Investigation of *Terminalia arjuna* as potential IL-4 and IL-13 modulator for the prevention of autoimmune diabetes: A Pharmacoinformatics-based study

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Abstract

Cytokines are proteins that play a critical role in immune cells' development, maturation, and functional activities. For the first time, we have investigated the potential role of *Terminalia arjuna* as IL-4 and IL-13 modulators for preventing T1DM, i.e., autoimmune diabetes. It has been well documented that the stimulation of IL-4 and IL-13 can regulate the level of type 2 cytokines which can be maintained with the level of type 1 cytokines. The present study investigated gallic acid, arjunolic acid, luteolin, ellagic acid, and arjunone for their potential modulating activity of IL-4 and IL-13. The active amino acid residues identified for IL-4 are VAL51, HIS58, ASP87, THR30, GLN54, THR63, ARG64, LYS84, and GLU60. The active amino acid residues identified for IL-13 are H: GLU46, H: TRP47, H: GLN61, L: PHE98, L: VAL97, L: GLU162, L: THR163, H: ARG105, L: GLN38, L: ASP85, H: GLY42, L: GLY41, H: PRO41, H: TRP47, and L: PHE98. The phytoconstituents demonstrated better modulating activity towards IL-13 than IL-4. Luteolin displayed better potential for both IL-4 and IL-13, and therefore we concluded that it could be used to modulate the activity of IL-4 and IL-13 for the prevention of autoimmune diabetes.

Keywords: IL-4, IL-13, T1DM, *Terminalia arjuna*, luteolin, autoimmune diabetes

1. Introduction

Cytokines are proteins that are vital in immune cell development, maturation, and function (Hill & Artis, 2010). The type 1 and type 2 cytokines are frequently antagonistic to one another in their activities (Zaccone *et al.,* 1999). Type 2 anti-inflammatory cytokines such as

interleukin-13, interleukin-10, interleukin-6, interleukin-5, and interleukin-4, which are not cytotoxic, promote humoral immune responses, aid in the formation of IgE, IgG1, and IgG2b (Swain, 1995), and induce a functional state of T cells and macrophages. The balance of the two types of cytokines is tightly regulated. Type 1 proinflammatory cytokine dysregulation may play a role in the clinical course, and possibly even the emergence, of organ-specific autoimmune diseases such as thyroiditis, multiple sclerosis, and type 1 diabetes mellitus (T1DM) (Mandrup-Poulsen *et al*., 1996), (Rabinovitch, 1994), (Zaccone *et al*., 1999).

T1DM is an autoimmune disorder characterised by the gradual and insidious depletion of insulin-producing beta cells. Current T1DM treatment is mainly based on exogenous insulin replacement, emphasising the need for immunotherapies to both restrict the progression of the illness and enhance clinical results in the long term (Bach, 1994). Growing data indicates that in humans and rodents with type 1 diabetes, an elevated type 1 cytokine response is encouraged by a deficient generation of type 2 cytokines. On the other hand, non-obese diabetic (NOD) mice generate less IL-4 and more IFN-γ in vitro due to reduced CD1-restricted T-cells. Furthermore, in humans and BioBreeding (BB) rats with type 1 diabetes, blood levels of type 1 cytokines, but not type 2 cytokines, increase during the disease's beginning (Zaccone *et al.,* 1999).

A type 2 cytokine profile defined by elevated levels of IL-4 and decreased levels of IFN-g is seen in the insulitis lesions of both male and female NOD mice with low T1DM incidence (Fox & Danska, 1997), (Rabinovitch *et al.,* 1995). It could be concluded that IL-4 (Cameron *et al.,* 1997), (Rapoport *et al.,* 1993), pancreatically generated IL-4, and IL-10 (Pennline et al., 1994) all prevent T1DM in female NOD mice, and the effects of IL-4 are linked to a shift in cytokine production toward a type 2 phenotype. IL-13 is an essential anti-inflammatory cytokine produced primarily by Th-2 lymphocytes. It is produced in significant quantities by lymphocytes of the Th-2 subset (Zaccone *et al.,* 1999). The effect of IL-13 treatment on the development of T1DM in diabetic NOD mice was studied. Long-term therapy of recombinant human IL-13 significantly reduced the incidence of spontaneous T1DM in mice (De Vries, 1998).

