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Evaluating the Therapeutic Effects of *Asystasia gangetica* (Chinese Violet) on the Chronic Respiratory Condition – Asthma

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Abstract

Asthma is a chronic condition affecting the respiratory system. Its symptoms affect the immune pathways via helper T-cells. The plant Asystasia gangetica is prevalent throughout tropical regions around the world. It has been used in some herbal remedies to relieve symptoms of asthma. This study used computational techniques to assess the effectiveness of several phytocompounds present in A. gangetica in relieving symptoms of Asthma. Three proteins associated with asthma, namely IL-5, TRPA1 and IL1RL1, were selected. The phytocompound (1aR,4S,4aS,7R,7aS,7bR)-1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol [Palustrol], was discovered to dock with the best binding affinity to all three proteins. In-silico pharmacological studies also confirmed its drug-likeness. This ligand should be further tested to understand its mode of action, ease of use and effectiveness against asthma symptoms.

Keywords: Asthma, *Asystasia gangetica*, IL-5, TRPA1, IL1RL1, Palustrol

Abbreviations

IL1RL1	:	Interleukin-1 receptor-like 1,
IL-5	:	Interleukin-5
PDB	:	Protein Databank
TRPA1	:	Transient receptor potential cation channel member A1

INTRODUCTION

Asthma is chronic respiratory syndrome. It affects the airways due to inflammation and hyperresponsiveness. Symptoms include breathlessness, wheezing, coughing and chest-tightening etc. All these symptoms do not merely affect the patient in the short term. Longer term effects include lowered quality of life because patients may have poor sleep, fatigue and low concentration for tasks. [1, 2]. The WHO (World health organization) considers asthma to be a major non-communicable disease affecting both adults and children. In 2019, 262 million people were suffering from asthma and it caused 4,55,000 deaths [3]. It is not just allergens, but urbanization, increasing pollution, age, lifestyle issues, such as obesity among the factors driving the prevalence and treatment course for asthma [1]. There is also a higher chance that those in lower or middle-income countries are less likely to be diagnosed with asthma where treatments might also be costlier. In the US alone, 28 million people are suffering from asthma according to 2024 investigations by CDC (Centre for Disease Control).

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Since asthma is heterogenous in nature, it also shows individual variation. Thus, determining specific biomarkers for the disease is hard. Asthma has most often been categorized based on the level of Type-2 Cytokine driven inflammation [1, 4]. Helper T-cells (Th2) produce specific type 2 cytokines like Interleukins (IL) 4, 5, 9 and 13 upon encountering an allergen which leads to the accumulation of eosinophils in the airway. This "type-2 high asthma" sees an overproduction of mucus and IgE (immunoglobulin E) by allergen specific B-cells. In contrast epithelial cell (EC) damage is common across all forms of asthma and EC's role in asthma pathogenesis [1]. Recent advances to understand the pathways of asthma pathogenesis have discovered elevated levels of TRPA1 agonists (Transient receptor potential cation channel member A1) in airways of asthmatic patients [4, 5]. TRPA is a sensory nerve receptor for a diverse set of chemical and physical irritants and is associated with inducing airway inflammation in rodents furthering its role in asthma symptoms. With the advent of -omics technologies and GWAS (Genome wide association studies), new associations between asthma and genes, such as IL1RL1 (interleukin 1 receptor-like 1) have also come to light [2, 6].

Although asthma cannot be cured, its symptoms are manageable with appropriate medication. While avoiding triggers is ideal, it is impractical. Therefore, most asthmatic patients subscribe to therapeutic regimens that include bronchodilators, corticosteroids and approved biologics (monoclonal antibodies) [7, 8]. While modern western medicine has made strides in the control and treatment of asthma, natural remedies too have persisted for such a prevalent and chronic condition. One such treatment has been the use of leaf extracts of the plant *Asystasia gangetica* for its anti-asthmatic properties in Nigerian folk-medicine [9]. *A. gangetica* (acanthaceae) or Chinese violet is a fast-growing herb, tolerant of low-fertility soils and capable of spreading over large area (Image 1). It is present in large parts of tropical Africa and Asia. The plant reaches a height of up to 60 cm, has cream colored small flowers with purple markings. It is a good cover crop to avoid soil-erosion and presents a good source of nutrition for fodder. Additionally, it seems to be used in alternative medicines in Africa and Asia for its anti-microbial and anti-helminthic properties as well as providing relief for kidney stones [10–13]. While the aforementioned studies have extracted and quantified certain phytocompounds of *A. gangetica*, specific dynamics of the disease, its symptoms and the mechanism of treatment have not been tested. Advances, such as in-silico pharmacology and molecular docking techniques allow for a cost-effective method to analyse the prospects of certain ligands as drug compounds [14–16].

