Section A-Research paper
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CASCADING THE INSULIN RESISTANCE THROUGH MULTIPLE INSILICO TARGETTING *BY JUSTICA ADHATODA*

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ABSTRACT:

Justica Adhatoda also known as Malabar nut, has its several applications in the medicinal utility. It is found in most of the Asian countries. The leaves and roots of the adhatoda are particularly used for the respiratory tract aliments. The alkaloid vasicine has been employed as an oxytocic and abortifient agent over more than 20 years. The present study is designed to bring the ability of the constituents to tackle with the prevailing insulin resistance. The structures were found by literature review, and drawn by Marvin Sketch. They were docked using AutodockVina with the certain targets of inflammatory cytokines such as Tumour necrosis factor (TNF-α), Interleukin (IL-6), and also with the insulin receptor substrate IRS-1, protein kinase B (AKT),and mammalian target of rapamycin (mTOR). The constituents were found to bind with the targets by van der waals interaction with the docking score in the range of -3 to -8 kcal/mol. The toxicity levels were also analyzed. Our research leads us to believe that screened phytochemicals found

in Justica adhatoda can be employed as therapeutic medication candidates to treat insulin resisance.

Keywords: Justica Adhatoda, *insilico*, docking, insulin resistance, inflammatory cytokines.

1 INTRODUCTION:

The insulin resistance is the loss or the failure of the cells to respond to the insulin. The resistance develops over a period of time by the body due to the obesity, cardiovascular diseases, inflammations, etc. The obesity I the most common cause for the insulin resistance. About 30% of the people in the world are facing obesity. About 537 million people affected by diabetes in the year 2021 and estimated to increase by 643 million by 2030 [1, 2]. The insulin resistance is an apparent effect of the pro-inflammatory cytokines and the chemokines released during an inflammation caused in the body. The pro-inflammatory cytokines include, interleukin-1 beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and other various cytokines. Nuclear factor kappa-B kinase (IKK- β) activates the release of inflammatory cytokines during acute inflammation. It causes obesity-induced insulin resistance [3]. Obesity leads to the increased levels of pro-inflammatory cytokines [2]. The interleukin IL-6 induces the JAK/STAT pathway for the STAT phosphorylation, dimerization and transcription of genes. IL-6 induces the suppressor gene SOCS-3 that inhibit the insulin-signaling pathway. IL-6 develops the insulin resistance by interrupting the phosphorylation of insulin receptor and its substrates [4, 5]. The TNF- α also mediates the insulin signaling pathway by inducing the serine phosphorylation of IRS-1. This inhibits the tyrosine phosphorylation of IRS-1. [6, 7]

The blood glucose level depends on the production of insulin in the beta islets of pancreas. Insulin exhibits a cascade mechanism involving the auto phosphorylation of tyrosine residues of the insulin receptors and its substrates. The IRS-1 and IRS-2 substrates activates the PI3K (PI3K: phosphoinositide 3-kinase)/Akt pathway and the MAPK (MAPK: mitogen-activated protein kinase) pathway.The AKT kinases, involved in the insulin-signaling pathway, mediates the glucose metabolism and transportation. The mammalian target of rapamycin (mTOR) has several roles in the cellular growth, differentiation and metabolism activities [8-11]. The mTOR has two forms of complexes, based on the presence of regulatory-associated protein of mTOR (raptor) and rapamycin insensitive companion of the mammalian target of rapamycin (rictor). The mTORC1 activation results from the indirect stimulation by akt phosphorylation through the phosphorylation of TSC and PRAS40. The mTORC1 shows the negative feedback mechanism to the IRS-1 substrates phosphorylation. mTORC2 regulates the glucose homeostasis through the AKT kinases [12]. The mTORC2 activates AKT kinases and phosphorylates the insulin growth factor (IGF-1R) and insulin receptors. This AKT increases insulin sensitivity and improves GLUT4 mediated trnslocation. The GLUT4 is involved in the translocation of glucose to cell membrane that break into cellular components. The GLUT4 aids in the storage of glycogen in the adipose tissues as a part of insulin signaling pathway [8-12].

