INSILICO ANALYSIS OF COMPOUNDS FROM MEDICINAL PLANTS AGAINST ATOPIC DERMATITIS [AD]

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***Abstract*:** Skin is the most extensive and diverse organs of the human body. It provides contact to the environment and protects the human body from unfavourable external factors. Atopic Dermatitis (AD) is a chronic inflammatory skin disease. It is characterized by strong itchy and inflamed skin condition with scaly plaques. The patient suffering from AD eventually develops asthma and allergic rhinitis in a process called 'Atopic March' (AM). The protein Thymic Stromal Lymphopoietin (TSLP) is thought to drive AD and AM. TSLP is highly expressed in human cutaneous epithelial cells. The medicinal plants are claimed to have anti-inflammatory and anti-itching properties that can be used to treat AD. The paper presents the results of the in silico docking study regarding the interactions between TSLP and the compounds from the medicinal plants like *Trigonellafoenum, Rosmarinusofficinalis, Ficuscarica, Althaeae radix, Prunuspersica, Achyranthesaspera, Allium cepa, Curcuma longa, Camellia sinensis, Lycopersiconesculentum, Cannabis sativus, Oenotherabiennis*and *Matricaria chamomile.* The compounds were analyzed for their significant interaction with the target, Absorption, Distribution, Metabolism, Excretion and Toxicology (ADMET) properties, drug-likeness using the Schrondinger software. The docking results were observed which indicates that 10 compoundswere found to be active. Among 10 compounds, thecompoundRosmarinic acid from *Rosmarinusofficinalis*showed significant result with G.score of -6.28kcal/mol. The compound interacts with the residues Glutamic acid (GLU-78), Methionine (MET-28), Serine (SER-114) and Glutamine (GLN-80). This study may pave way for the future studies to predict the stability of the complexes formed and toconsider them as potential candidates to develop a drug out of them.

***Keywords*:** Atopic Dermatitis, Thymic Stromal Lymphopoietin(TSLP), *In silico* docking studies, ADMET properties, *Rosmarinus officinalis*, Rosmarinic acid.

I. INTRODUCTION:

Patients afflicted with skin diseases suffer greatly as it affects their routine life. Atopic Dermatitis (AD) is a chronic itch and inflammatory disorder of the skin that affects the general population of about one in ten people. AD is characterized by intolerable and incurable itch.[1]AD patients go on to develop asthma along with allergic rhinitis in a process known as 'Atopic March' (AM). [2] several studies suggest that approximately half of the AD patients may develop asthma and two-third may develop allergic rhinitis. [3] It affects a large number of children and adults in industrialized countries. Around 10-20% of infants and 3-5% of adults were affected by AD.[4] In 45% of children, the onset of AD occurs during the first 6 months of life, 60% of children were affected during the first year of life, and before the age of 5 years at least 85% of children were affected.[5] In those children with onset before the age of 2 years, 20% will have persisting manifestations of the disease and an additional 17% will have intermittent symptoms by the age of 7 years.[6] In adults with AD, only 16.8% had onset after adolescence.[7]The fundamental lesion in AD is a defective skin barrier that results in dry itchy skin, and is aggravated by mechanical injury inflicted by scratching. This allows the entry of pathogens via the skin and creates a milieu that shapes the immune response to those pathogens.[8]

AD and AM share a common immunological response, involving a T helper type 2 (Th2) cell-mediated allergic inflammation.The important role of Th1 and Th2 cytokines that play in the skin inflammatory response has been demonstrated in experimental models of allergen-induced allergic skin inflammation in mice.[9] This inflammation is characterized by the secretion of cytokines (IL-4, IL-5, IL-13 and TNFα) by CD_4+ T-cells, which triggers increased IgE antibody-production by B-cells. In turn IgE binds to mast cells and facilitates initiation of allergic reactions to drive infilteration of leucocytes into the skin dermis. The Dendritic Cells (DC) control polarization of the native CD_4+ T-cells to differentiate into TH2 lymphocytes.[10] The signaling between the epithelial cells and the immune cells via the cytokine Thymic Stromal Lymphopoietin (TSLP) is thought to drive AD and AM.TSLP is highly expressed by keratinocytes in skin lesions of patients with atopic AD and is associated with dendritic cell activation, suggesting that TSLP might be a master switch for allergic inflammation at the epithelial cell–dendritic cell interface.[11]