In this study, I identified three proteins, IL-5, TRPA1 and IL1RL1 from previous studies on asthma, in this study, I identified three proteins, IL-5, TRPA1 and IL1RL1 based on previous studies on asthma; its causes, symptoms, and cures. These proteins are part of three different pathways but are all implicated in asthma pathogenesis though their exact roles or modes of action may not be known [2, 4, 17]. The structures of these three proteins were further studied. Using molecular docking, modified proteins were used as receptors for phytocompounds present in *A. gangetica*. The study showed one specific ligand (CID 9794494) with the best docking ability with all three proteins and can be considered a candidate for drug-trials.

METHODS

Protein Preparation

We selected three proteins implicated in Asthmatic symptoms and pathologies. The 3-D protein structures of the proteins (i) TRPA1 (PDB ID- 7JUP), (ii) IL-5 (PDB ID- 8TLD), and (iii) IL1RL1 (PDB ID- 4KC3) were downloaded in .pdb format from the RCSB Protein Data Bank [18]. All three proteins were viewed in Biovia Discovery Studio Visualiser v.24.1.0.23298 [19]. Each protein's heteroatoms and water molecules were removed as were the chains which did not contain any amino acids. Polar hydrogens were added to this molecule, and the purified protein structure was saved in .pdb format for further analysis.

Protein Structure Analysis

Ramachandran plots were made for the three purified proteins using PDBSumGenerate's PROCHECK tool [20]. This helped better understand the best conformations for the amino acid residues and validate the protein's backbone.

Ligand Selection

Fifty-four unique phytocompounds associated with *Asystasia gangetica* were retrieved from the Indian medicinal plants, phytochemistry, and therapeutics (IMPPAT). This manually curated online database contains a repertoire of many Indian plants and their pharmacological properties as cited in other scientific literature [21]. Further, we retrieved used Pubchem [22] to retrieve their Pubchem IDs and canonical smiles. Another online tool SWISSADME [23] was used to gauge the pharmacokinetics and drug likeness of the 54 phytocompounds. The phytocompounds were then manually vetted based on the following criteria:

1. Molecular weight <500 Dalton.
2. No. of H-bond acceptors <10.
3. No. of H-bond donors <5.
4. Molecular refractivity 40–130.
5. iLogP < 5.
6. Lipinski rule – 0 flags.
7. GI absorption – High.

Based on the above criteria eight phytocompounds remained. The compounds were retrieved as 2-D structures in .sdf format from Pubchem. Toxicity class and LD_{50} were ascertained for these compounds using the online Protox3.0 database [24].

Molecular Docking

A virtual screening tool PyRx (v 0.8) [25] was used to assess the ligand with the best likelihood for binding to each of the three proteins associated with asthma. Each purified protein was uploaded and converted into a macromolecule for docks. The ligands were uploaded, their energies minimized converted into the ".pdbqt" format. Once the protein and ligands were processed, the Vina wizard tool was used to generate a table containing nine conformations or poses of each ligand when docked to a protein. The ligand with the highest binding affinity were subsequently selected for visualization in 2-d and 3-d forms with the Biovia Discovery Tool.

RESULTS

Protein Visualization and Validation

The three purified proteins, IL-5, TRPA1 and IL1RL1 saved in 3-d form were used to generate Ramachandran plots as presented in Figure 1. Further details are presented in Table 1. Secondary structures associated with each protein are presented in Supplementary Figure 1.