Terminalia arjuna has been used as a cardiotonic in treating ischemic heart disease, heart failure, myocardium necrosis, cardiomyopathy, atherosclerosis, and a variety of human diseases such as anaemia, viral disease, and venereal disease, as well as for general health maintenance. It has antibacterial, hypocholesterolemic, antitumoral, antimicrobial, antiallergic, antioxidant, and antifeedant properties, as well as antifertility and anti-HIV properties (Amalraj & Gopi, 2017), (Bachaya *et al.,* 2009), (Ram *et al.,* 1997). Terminalia arjuna has been proven to have significant hypolipidemic properties and is used to treat ulcers, fractures, and hepatic disease. *Terminalia arjuna's* saponin glycosides are believed responsible for its inotropic effects. At the same time, flavonoids/phenolics may provide antioxidant potential in addition to vasodilation action, validating the plant's multiple activities for its cardioprotective function (Dwivedi, 2007). In animal models, *Terminaliashown*hown has anti-diabetic activity (Kapoor *et al.,* 2014).

It has been well documented that the stimulation of IL-4 and IL-13 can regulate the level of type 2 cytokines which can be maintained with the level of type 1 cytokines. For the first time, we have investigated the potential role of *Terminalia arjuna* as IL-4 and IL-13 modulators for preventing T1DM, i.e., autoimmune diabetes. We have screened the major

phytoconstituents using the Lipinski rule of five, ADMET properties, toxicity prediction, and binding affinity towards IL-4 and IL-13.

2. Material and Methods

2.1 Pharmacokinetics and toxicity prediction of phytoconstituents

The Lipinski rule and the pharmacokinetic characteristics of phytoconstituents were investigated using PubChem (Kim *et al.,* 2021), molinspiration ("Molinspiration cheminformatics," 2006), (Unnisa *et al.,* 2020), and SwissADME (Daina *et al.,* 2017), (Unnisa *et al.,* 2020) servers. The major phytoconstituents have been selected from each class of chemicals in *Terminalia arjuna*. The toxicity of the phytoconstituents has been predicted using ProTox-II, an accessible webserver for *in silico* toxicity predictions of new derivatives (http://tox.charite.de/protox_II).

2.2 Molecular docking studies

To perform molecular docking, the Autodock vina 1.1.2 with PyRx Virtual Screening Tool 0.8 software of the Chimera version 1.10.2 (Dallakyan & Olson, 2015), (Dian *et al.*, 2021) and the Biovia Discovery studio were used (Miyata, 2015). The structure of selected phytoconstituents (SDF File) was obtained from the US National Library of Medicine PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Figure 1 depicts the structures of all of the selected phytoconstituents. Universal Force Field (UFF) (Rappé *et al.,* 1992), performed the energy minimisation. The RCSB-PDB (https://www.rcsb.org/) was used to obtain the crystal structures of IL-4 and IL-13. Figure 2 depicts the 3D structures of the enzymes along with their PDB IDs. The three-dimensional grid box of known size (IL-4, size x = 52.3300A^o, size y = 64.5243A^o, size z = 57.4915A^o; IL-13, size x = 48.9548A^0, size y = 44.6697A^o, size z = 64.2511A^o) was adjusted with an exhaustiveness value of 8 for MD simulation. The entire molecular docking procedure was performed exactly as described by S. L. Khan *et al.* . (S. Khan *et al.,* 2020), (Khan *et al*., 2021), (S. L. Khan *et al*., 2021).

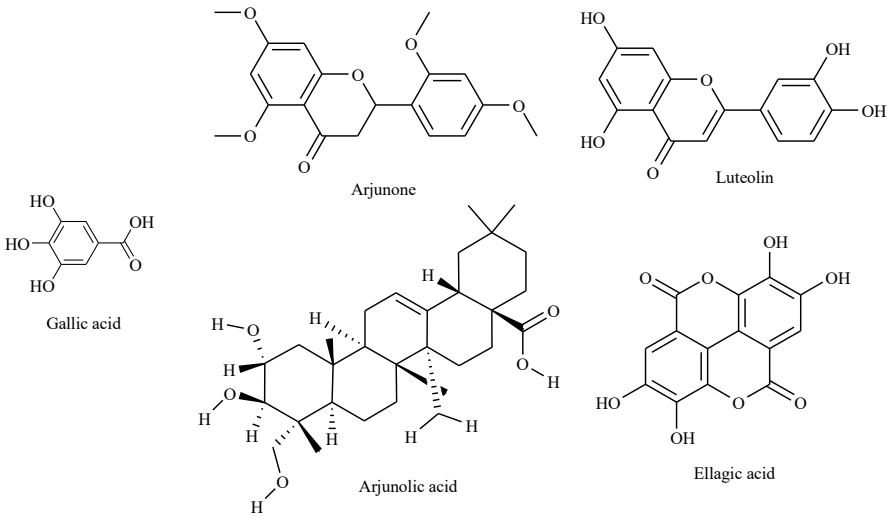

Fig. 1. The structures of selected major phytoconstituents from *Terminalia arjuna*