TSLP is a novel 1L-7 like cytokine, originally cloned from murine thymic stromal cell lines, that supports the growth and differentiation of B-cells and proliferation of T-cells.[12] TSLP is highly expressed in human cutaneous epithelial cells in AD and bronchial epithelial cells in asthma.[13] Over expression of TSLP in keratinocytes, triggers robust itch-evoked scratching, the development of an AD-like phenotype and ultimately asthma-like lung inflammation in mice.[14] A TSLP binding protein identified in mouse, referred to as TSLP-receptor (TSLPR) binds to TSLP with low affinity and the high affinity a heterodimer of TSLPR and interleukin 7 receptor α (IL-7Rα).[15]

In case of skin lesion or damages, cytokines are found at high levels (IL-1β, TNF-α, IL-4 and IL-13). This can synergize to induce the TSLP expression by keratinocytes. TSLP activates various cell populations and predominantly, dendritic Cells (DC). After the activation by TSLP, DC proliferates, differentiates and then migrates to the lymph nodes where the antigen is presented to immature T-lymphocyte. The presentation of antigen enables differentiation of the T-cells to Th2 cells, which are essential for the

immune responses of AD termed ("Th2 cell mediated allergic inflammation").[16]The TSLP inhibitors are often recommended before the onset of AD complications. In severe cases, the efficiency of the inhibitors would be reduced.

Since time immemorial, people relied on nature as cure for their diseases.[17] The secondary metabolites synthesized by the plants involve in important biological functions, and also as defense against predators. This paper presents the study of anti-inflammatory activity of the plant raw material related to its influence on the skin. Medicinal plants used in treatment of Atopic Dermatitis-an inflammatory disease of the skin are *Trigonella foenum, Rosmarinus officinalis, Ficuscarica, Althaeae radix, Prunus persica, Achyranthes aspera, Allium cepa, Curcuma longa, Camellia sinensis, Lycopersicon esculentum, Cannabis sativus, Oenothera biennis, Matricaria chamomile,* to name a few. The compounds from these plants were analyzed for their binding efficiency with the target protein TSLP through molecular docking in this study. Further, the compounds targeting TSLP may be used as a model for the development of synthetic drugs.

II. MATERIALS AND METHODS:

The 3D structure of the protein target TSLP was retrieved from the Protein Data Bank (PDB) database (PDB ID: 5J11) (https://www.rcsb.org/pdb/home/home.do) which comes under the classification-Signaling protein and the source organism is *Homosapiens*. The phytochemical compounds of nearly 50 plants were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The plants taken were *Trigonella foenum, Rosmarinus officinalis, Ficuscarica, Althaeae radix, Prunus persica, Achyranthes aspera, Allium cepa, Curcuma longa, Camellia sinensis, Lycopersicon esculentum, Cannabis sativus, Oenothera biennis, Matricaria chamomile,* to name a few. 83 compounds were retrieved from these plants. The Absorption, Distribution, Metabolism, Excretion and Toxicology (ADMET) properties of the compounds were predicted through QikProp module of Schrondinger software. Finally the compounds exhibiting drug-likeness were taken into account for docking studies with the target protein using Glide module of the Schrondinger software. The interactions between the target and the compounds were observed using PyMol software which can produce a high-quality of 3D images with small molecules and macromolecules, such as proteins.