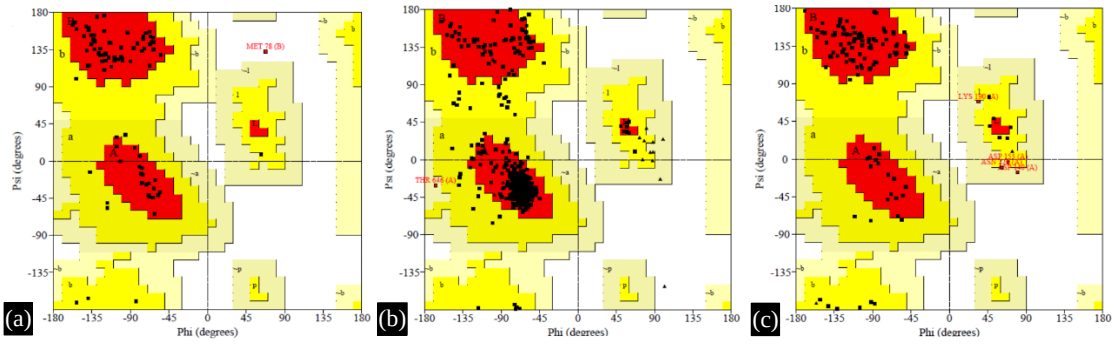

Figure 1. The Ramachandran plots for proteins (a) Interleukin 5 (IL-5), (b) Transient receptor potential cation channel member A1 (TRPA-1), and (c) Interleukin 1 receptor like 1 (IL1RL1) were generated using PROCHECK of PDBSumGenerate. The plot helps to visualize the protein backbone and assess best conformations for polypeptide chains. The red and yellow portions show "allowed regions" that correspond to energetically viable conformations of the proteins. All three proteins had >80% of the residues in "allowed regions" indicating their stability.

Table 1. Details of the Ramachandran plot for all three proteins generated via the PROCHECK tool. All three proteins show more than 80% of the residues in the most allowed regions of the plot (Figure 1).

Protein	IL-5		TRPA1		IL1RL1	
	Number	Percent	Number	Percent	Number	Percent
Total number of residues	94		557		134	
Residues in most favored regions [A,B,L]	71	*87.70%*	465	*91.70%*	94	*81.70%*
Residues in additional allowed regions [a,b,l,p]	9	11.10%	41	8.10%	17	14.80%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.00%	1	0.20%	4	3.50%
Residues in disallowed regions	1	1.20%	0	0.00%	0	0.00%
Number of non-glycine and non-proline residues	81	100.00%	507	100%	115	100%
Number of end-residues (excl. Gly and Pro)	3		9		10	
Number of glycine residues (shown as triangles)	1		24		3	
Number of proline residues	9		17		6	

Ligand Selection

In-silico pharmacology methods helped reduce and select phytocompounds best suited for subsequent docking studies. The eight ligands and some of their physico-chemical properties are outlined in Tables 2 and 3.

Table 2. These eight phytocompounds were chosen as ligands based on criteria outlined in this table.

Ligand	Mol. Weight (da)	H-Acceptors	H-Donors	MR	iLOGP	GI Absorption	Lipinski Violations
11-Phenoxyundecanoic acid	278.39	3	1	82.78	3.79	High	0
4-(4-Fluorophenyl)-1H-pyrazol-5-amine	177.18	2	2	48.39	1.02	High	0
Oct-1-EN-2-OL	128.21	1	1	41.67	2.34	High	0
Acetylcholine	146.21	2	0	39.42	–2.25	High	0
1-Octen-3-OL	128.21	1	1	41.26	2.33	High	0
Luteolin	286.24	6	4	76.01	1.86	High	0
4-Methoxy-3-[(quinolin-8-yloxy)methyl]benzaldehyde	293.32	4	0	84.6	2.57	High	0
(1aR,4S,4aS,7R,7aS,7bR)-1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol	222.37	1	1	68.82	3.02	High	0

Table 3. The chemical formula and canonical smiles of the filtered ligands and their CID were retrieved from PubChem. Toxicity was retrieved using the online tool Protox3.0.

Ligand	Canonical SMILES	CID	Toxicity Class	LD50 mg/kg
11-Phenoxyundecanoic acid	OC(=O)CCCCCCCCCCOc1ccccc1	81597	5	4000
4-(4-Fluorophenyl)-1H-pyrazol-5-amine	Fc1ccc(cc1)c1cn[nH]c1N	201772	4	400
Oct-1-EN-2-OL	CCCCCCC(=C)O	15372159	4	1500
Acetylcholine	CC(=O)OCC[N+](C)(C)C	187	5	5000
1-Octen-3-OL	CCCCCC(C=C)O	18827	4	340
Luteolin	Oc1cc(O)c2c(c1)oc(cc2=O)c1ccc(c(c1)O)O	5280445	5	3919
4-Methoxy-3-[(quinolin-8-yloxy)methyl]benzaldehyde	COc1ccc(cc1COc1cccc2c1nccc2)C=O	590877	4	1250
(1aR,4S,4aS,7R,7aS,7bR)-1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol	C[C@@H]1CC[C@]2([C@@H]1[C@H]1[C@H](C1(C)C)CC[C@@H]2C)O	9794494	4	2000