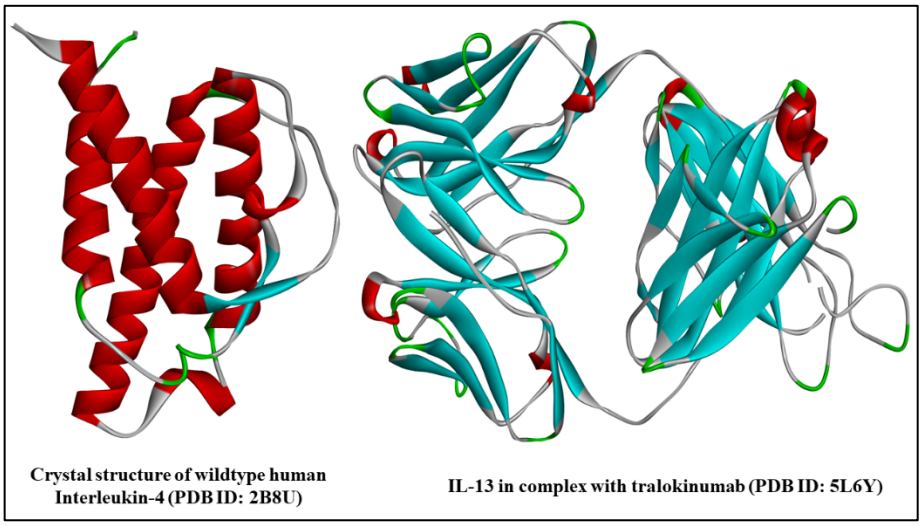

Fig. 2. 3D ribbon view with PDB IDs of the enzymes obtained from the RCSB Protein Data Bank

3. Results

3.1 Pharmacokinetics and toxicity prediction of phytoconstituents

Pharmacokinetic characteristics are a critical component of drug development because they allow researchers to determine the biological features of drug candidates. To determine whether or not the substance was drug-like, Lipinski's rule of five and Veber's rules were used (Table 1). To understand their pharmacokinetics profile, phytoconstituents were investigated for their ADMET properties better (Table 2). Oral acute toxicity has been predicted along with LD_{50} (mg/kg), toxicity class, hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity (Table 3).

Table 1. Physicochemical Properties of the Major Chemical Constituents of *Terminalia arjuna*

Name of Compound	Lipinski's Rule of 5					Veber's Rule	
	Log P	Mol. Wt.	Hydrogen Donor	Hydrogen Acceptor	No. of Violations	Total polar surface area (Å^2)	No. of rotatable bonds
Gallic acid	0.59	170.12	4	5	0	97.99	1
Arjunolic Acid	4.63	488.70	4	5	0	97.99	2
Luteolin	1.97	286.24	4	6	0	111.13	1
Ellagic Acid	0.94	302.19	4	8	0	141.34	0
Arjunone	3.24	344.36	0	6	0	63.22	5

Table 2. The ADMET properties of the Major Chemical Constituents of *Terminalia arjuna*

Name of Compound	Absorption			Distribution			Toxicity	
	Solubility Log S (log mol/L)	Caco-2 perm. (nm/sec)	Intestinal. Absorption (% Abs)	Skin perm. (logKp, cm/hour)	BBB pen. (C.brain/C.blood)	PPB (%)	Metabolism	Carcinogenicity (Mouse)	Ames Toxicity
Gallic acid	-2.34	13.8492	53.69685	-3.62686	0.348084	65.38	CYP3A4 inhibitor	Negative	Mutagenic
Arjunolic Acid	-7.67	20.9815	91.23332	-3.57106	0.588608	97.04	---	Positive	Non-mutagenic
Luteolin	-4.51	4.53973	79.42723	-4.28017	0.367582	99.71	CYP1A2, CYP2D6, CYP3A4 inhibitor	Positive	Mutagenic
Ellagic Acid	-3.66	20.5635	50.89231	-5.09432	0.299777	43.82	CYP1A2 inhibitor	Positive	Non-mutagenic
Arjunone	-3.91	57.0533	98.54008	-3.50358	1.5995	88.41	CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 inhibitor	Negative	Mutagenic

BBB: Blood-brain barrier, CYP: Cytochrome, PPB: Plasma-protein binding, perm: permeability.