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Justica adhatoda, which is synonymously known as Adhtodavasica and Adhatodazeylanica, belongs to family Acanthaceae, subclass Asteridae and specie *Adhatoda.* It is a known medicinal plant, used in the siddha and unani medicine. It is commonly known as Malabar nut or vasaka. World Health Organisation supports the treatment of cough, asthma and bleeding piles with the preparation of Adathoda species [13]. The major alkaloid called the vasicine is extensively used as an oxytocic and an abortifacient agent. The major alkaloids of the plant include vasicinone, Vasicoline, Amrinone, Anisotine, Adhatodine and vasicine. The alkaloids are normally reported to have the anti-tubercolar activity and anti-viral property. Other constituents include Sulforaphane, Pyrazinamide, Squalene, Stigmasterol, Hexadecanoic acid, and Ethambutol. The extract from the leaves of this plants were found to decrease the glycemic level in the blood of the rats (by) but the mechanism of its action is known in the anti-diabetic activity[14-16]. The purpose of this study is to evaluate the efficiency on the improvement of the insulin resistance by various cascade that involve the pro-inflammatory cytokines and also the insulin receptor mediated protein kinases. It is performed by bioinformatics tools to understand it potential targeting criteria.

2 MATERIALS AND METHODS:

2.1 Ligand identification:

Justica adhatoda also known as Malabar nut was chosen as the source of ligands. The major constituents of adhatoda containing vasicinone, Vasicoline, Amrinone, Anisotine, Adhatodine, vasicine, Sulforaphane, Pyrazinamide, Squalene, Stigmasterol, Hexadecanoic acid, and Ethambutanol. All the phytochemical constituents were drawn using ChemAxon sketching tool and saved in .mol format. These ligands were minimized using Chem3DUltra software. (Table.1)

2.2 Screening of ligands:

Swiss ADME, used to predict the pharmacokinetics, drug-likeness, and physicochemical parameters of the ligands. This tool performs by uploading SMILES notation or by drawing structures. The ligands are analysed for the drug likeliness properties for the further clinical studies. The tool performs Lipinski's rule, veber filter, Ghose filter, Eager filter, etc for evaluation. Toxicity of the compounds are also predicted using PROTOX-II software.

2.3 Target identification:

According to the literature review, the targets were identified based on the improvement of the insulin resistance in the subjects. The resistance developing inflammatory cytokines, nuclear factor and the insulin receptor substrates were chosen as the targets. Targets tumor necrosis factor alpha (TNF-α) (PDB ID : 5TSW), interleukin IL-6 (PDB ID : 1ALU), Insulin receptor substrates (IRS-1) (PDB ID : 1IRK), AKT kinases (PDB ID : 1GZO), Mammalian target of rapamycin (mTORC2) (PDB ID : 4JSV)were obtained from the PDB database based on litreatures. These targets were prepared for docking by removing water, cofactors, and ligands. It is saved in .pdb format.

2.4 Molecular docking:

The AutoDockVina platform wasutilisedfor the docking of ligands. The ligands were imported as the combined ligand file (.sdf) as a Chemical Table SDF file. Using OpenBabel, the molecules' energy was reduced, and then they were converted to AutoDock Ligand format (. pdbqt). The protein was uploaded, Vina Wizard was applied to dock each of the ligands. The search space in the protein must be defined using a grid box in AutoDockVina. The area of interest (active site) in the macromolecule must be encircled by this grid box. The protein's active sites were determined using a literature review. The docking results were visualized using Biovia Discovery Studio.

3 RESULTS:

The pharmacokinetics analyses of each of J. adhatoda's eleven compounds were performed by the SwissADME software (Table.2). The drug-likeliness properties were listed in the table.3 The ligands were found to have molecular weights (MW) between 187.2 and 412.69,which are below 500 Daltons. Lipinski's requirements are the number of hydrogen bond donors (H-Do) and the number of hydrogen bond acceptors (H-Ac) was less than 5, Topological Polar Surface Area to be less than 140Å and partition coefficient (LogP) must be less than 5. All the ligands except squalene, stigmasterol and hexadecanoic acid were found to pass the lipinski's rule which is the most important criteria for a drug. The ligands resulted with the topological polar surface areas (TPSA) in the range between 18.8 and 80.7Å. The bioavailability score predicted by Swiss ADME was found to be 0.55 for all the ligands. The intestinal absorption reported to be high for all the ligands except pyrazinamide (A5) and squalene (A6). These factors determines the potent pharmacokinetic aspects of the drug [17].