Molecular Docking

Vina wizard tool from PyRx helped generate tables for each protein consisting of 72 lines- 9 "poses" or conformations of the 8 ligands. Three ligands with the highest binding affinity and RMSD value of zero are tabulated (Table 4). The ligand (1aR,4S,4aS,7R,7aS,7bR)–1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol (CID 9794494 syn Palustrol) had the best binding affinity with all three proteins. The results of this docking were visualized with Biovia Discovery tool showcasing both 2-dimensional and 3-dimensional structures (Figures 2–4).

Table 4. Results of molecular docking for each of the three chosen proteins associated with Asthma. Docking was tested for eight ligands that passed in-silico pharmacology filters. The lowest numbers for binding affinity show the most stable protein-ligand complex.

Protein	Ligand	Binding Affinity	rmsd/ub	rmsd/lb
IL5	(1aR,4S,4aS,7R,7aS,7bR)-1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol	–7.1	0	0
IL5	Luteolin	–7	0	0
IL5	4-Methoxy-3-[(quinolin-8-yloxy)methyl]benzaldehyde	–6.5	0	0
TRPA1	(1aR,4S,4aS,7R,7aS,7bR)-1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol	–9.9	0	0
TRPA1	4-Methoxy-3-[(quinolin-8-yloxy)methyl]benzaldehyde	–9	0	0
TRPA1	Luteolin	–8.6	0	0
IL1RL1	(1aR,4S,4aS,7R,7aS,7bR)-1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol	–9.6		
IL1RL1	Luteolin	–6.6	0	0
IL1RL1	4-Methoxy-3-[(quinolin-8-yloxy)methyl]benzaldehyde	–6.4	0	0

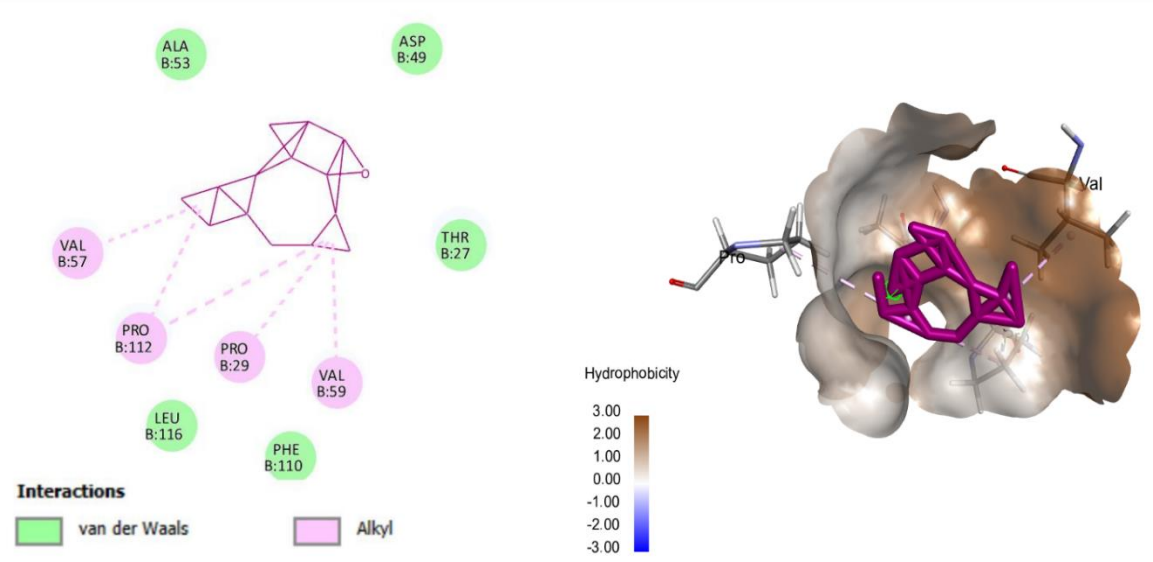

Figure 2. 2-dimensional (left) and 3-dimensional (right) representations of the molecular docking process were created for protein IL-5 (Interleukin 5) as the receptor. Its interaction with the ligand 9794494 (1aR,4S,4aS,7R,7aS,7bR)-1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol) had the best binding affinity. The interacting amino acids on the protein chain are represented by three letter codes. The hydrophobicity of the complex is presented. Details of the ligand-receptor complex are presented in Table 4.