III. RESULT AND DISCUSSION:

The Absorption, Distribution, Metabolism, Excretion and Toxicology (ADMET) properties are important for drugs, and prediction of these properties in advance will save the cost of drug discovery substantially. Around 23 compounds (bold in the Table 3.1) have high oral absorption percent which is considered as important criteria for the development of potential drug. The ability to penetrate the blood–brain barrier is critical for drugs targeting central nervous system, which is represented by the ratio of its concentration in brain and in blood.[18]Though the compounds are subjected to predict the brain and blood partition coefficient, most of the drugs for skin diseases have topical application like lotions or creams. Lipinski's rule of five also known as the Pfizer's rule of five or simply the rule of five (RO5), is a thumb rule to evaluate drug-likeness i.e. the ability of chemical compound having specific physical and chemical properties relate to itspharmacological or biological activity. The compound Reserpine had satisfied only 2 components of the Lipinski's rule of five.The molecular weight, hydrogen bond acceptors and hydrogen bond donors are the other factors are taken into account in this study. The plants compounds that didn't fall in the normal range were omitted for further processes and study. Nearly 40% of drug candidates fail in clinical trials due to poor Absorption, Distribution, Metabolism, Excretion and Toxicology (ADMET) properties. The compounds from the selected plants were first subjected for analyzing (ADMET) properties. Among 83 compounds retrieved, only 52 compounds showed drug likeness for ADMET properties analysis using QikProp module of the Schrondinger software. The analysis of ADME properties of the plant compounds were tabulated (Table 3.1).The docking studies of the retrieved plant compounds showed drug likeness in ADMET properties analysis, with the target protein TSLP, using Schrondinger software in 10 compounds which were found to be active. The interactions were viewed using the PyMol tool. The interactions of phytochemical compounds with TSLP were tabulated representing their G-score, number of hydrogen bonds, bond length and interacting residues (Table 3.2).

Table 3.1 Analysis of ADME properties of the plant compounds

S.No.	Compound Name &PubChem ID	Molecular weight (Da)	Donor - Hydrogen Bonds	Acceptor - Hydrogen Bonds	QlogP o/w	Predicted brain/blood partition coefficient	Human Oral Absorption	Percent Human Oral Absorption	Rule of Five	Rule of Three
	Normal Range	**130 - 725**	**0 - 6**	**2.0 - 20.0**	**–2.0 – 6.5**	**–3.0 – 1.2**	**1- Low, 2- Medium, 3- High**	**>80% is high <25% is poor**	**Maximum 4**	**Maximum 3**
1	Reserpine (5770)	608.7	1	10.7	4.6	-0.7	2.0	70.8	2	2
2	Aloin A (12305761)	418.4	5	11.7	-0.6	-2.8	1.0	28.5	1	2
3	Salannin(6437066)	596.7	0	9.9	4.9	-0.5	3.0	**100.0**	1	1
4	Salicylate (54675850)	138.1	1	1.75	2.3	-0.7	3.0	76.6	0	0
5	Benzoate (242)	122.1	1	2	1.9	-0.3	3.0	80.9	0	0