About 11 phytoconstituents of Justica adhatoda were predicted for their toicity profile in PROTOX II software. The prediction results is given in the Table.4 the constituents Amrinone (A1), Anisotine (A2), Sulforaphane (A3), Pyrazinamide (A5), Stigmasterol (A7), Vasicinone (A8), Hexadecanoic acid (A10), and Ethambutol (A12) resulted in class 4 toxicity (i.e., harmful if swallowed beyond the range $300 < LD50 \leq 2000$) with the lethal dose ranging from 290-1100 mg/kg. Squalene (A6) gave class 5 toxicity with the lethal dose of 5000mg/kg. The compounds Sulforaphane (A3), Squalene (A6), Vasicinone (A8), Vasicoline (A9), and Hexadecanoic acid (A10) were found to be inactive in all the toxicity predictions. Amrinone (A1), Anisotine (A2), Pyrazinamide (A5), Adhatodine (A11), and Ethambutol (A12) have either carcinogenicity or hepatotoxicity or both. This cases determines the ligands to be categorized as harmful beyond the lethal dose.

The molecular docking of protein and ligand has the ability to determine the affinity between them that provides the lead to the drug discovery. The docking of the targets, tumor necrosis factor alpha (TNF-α) (PDB ID: 5TSW), interleukin IL-6 (PDB ID: 1ALU), Insulin receptor substrates (IRS-1) (PDB ID: 1IRK), AKT kinases (PDB ID: 1GZO), Mammalian target of rapamycin (mTORC2) (PDB ID : 4JSV) with all the 11 phytoconstituents were carried out

using autodockvina. Their interactions were visualized using biovia discovery studio for investigating the ligand binding sites with each of the target. The docking score and binding sites were listed in the Table.5 and Table 6 respectively. This docking is validated using the RMSD values calculated during docking. Lower the RMSD value, more significant the ligand binding with the target. If the RMSD value is more, the ligands have more binding site with the same target with similar binding affinities.

The target interleukin IL-6 was docked to eleven ligands resulted with the good binding score ranging between -3.1 and -6.9 kcal/mol. Sulforaphane (A3), pyrazinamide (A5) and hexadecanoic acid (A10) were found to be the best binding with the interleukin with the score of 3.1, 4.3, and 3.7 kcal/mol respectively.The binding interactions of the compound A5 (Pyrazinamide) was given in the Figure.1. Also to validate the docking, the RMSD value was 4.6, 4, and 4 Å respectively. This clearly states that the highest score docking pose compared to the 2nd highest pose showed similarities in binding affinity. The sulforaphane (A3) is involved majorly with the sites GLU A(106), LYS A(46), PHE A(105), THR A(43), THR A(163), ARG A(104), and ASP A(160) by vanderwaals interaction. But the compound pyrazinamide (A5) forms hydrogen bonding with ARG A(104), GLU A(106), and SER A(108). Hexadecanoic acid (A10), inspiteofformingvanderwaals, it also forms hydrogen bond with GLU A(106).

The binding affinity of the ligand sulforaphane (A3), pyrazinamide (A5), and squalene (A6) were found to be -3.7, -5.4, and -5.6 kcal/mol. These ligands were found to be the best with the tumour necrosis factor-alpha at their best docking pose. Of these ligands the squalene is found to be the best with the RMSD value of 1.8 Å with the TNF-α. The binding interactions of the compound A6 (Squalene) was given in the Figure.2. The best docked ligands were involved more in the vanderwaals's interaction. Especially squalene (A6) being a terpenoid, involved both the van der waals and an alkyl bond with ARG E(103), ARG F(103), LYS F(112), and CYS F(69). Also the pyrazinamide (A5) forms hydrogen bnding with TYR B(119), and PRO C(117).

The mammalian target of rapamycin (mTOR) was docked with 11 all the phytoconstituents. The affinity ranged between -3.7 and -8.4 kcal/mol. The ligands squalene (A6) and vasicinone (A8) were found to be the best docked with the score of -6.6 and -7.9 kcal/mol respectively. Also gave the RMSD value of 7.9 and 2.6 Å respectively.Both the ligands are extensively bonded by vanderwaals. In addition, hydrogen bonds, alkyl bonds and steric bonds were also formed in vasicinone.The binding interactions of the compound A8 (Vasicinone) was given in the Figure.3.

The docked score results of the compounds with the insulin receptor substrate (IRS-1) ranged from -4 to -8.8kcal/mol. The ligand Sulforaphane (A3), pyrazinamide (A4) and hexadecanoic acid (A10) gave the best docking score of -4, -5, and -5.1 kcal/mol. These ligands also gave the RMSD value of 22.5, 2.3, and 6.8 Å. This signifies the availability of multiple sites of each of the compound on the same target that are similar to one another in affinity. These best docked ligands formed vanderwaals, hydrogen bonding interaction most prominently. The amino

acid residues, tyrosine and aspartate were bonded in all the three ligands. The compounds selectively produced interaction with any of the following amino acids alanine, phenylalanine and glutamate. The binding interactions of the compound A5 (Pyrazinamide) was given in the Figure.4.