Table 3. The predicted acute toxicity of phytoconstituents

Parameters	Molecule Name				
	Gallic acid	Arjunolic Acid	Luteolin	Ellagic Acid	Arjunone
LD50 (mg/kg)	2000	2000	3919	2991	2000
Toxicity class	4	4	5	5	4
Prediction accuracy (%)	70.97	70.97	70.97	70.97	69.26
Hepatotoxicity (Probability)	I (0.61)	I (0.92)	I (0.69)	I (0.83)	I (0.66)
Carcinogenicity (Probability)	A (0.56)	I (0.63)	A (0.68)	A (0.59)	I (0.54)
Immunotoxicity (Probability)	I (0.99)	I (0.70)	I (0.97)	I (0.81)	A (0.85)
Mutagenicity (Probability)	I (0.94)	I (0.84)	A (0.51)	I (0.84)	I (0.71)
Cytotoxicity (Probability)	I (0.91)	I (0.96)	I (0.99)	I (0.90)	I (0.83)

Where: I, Inactive; A, Active

3.2 Molecular docking

The 2D- and 3D-docking poses of phytoconstituents with IL-4 are depicted in Figure 3, whereas IL-13 are illustrated in Figure 4. The active amino residues, atoms responsible for interactions from ligand molecules, bond type, bond category, and bond length (A^0) involved in the interactions are tabulated in Table 4. The ligand energies (kcal/mol), PubChem CID, and binding affinities (kcal/mol) of ligands with IL-4 and IL-13 are tabulated in Table 5.

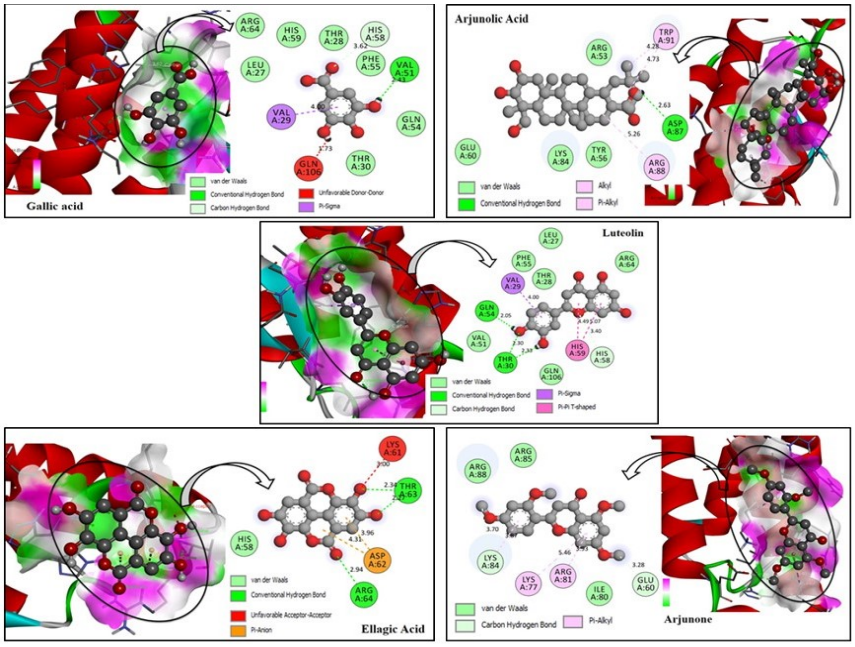

Fig. 3. 2D-and -3D molecular interactions of Gallic acid, Arjunolic acid, Luteolin, Ellagic acid and Arjunone with IL-4