6	Allicin(65036)	162.3	0	4	1.4	0.1	3.0	75.1	0	0
7	AllylTrisulfide(16315)	178.3	0	0.5	4.2	0.4	3.0	**100.0**	0	0
8	Allyl Sulfide (11617)	114.2	0	0.5	2.9	0.4	3.0	**100.0**	0	0
9	Andrographis Extract (6436016)	350.5	3	8.1	1.4	-1.4	3.0	76.9	0	0
10	Nicotine (89594)	162.2	0	3.5	1.2	0.7	3.0	**89.4**	0	0
11	Faradiol(397486)	442.7	2	3.4	6.0	-0.2	1.0	**100.0**	1	1
12	Quercetin(5280343)	302.2	4	5.25	0.4	-2.3	2.0	52.9	0	1
13	Chloramphenicol (5959)	323.1	3	6.9	1.1	-1.5	3.0	66.0	0	0
14	Dronabinol(16078)	314.5	1	1.5	5.7	-0.1	1.0	**100.0**	1	1
15	Delta 8-Tetrahydro cannabinol(2977)	314.5	1	1.5	5.7	-0.1	1.0	**100.0**	1	1
16	Oleanic Acid (10494)	456.7	2	3.7	6.2	-0.4	1.0	**94.5**	1	1
17	Ursolic Acid (64945)	456.7	2	3.7	6.1	-0.5	1.0	**93.6**	1	1
18	Caryophyllene(5281515)	204.4	0	0	4.9	1.0	3.0	**100.0**	0	1
19	Eugenol(3314)	164.2	1	1.5	2.7	-0.1	3.0	**100.0**	0	0
20	Transcrocetinate(52812 32)	328.4	2	4	4.9	-2.7	2.0	70.8	0	1
21	Curcuma Longa oleoresin (11980944)	368.4	2	7	2.9	-2.1	3.0	**85.5**	0	0
22	Curcumin(969516)	368.4	2	7	2.9	-2.1	3.0	**85.5**	0	0
23	Caffeic Acid (689043)	180.2	3	3.5	0.6	-1.6	2.0	54.2	0	1
24	Cianidanol(9064)	290.3	5	5.45	0.5	-1.8	2.0	61.0	0	1
25	Gallic Acid (370)	170.1	4	4.25	-0.6	-1.7	2.0	41.5	0	1
26	Wallichiiside A (54589907)	380.4	6	16.05	-1.6	-1.5	2.0	44.7	1	0
27	Syringic Acid (10742)	198.2	2	4.25	1.0	-1.0	3.0	66.7	0	0
28	Arabinose (66308)	150.1	3	7.8	-1.9	-1.7	2.0	47.4	0	0
29	Coumarin(323)	146.1	0	2.5	1.4	0.0	3.0	**94.3**	0	0
30	Kaempferol(5280863)	286.2	3	4.5	1.1	-1.8	3.0	64.7	0	0
31	Linalool (6549)	154.3	1	0.75	3.1	0.1	3.0	**100.0**	0	0
32	Lavandulyl Acetate (30247)	196.3	0	2	3.4	-0.3	3.0	**100.0**	0	0
33	Diclofenac Sodium (5018304)	296.2	2	2.5	4.5	-0.1	3.0	**100.0**	0	0
34	Quinone (4650)	108.1	0	4	-0.8	-0.4	2.0	72.7	0	0
35	Ascorbic Acid (54670067)	176.1	4	7.9	-1.9	-1.7	2.0	45.0	0	0
36	Shikimic Acid (8742)	174.2	4	7.1	-1.0	-1.5	2.0	41.6	0	1
37	Benzydamine(12555)	309.4	0	3	4.7	0.3	3.0	**100.0**	0	0
38	Quercitrin(5280459)	448.4	6	12.05	-0.5	-2.8	2.0	16.7	2	2
39	Scillavone A (101863378)	344.3	2	5.5	2.1	-1.3	3.0	81.5	0	0
40	Scillavone B (91365537)	346.3	2	5.5	2.4	-1.4	2.0	84.9	0	1
41	Chlorogenic Acid (1794427)	354.3	6	9.65	-0.2	-3.1	1.0	20.3	1	1
42	Camphor (2537)	152.2	0	2	1.9	0.3	3.0	**100.0**	0	0
43	Rosmarinic Acid (5281792)	360.3	5	7	1.2	-3.4	1.0	40.6	0	1
44	Sesquiterpene lactone CP-2 (98958)	248.3	1	4.7	1.9	-0.4	3.0	**92.6**	0	0
45	D-Galacturonic Acid (439215)	194.1	4	9.5	-1.8	-1.8	2.0	31.3	0	1
46	Gamolenic Acid (5280933)	278.4	1	2	5.2	-1.1	3.0	**89.1**	1	0

47	Linoleic Acid (5280450)	280.5	1	2	5.3	-1.2	3.0	**89.4**	1	0
48	Quercetin(5280343)	302.2	4	5.25	0.4	-2.3	2.0	52.9	0	1
49	Ajoene(5386591)	234.4	0	5	2.6	0.0	3.0	**82.2**	0	0
50	20-OH ecdysone(5459840)	480.6	6	9.35	2.2	-2.2	2.0	60.4	1	1
51	Methyl AloesinylCinnamate(9958939)	540.6	4	13.75	2.1	-2.3	2.0	63.4	1	1
52	Carminic Acid (10255083)	492.4	6	14.5	-1.5	-4.3	1.0	0.0	2	2