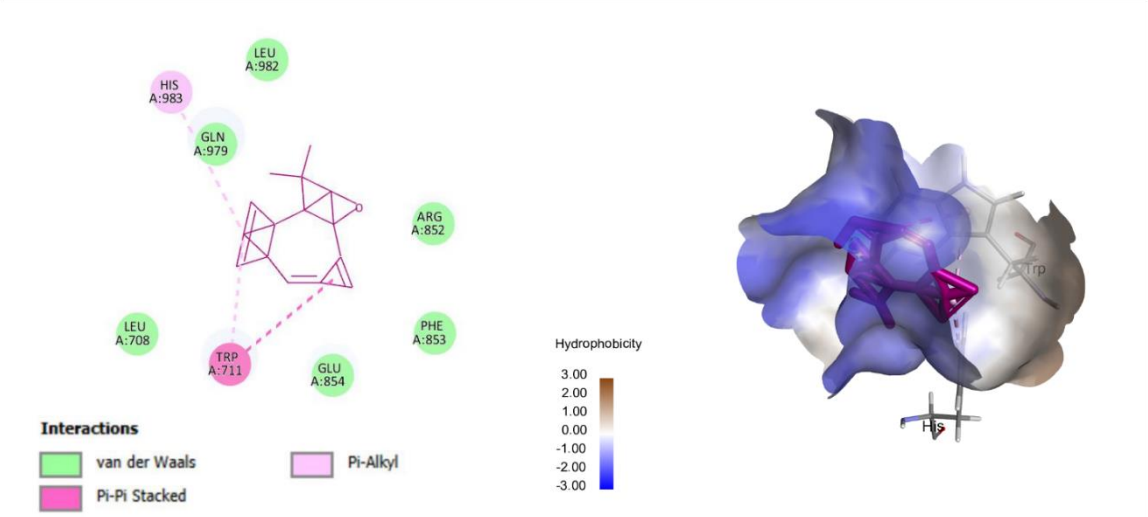

Figure 3. 2-dimensional (left) and 3-dimensional (right) representations of the molecular docking process were created for protein TRPA1 (Transient receptor potential cation channel member A1) as the receptor. Its interaction with the ligand 9794494 (1aR,4S,4aS,7R,7aS,7bR)-1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol) had the best binding affinity. The interacting amino acids on the protein chain are represented by three letter codes. The hydrophobicity of the complex is presented. Details of the ligand-receptor complex are presented in Table 4.