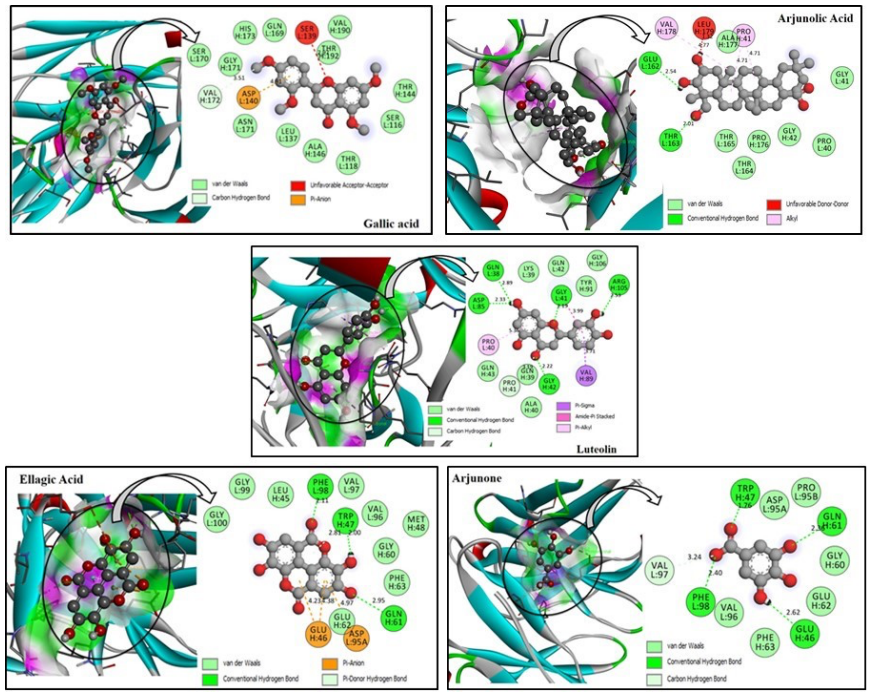

Fig. 4. 2D-and -3D molecular interactions of Gallic acid, Arjunolic acid, Luteolin, Ellagic acid and Arjunone with IL-13

Table 4. The interactions of ligands with IL-4 and IL-13

Active amino acid residue	Atom from ligand	Bond length (A^0)	Bond type	Bond category
		IL-4		
		Gallic acid		
VAL51	H	2.43261	Hydrogen Bond	Conventional Hydrogen Bond
HIS58	O	3.61654		Carbon Hydrogen Bond
VAL29	Pi-orbitals	3.99997	Hydrophobic	Pi-Sigma
		Arjunolic acid		
ASP87	H	2.6309	Hydrogen Bond	Conventional Hydrogen Bond
ARG88	Alkyl	5.25841		Alkyl
TRP91	Alkyl C C	4.73276 4.06776 5.17395	Hydrophobic	Pi-Alkyl
		Luteolin		
THR30	H	2.32649	Hydrogen Bond	Conventional Hydrogen Bond
GLN54	H	2.04949		
THR30	O	2.30473		
HIS58	O	3.4019		Carbon Hydrogen Bond
VAL29	Pi-orbitals	3.99582	Hydrophobic	Pi-Sigma
HIS59	Pi-orbitals Pi-orbitals	4.49401 5.06782		Pi-Pi T-shaped
		Ellagic acid		
THR63	O	2.33726	Hydrogen Bond	Conventional Hydrogen Bond
	O	2.27405		
ARG64	O	2.93604		
ASP62	Pi-orbitals Pi-orbitals	3.96054 4.31447	Electrostatic	Pi-Anion
		Arjunone		
LYS84	C	3.69721	Hydrogen Bond	Carbon Hydrogen Bond
GLU60	C	3.27655		
LYS77	Pi-orbitals	5.46366	Hydrophobic	Pi-Alkyl
ARG81	Pi-orbitals	3.92705		
LYS84	Pi-orbitals	3.87081		
		IL-13		
		Gallic acid		
H: GLU46	H	2.61626	Hydrogen Bond	Conventional Hydrogen Bond
H: TRP47	O	1.75911		
H: GLN61	O	2.35868		
L: PHE98	O	2.40313		
L: VAL97	O	3.24093		Carbon Hydrogen Bond

Arjunolic acid				
L: GLU162	H	2.54362	Hydrogen Bond	Conventional Hydrogen Bond
L: THR163	O	2.01198		
H: VAL178	C	4.77331		
H: PRO41	Alkyl	4.71425	Hydrophobic	Alkyl
	Alkyl	4.71373		
Luteolin				
H: ARG105	H	2.53011	Hydrogen Bond	Conventional Hydrogen Bond
L: GLN38	H	2.88796		
L: ASP85	H	2.32687		
H: GLY42	O	2.22481		
L: GLY41	O	2.18872		
H: PRO41	O	3.18868		Carbon Hydrogen Bond
H: VAL89	Pi-orbital	3.70973		Pi-Sigma
L: GLY41; GLN42	Pi-orbital	3.99091	Hydrophobic	Amide-Pi Stacked
L:PRO40	Alkyl	5.36427		Pi-Alkyl
Ellagic acid				
H: TRP47	H	2.00407	Hydrogen Bond	Conventional Hydrogen Bond
H: GLN61	O	2.95449		
L: PHE98	O	2.10703		
H: GLU46	Pi-orbital	4.22682	Electrostatic	Pi-Anion
	Pi-orbital	4.38107		
L:ASP95	Pi-orbital	4.97265		
H: TRP47	Pi-orbital	2.81399	Hydrogen Bond	Pi-Donor Hydrogen Bond
Arjunone				
H: VAL172	C	3.50648	Hydrogen Bond	Carbon Hydrogen Bond
L: ASP140	Pi-orbital	4.91005	Electrostatic	Pi-Anion

Table 5. The ligand energies (kcal/mol), PubChem CID, and binding affinities (kcal/mol) of ligands with IL-4 and IL-13