Table 3.2: Interactions of the Plant Compounds with the target protein Thymic Stromal Lymphopoietin (TSLP)

S.No.	Plant Name	Compound Name	PubChem ID	Interacting residues	Bond Length(Å)	No. of Hydrogen Bonds	G.Score (kcal/mol)
1.	*Rosmarinusofficinalis*	Rosmarinic acid	5281792	GLN78	2.0(O-H)	7	-6.28
					1.6(O-H)		
				SER114	1.8(H-O)		
					1.7(O-H)		
					1.6(O-H)		
				MET28	2.2(H-O)		
				GLN80	2.6(H-O)		
2.	*Prunuspersica*	Chlorogenic acid	1794427	TYR46	2.1(H-O)	2	-4.77
				LYS38	2.2(O-H)		
3.	*Achyranthesaspera*	20-Hydroxy ecdysone	5459840	MET28	2.1(H-O)	5	-4.06
					1.7(H-O)		
				GLU78	1.8(O-H)		
					1.7(O-H)		
				TYR29	2.7(O-H)		
4.	*Allium cepa*	Salicylate	54675850	GLN158	2.8(H-O)	3	-4.05
					2.0(H-O)		
				ARG153	2.0(H-O)		
5.	*Curcuma longa*	Curcuma longa oleoresin	11980944	MET28	2.7(H-O)	6	-4.00
					1.9(H-O)		
				TYR29	2.7(O-H)		
					2.2(H-O)		
				GLN80	2.1(H-O)		
				SER92	2.5(H-O)		
6.	*Curcuma longa*	Curcumin	969516	ASN71	2.5(H-O)	4	-3.57
					2.0(O-H)		
				MET28	1.8(H-O)		
				GLN80	2.8(H-O)		
7.	*Camellia sinensis*	Chloramphenicol	5959	ARG150	2.6(H-O)	5	-2.5
					2.5(H-O)		
					2.2(H-O)		
				ARG149	2.1(H-O)		
				ASN65	2.2(O-H)		
					2.5(H-O)		
				TYR29	2.1(O-H)		
8.	*Cannabis sativus*	Delta 8-Tetrahydrocannabinol	2977	SER48	1.8(O-H)	1	-1.60
9.	*Oenotherabiennis*	Linoelic acid	5280450	LYS101	2.6(H-O)	2	-0.58
					1.6(H-O)		
10.	*Matricaria chamomile*	Benzydamine	12555	ASN85	1.9(O-H)	2	-0.48
					2.1(H-O)		

Among 10 compounds, Rosmarinic acid (PubChem CID: 5281792) (Fig.3.1) from *Rosmarinusofficinalis*(Rosemary) showed the least G.score of -6.28 kcal/mol and had 7 hydrogen bonds.

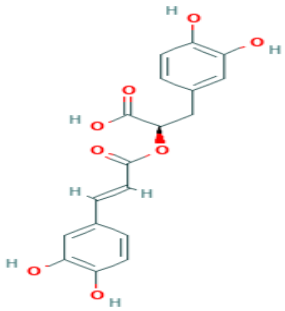

Fig.3.1: Chemical structure of Rosmarinicacid.

The compound binds with four active residues of the target protein. The residues Glutamic acid (GLU78) had two hydrogen bonds of bond lengths 1.6, 2.0Å. The residue Serine (SER114) had three hydrogen bonds of bond lengths 1.8, 1.7 and 1.6Å. The residues Methionine (MET28) and Glutamine (GLN80) had single hydrogen bond with bond length 2.2 and 2.6Å respectively. The interaction of rosmarinic acid has been shown below (Fig.3.2).

