), 183-195.
64. Xu, W. L., Huang, Y. B., Qian, J. H., Sha, O and Wang, Y. Q. (2005). Separation and purification of stigmasterol and β-sitosterol from phytosterol mixtures by solvent crystallization method. Separation and purification technology, 41(2), 173-178.
65. Yang, J., Nie, J., Ma, X., Wei, Y., Peng, Y., & Wei, X. (2019). Targeting PI3K in cancer: mechanisms and advances in clinical trials. Molecular cancer, 18(1), 1-28.
66. Yasmeen, Z., Basit, A and Tahir, S. (2020). Traditional uses and pharmacological effects of *Anagallis arvensis*: A Review: *Anagallisarvensis*: a review. The International Journal of Frontier Sciences, 4(2), 97-100.

67. Zhao, C., Hu, L., He, X., Li, L., Yin, M., Tettey, A. T and Li, X. (2022). TPN171H alleviates pulmonary hypertension via inhibiting inflammation in hypoxia and monocrotaline-induced rats. Vascular Pharmacology, 145, 1-3.

Abbriviations

Ala= Alanine; Arg= Arginine; Asn= Asparagine; Asp= Aspartic acid; ADME= Absorption, Distribution, Metabolism and Excretion AA= Arachidonic acid; BBB= Blood brain barrier; COX= Cyclooxygenase; cGMP= Guanosine-mono phosphate; cGKs = cGMP-dependent protein kinases; CYP1A2 Gene = Cytochrome P450 Family 1 Subfamily A Member 2; CYP2C19= Cytochrome P450 2C19; CYP2C9= Cytochrome P450 family 2 subfamily C member 9; CYP2D6= Cytochrome P450 2D6; CYP3A4= Cytochrome P450 3A4; Cys= Cysteine; FDA= Food and Drug Administration; GRs= Glucocorticoid receptors; GI absorption= Gastro-intestinal absorption; Glu= Glutamic acid; Gln= Glutamine; Gly= Glycine; HAPE= High altitude pulmonary edema; HAPH= High Altitude Pulmonary Hypertension; His= Histidine; HVR= Hypoxic Ventilatory Response; Ile= Isoleucine; LTs= leukotrienes; Log $P_{o/w}$ (iLOGP)= n-octanol/water partition coefficient; Lys= Lysine; Leu= Leucine; mPAP= Mean Pulmonary Artery Pressure; MD= Molecular Dynamics; MW= Molecular weight; Met= Methionine; NF-$\varkappa$B= Nuclear factor kappa-light-chain-enhancer of activated B cells; PDE-5= Phosphodiesterase-5 enzyme; PGs= Prostaglandins; PI3K= Phosphotidyl inositol 3-Kinase; P-gp= P- glycoprotein; Phe= Phenylalanine; Pro= Proline; RMSD= Root Mean Square Deviation; RMSF= Root Mean Square Fluctuation; Ser= Serine; SDF= 3D structure-data files; TPSA= Tissue Polypeptide Specific Antigen; TPSA= Topological polar surface area; Thr= Threonine; Trp= Tryptophan; Tyr= Tyrosine; Val= Valine