Name of Compound	PubChem CID	Ligand energy (kcal/mol)	Binding affinity (kcal/mol)	
			IL-4	IL-13
Gallic acid	370	76.79	-5.6	-6.2
Arjunolic Acid	73641	1250.56	-6.2	-8.1
Luteolin	5280445	195.94	-7.2	-8.1
Ellagic Acid	5281855	215.78	-5.8	-7.9
Arjunone	14034821	313.16	-5.9	-7.3

4. Discussion

4.1 Pharmacokinetics and toxicity prediction of phytoconstituents

Following Lipinski's(Barret, 2018) and Veber's criteria(Veber *et al.,* 2002) (Table 1), all of the chosen phytoconstituents presented in Figure 1 demonstrate drug-likeness characteristics without violating any of the requirements. The Log P values of all the selected phytoconstituents fall between 0.59 and 4.63, which is within an acceptable range. The phytoconstituents have a molecular weight of less than 500, a number of H-donors less than 5, and a number of H-acceptors less than 10, which are within the range of acceptable values. Veber's rules are followed by selected phytoconstituents, with total polar surface area (TPSA, should be less than 140) values and the number of rotatable bonds (should be less than 10) falling within the acceptable range for oral availability.

The Caco-2 cell line, derived from a human colon epithelial cancer cell line, is used to study medication absorption in the human digestive tract. This model may be used to determine if a molecule is appropriate for oral administration, predict intestinal permeability, and study drug efflux. The criteria for assessing drug absorption were solubility and Caco-2 permeability, given in Table 2 for reference. The solubility of gallic acid, luteolin, ellagic acid, and arjunone varies from -2 to -4 log mol/L, showing that chosen phytoconstituents have acceptable solubility. However, arjunolic acid displayed -7.67 log mol/L solubility showing poor solubility. It might be due to its steroidal structure. The Caco-2 permeability of selected phytoconstituents was high except for luteolin which displayed low permeability. The gastrointestinal absorption of arjunolic acid, luteolin, and arjunone was more than 75%, whereas gallic acid and ellagic acid showed 53.69 and 50.89%, respectively. The skin permeation values of selected phytoconstituents are optimum. The distribution of drug candidates considers the permeability of the blood-brain barrier (BBB) and the plasma proteins binding (PPB). The selected phytoconstituents did not show BBB permeability. However, arjunone displayed BBB permeability. The % PPB of the phytoconstituents was significantly high. The metabolism and toxicity assessment of phytoconstituents was performed. Arjunolic acid, luteolin, and ellagic acid showed carcinogenicity. Galli acid, luteolin, and arjunone were found to be mutagenic.

In acute toxicity predictions, gallic acid, arjunolic acid, and arjunone fall under toxicity class IV, indicating that the molecules are harmful after swallowing ($300< LD_{50}\leq2000$). Luteolin and ellagic acid fall under the class V, which indicate may be detrimental after consuming ($2000< LD_{50}\leq5000$)(Drwal *et al*., 2014). The prediction accuracy was 70.97% for all the molecules except for arjunone was 69.26%. Medication-induced hepatotoxicity is a leading cause of abrupt liver failure, and one of the most common reasons for drug recalls. In the present investigation, none of the phytoconstituent displayed hepatotoxicity. Carcinogens are chemicals that can cause cancer or raise the risk of cancer. Mutagens are chemicals that produce aberrant genetic mutations, such as alterations in a cell's DNA. Immunotoxicity is the detrimental impact of xenobiotics on the immune system. The ability to predict cytotoxicity is critical for screening chemicals that might induce both unwanted and desired cell harm, the latter being particularly relevant in the case of tumour cells (Drwal *et al*., 2014). Many molecules did not exhibit hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and

cytotoxicity but displayed the activity with a much lower probability score (Table 3). The ADMET profile and toxicity prediction of a chosen phytoconstituent are acceptable, making it appropriate for *in silico* investigations with IL-4 and IL-13.