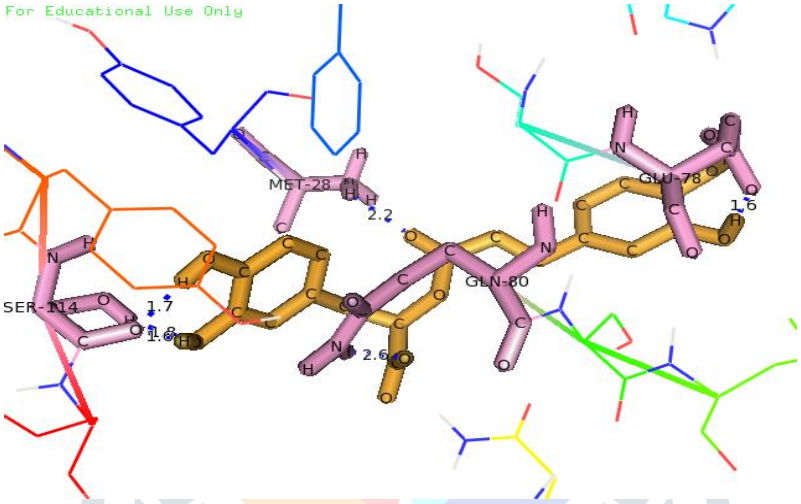

Fig.3.2: The PyMol view of interaction of Rosmarinic acid with TSLP

The compound Chlorogenic acid from *Prunuspersica* (Peach) with G.score of -4.77kcal/mol had 2 hydrogen bonds with residues Threonine (THR46) and Lysine (LYS38) of the target protein.The compounds 20-Hydroxy ecdysone from *Achyranthesaspera*(Chaff Flowers) had G.score of -4.06kcal/mol which is more close to the compound Salicylate from *Allium cepa*with G.score of -4.05kcal/mol. But Salicylate had only three bonds whereas 20-Hydroxy ecdysone had five bonds. The compound Curcuma longa oleoresin from *Curcuma longa* had showed the G.scoreof-4kcal/mol whereas the compound Curcumin from the same plant interacted with the target at residues Methionine (MET28), Asparagine (ASN71) and Glutamine (GLN80), forming 4 hydrogen bonds with G.score value of -3.57kcal/mol. The compound Chloramphenicol, though with G.score -2.51 kcal/mol, had 6 hydrogen bonds. The compound Delta 8- Tetrahydrocannabinol from *Cannabis sativus*(Hemp) had interacted with the target resulted in the formation of a single hydrogen bond. It bound to the Serine (SER114) with a bond length of 2.1 Å showed the G.score of -1.6kcal/mol. The compounds Linoelic acid from *Oenotherabiennis* (Evening Primrose) and Benzydamine from *Matricaria chamomile* (Chamomile) had two hydrogen bonds with the target but with the G.score more the -1kcal/mol.

The bond length and the bond strength are inversely related to each other. “The greater the bond length is, the weaker the bond strength”.The above compounds bind to the target protein with the bond lengths ranging from 1.5 Å to 2.5 Å. The compounds Salicylate and Curcumin had formed the bond of bond length 2.8Å with residues GLN158 and GLN80 respectively. Thus the bonds with bond length 2.8 Å can be represented as the weakest bond among the other bonds formed. But Curcumin serves as an antioxidant and a natural anti-inflammatory compound whereas Salicylate is widely used to treat skin and foot diseases. The above mentioned compounds showing interactions can be subjected to further studies in order to bring new insights in drug development.

IV. CONCLUSION:

The present study indicates the efficient binding of the plant compounds with the active amino acid residues of the target TSLP. The compounds showing drug-likeness were selected using the ADMET property analysis. Among several compounds retrieved from medicinal plants, only 10 compounds showed significant binding in the docking analysis using Schrodinger software. The compound Rosmarinic acid had significant G.score value of -6.28 kcal/mol and interactions with the active site residues (GLU78, MET28, SER114 and GLN80) of TSLP. As TSLP represents a master switch of allergic inflammation at the epithelial cell-Dendritic Cell (DC) interface in Atopic Dermatitis, the plant compounds should be explored more in order to develop a new potential drug molecule.

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