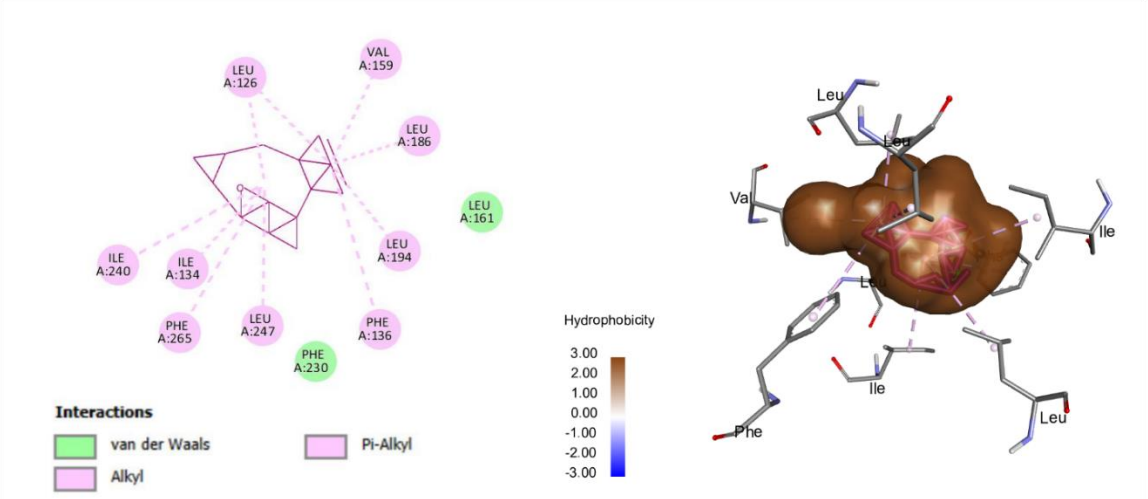

Figure 4. 2-dimensional (left) and 3-dimensional (right) representations of the molecular docking process were created for protein IL1RL1 (Interleukin 1 receptor like 1) as the receptor. Its interaction with the ligand 9794494 (1aR,4S,4aS,7R,7aS,7bR)-1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol) had the best binding affinity. The interacting amino acids on the protein chain are represented by three letter codes. The hydrophobicity of the complex is presented. Details of the ligand-receptor complex are presented in Table 4.

DISCUSSION

This study explored the phytocompounds of *A. gangetica* in terms of their therapeutic effects and drug-likeness using in-silico pharmacology techniques and molecular docking. Since asthma is a chronic respiratory syndrome driven by multiple genetic, lifestyle and environmental factors, I focused on three proteins which regulate inflammation of the airways via different pathways. Having three targets allowed me to look for phytocompounds in *A. gangetica* which may act to ameliorate the downstream effects of all three proteins albeit in different ways.

IL-5 (downstream in the IL-17 signaling pathway), TRPA1 (one of the inflammatory mediators regulated TRP channels) and IL1RL1 (featured in the cytokine-cytokine receptor interaction and MAPK pathways) proteins were purified and their backbones assured to allow stable conformations. The phytocompounds were retrieved and filtered based on their drug-likeness and toxicity. The same three ligands had the most stable and highest binding affinity with all three proteins. They were (1aR,4S,4aS,7R,7aS,7bR)-1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol, Luteolin and 4-Methoxy-3-[(quinolin-8-yloxy)methyl]benzaldehyde. Since (1aR,4S,4aS,7R,7aS,7bR)-1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol (CID 9794494 syn. Palustrol) had the best affinity with all three ligands, we should conduct further empirical and in-vitro tests on this specific phytocompound derived from *A. gangetica.* Palustrol is an aromadrendrane sesquiterpenoid which has been found in *Rhododendron tomentosum* (syn *Ledum palustre* wild rosemary) [26], in conifer oleoresins [27] and is a compound in the essential oil of clove *Syzegium aromaticum* [28]. While specific effects of the phytocompound on specific proteins have never been tested before, plants containing Palustrol have shown anti-inflammatory properties and are also used in alternative medicine [29]. For instance oedema induced in rats showed significant reduction when treated with oil-extracts of *L. palustre* [30]. Similarly, the anti-inflammatory potential of extracts from *Baccharis* plants were tested on human cells showing how palustrol may also play a minor role in controlling inflammation [31].

Compared to the other plants containing Palustrol, *A. gangetica* is more widespread and easier to culture and spread. It has also been employed for its anti-helminthic, anti-microbial as well as anti-asthmatic features previously [9, 10, 12, 32]. In this study I have also been able to test and visualize its therapeutic potential in asthma patients who deal with inflammation in their airways causing coughing, wheezing, breathlessness, etc. Future studies can be two pronged. One should determine the exact amount of palustrol that can be extracted from *A. gangetica,* study its chemical aspects and effectiveness to bind not just to the three proteins tested here but other proteins associated with asthma symptoms as well. Meanwhile, empirical testing can ascertain the development and use of *A. gangetica* as a drug to ameliorate respiratory syndromes associated with asthma.

CONCLUSIONS

This study explored the possibility of utilizing *A. gangetica* to remedy symptoms associated with Asthma. For three different proteins associated with the disease, one single ligand-the phytocompound (1aR,4S,4aS,7R,7aS,7bR)-1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol) (syn Palustrol) showed great potential as a drug compound. Asthma is chronic, affecting the respiratory system and ranging in severity. Since *A. gangetica* is found growing wild in the tropics, it can certainly be cultured and utilized. Future studies will need to be more nuanced. We need to ascertain the amount of palustrol we can extract from *A. gangetica*, understand its mode of action after binding to proteins of interest and run experiments to ascertain the concentrations that best relieve asthmatic symptoms.

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