4.2 Investigation of phytoconstituents as IL-4 modulators

There was no native ligand present in the PDB structure (PDB id: 2B8U) selected for docking studies. There was only one chain present in the structure. Gallic acid exhibited -5.6 kcal/mol binding free energy and formed one conventional and one carbon-hydrogen bond with VAL51 (2.43261A^0) and HIS58 (3.61654A^0), respectively. It has formed one Pi-sigma bond with VAL29 (3.99997A^0). The binding poses of gallic acid with IL-4 are illustrated in Figure 3. Arjunolic acid demonstrated -6.2 kcal/mol binding affinity and formed one conventional hydrogen bond with ASP87 (2.6309A^0). It has developed an alkyl bond with ARG88 (5.25841A^0) and a Pi-alkyl bond with TRP91 (4.73276A^0, 4.06776A^0, 5.17395A^0). The binding poses of arjunolic acid with IL-4 are depicted in Figure 4. Luteolin showed the most potent -7.2 kcal/mol binding free energy and formed three conventional hydrogen bonds with THR30 (2.32649A^0, 2.30473A^0) and GLN54 (2.04949A^0). It has formed one carbon-hydrogen bond with HIS58 (3.4019A^0). It has developed a Pi-sigma bond with VAL29 (3.99582A^0) and Pi-Pi T-shaped bonds with HIS59 (4.49401A^0, 5.06782A^0). The binding poses of luteolin with IL-4 are depicted in Figure 5. Ellagic acid displayed -5.8 kcal/mol binding affinity and formed three conventional hydrogen bonds with THR63 (2.33726A^0, 2.27405A^0) and ARG64 (2.93604A^0). It has developed electrostatic Pi-anion bonds with ASP62 (3.96054A^0, 4.31447A^0). The binding poses of luteolin with IL-4 are illustrated in Figure 6. Arjunone demonstrated -5.9 kcal/mol binding affinity and two carbon-hydrogen bonds with LYS84 (3.69721A^0) and GLU60 (3.27655A^0). It has developed three Pi-alkyl bonds with LYS77 (5.46366A^0), ARG81 (3.92705A^0), and LYS84 (3.87081A^0). The binding poses of arjunone with IL-4 are illustrated in Figure 7. From the above results, it was observed that luteolin has enough potential to modulate the activity of IL-4. It is a lead molecule for further development some IL-4 agonists or modulator.

4.3 Investigation of phytoconstituents as IL-13 modulators

The PDB structure selected for the docking studies of IL-13 was 5L6Y. There were two chains present in the target structure, i.e., chain L and chain H. Gallic acid displayed -6.2 kcal/mol binding affinity and formed four conventional hydrogen bonds with H: GLU46 (2.61626A^0), H: TRP47 (1.75911A^0), H: GLN61 (2.35868A^0), and L: PHE98 (2.40313A^0). It has formed one carbon-hydrogen bond with L: VAL97 (3.24093A^0). The binding poses of gallic acid with IL-13 are illustrated in Figure 8. Arjunolic acid exhibited -8.1 kcal/mol binding free energy and formed two conventional hydrogen bonds with L: GLU162 (2.54362A^0) and L: THR163 (2.01198A^0). It has developed hydrophobic bonds with H: VAL178 (4.77331A^0) and H: PRO41 (4.71425A^0, 4.71373A^0). The binding poses of arjunolic acid with IL-13 are depicted in Figure 9. Luteolin also displayed -8.1 kcal/mol binding free energy and formed five conventional hydrogen bonds with H: ARG105 (2.53011A^0), L: GLN38 (2.88796A^0), L: ASP85 (2.32687A^0), H: GLY42 (2.22481A^0), and L: GLY41 (2.18872A^0). It has formed one carbon-hydrogen bond with H: PRO41 (3.18868A^0). It has developed a Pi-sigma bond with H:

VAL89 (3.70973A^0), amide-Pi stacked bonds with L: GLY41; GLN42 (3.99091A^0), and Pi-alkyl bond with L: PRO40 (5.36427A^0). The binding poses of luteolin with IL-13 are demonstrated in Figure 10. Ellagic acid exhibited -7.9 kcal/mol binding free energy and formed three conventional hydrogen bonds with H: TRP47 (2.00407A^0), H: GLN61 (2.95449A^0), and L: PHE98 (2.10703A^0). It has developed an electrostatic bond with H: GLU46 (4.22682A^0, 4.38107A^0) and L: ASP95 (4.97265A^0). It has formed one Pi-donor hydrogen bond with H: TRP47 (2.81399A^0). The binding poses of ellagic acid with IL-13 are demonstrated in Fig. 11. Arjunone displayed -7.3 kcal/mol binding free energy but did not form any conventional hydrogen bond with the target. It has formed one carbon-hydrogen bond with H: VAL172 (3.50648A^0) and one Pi-anion bond with L: ASP140 (4.91005A^0). The binding poses of arjunone with IL-13 are demonstrated in Figure 12. From the above results, it was observed that luteolin has the potential to modulate the activity of IL-13. The selected phytoconstituents have displayed better modulating activity towards IL-13 than IL-4.

4.4 Structural Activity Relationship (SAR)

Among selected phytoconstituents, luteolin has been chosen as the most potent molecule that exhibited good binding affinity towards IL-4 and IL-13 with hydrogen and hydrophobic interactions. Arjunone and luteolin have a similar nucleus (4H-chromen-4-one), but both have different binding affinities and a number of hydrogen bonds. Even arjunone exhibited the least potent interaction with IL-13. This might be due to arjunone having methoxy substituents while lueolin has hydroxyl (polar) groups. Ellagic and gallic acid both have hydroxyl substituents but do not possess a 4H-chromen-4-one nucleus, which might be the reason behind their poor potency. In the case of arjunolic acid, it has a steroidal nucleus and exhibited equal binding affinity as luteolin with IL-13, but with IL-4, it displayed less potency than luteolin. It indicates polar substituents with benzo fused-4H-chromen-4-one nucleus and at least one free phenyl ring can exhibit the better modulatory activity of IL-4 and IL-13. The predicted SAR is demonstrated in Figure 5.

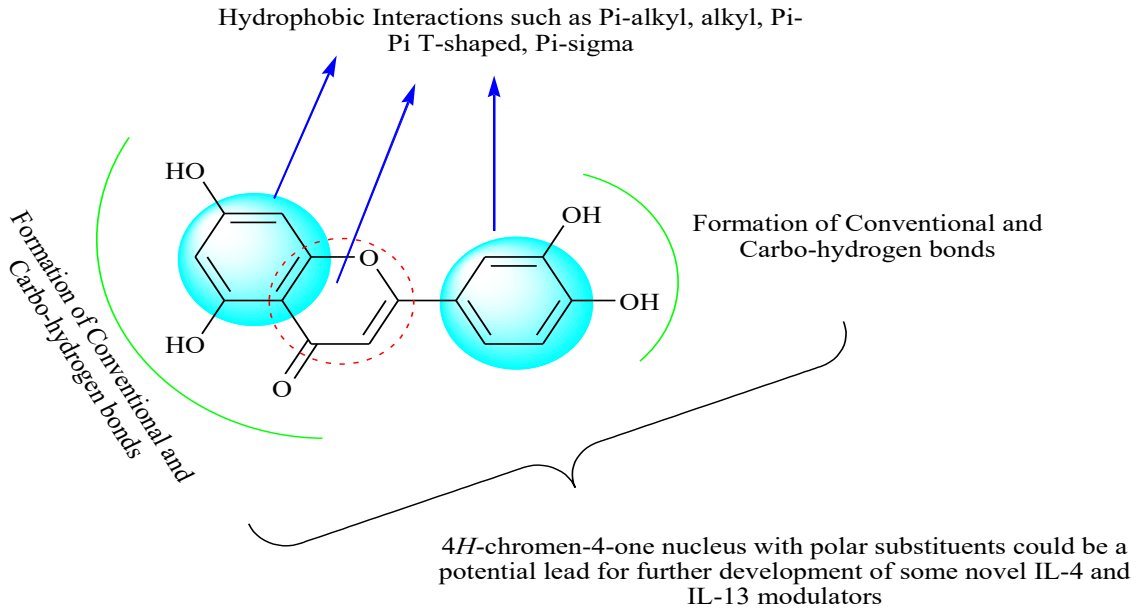

Fig. 5. Predicted SAR for the further development of some novel IL-4 and IL-13 modulators

5. Conclusion

The main phytoconstituents of *Terminalia arjuna* were evaluated *in silico* for their pharmacokinetic characteristics and were found to be druglike, indicating that they are potentially therapeutic. It is mandatory to balance cytokine type 1 and type 2; moreover, it has been observed that cytokine type 1 level was increased in autoimmune disorders. It has been well documented that the stimulation of IL-4 and IL-13 can regulate the level of type 2 cytokines which can be maintained with the level of type 1 cytokines. The present study investigated gallic acid, arjunolic acid, luteolin, ellagic acid, and arjunone for their potential modulating activity of IL-4 and IL-13. The active amino acid residues identified for IL-4 are VAL51, HIS58, ASP87, THR30, GLN54, THR63, ARG64, LYS84, and GLU60. The active amino acid residues identified for IL-13 are H:GLU46, H:TRP47, H:GLN61, L:PHE98, L:VAL97, L:GLU162, L:THR163, H:ARG105, L:GLN38, L:ASP85, H:GLY42, L:GLY41, H:PRO41, H:TRP47, and L:PHE98. The phytoconstituents demonstrated better modulating activity towards IL-13 than IL-4. Luteolin displayed better potential for both IL-4 and IL-13; therefore, we concluded that it could be used to modulate the activity of IL-4 and IL-13 for the prevention of autoimmune diabetes.

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