The eleven compounds gave docking score in the range from 3.6 to 7.8 kcal/mol with the protein kinase B (AKT) target. The compound vasicoline (A9) and vasicinone (A8) showed 3.1 and 4.5 Å of RMSD, with the docking score of -6.7 and -7.7 kcal/mol in their best docked pose.The binding interactions of the compound A8 (Vasicinone) was given in the Figure.5. The compound vasicinone (A8) binds with the target at ILE A(276), and GLY A(335) by hydrogen bonds. Vasicoline (A9) and vasicinone (A8) were involved in the vanderwaals interaction.

4 DISCUSSION:

There are several phytoconstituents in the J.Adhatoda, but the most prevailing constituents were discussed above. The leaves, stem, and roots were found to have potent anti-tumor activity. The constituents of this Adhatoda was found to be a potent inhibitor of inflammatory cytokines such as tumour necrosis factor TNF-α, interleukin -6, etc. this leads to the deterioration of the insulin resistance, thereby improving the phosphorylation of insulin receptor. Certain constituents like sulforaphane, pyrazinamide, vasicinone, vasicoline were found to interact with the insulin receptor substrate (IRS-1) and protein kinase B (AKT). This activates the tyrosine phosphorylation, and AKT pathway. through the AKT pathway the insulin signalling improves and increases the glucose absortion in skeletal muscles and the adipose tissues. This also activates the GLUT4 transporter to enter the glucose to the plasma. This kind of multiple activation is necessary for the insulin dependent patients. The improvement in the insulin signalling pathway as well as acting with inflammatory cytokines can cause the decrease in the resistance for the insulin at the receptors. Therefore, Justica Adhatoda can act as a potent multi-targeted constituent that improves the insulin resistance.

5 CONCLUSION:

In silico molecular docking of Justica Adhatoda revealed potential bio-molecular target phytochemicals of the plant, and it was found that the sulforaphane, pyrazinamide, vasicinone, vasicoline, and squalene contributes to the insulin resistance improvement. These findings could serve as the basis for the synthetic modification, de novo synthesis of structural motifs, and further research into phytochemicals.

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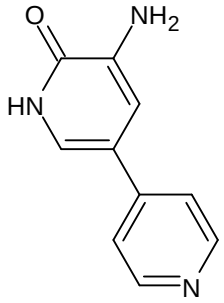
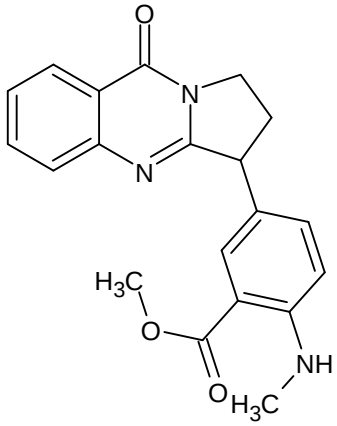
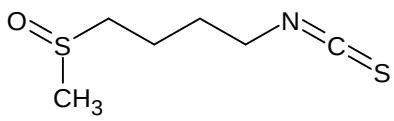
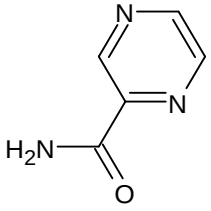
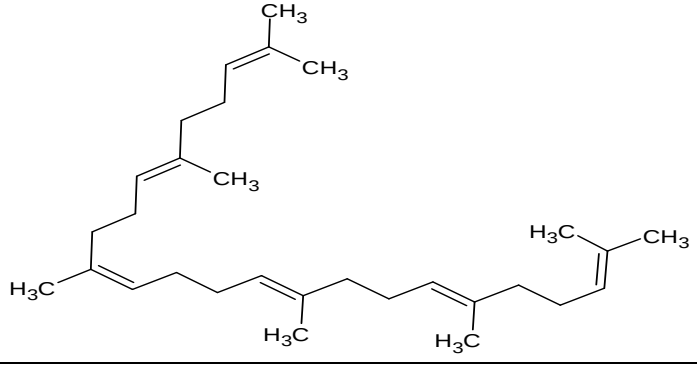
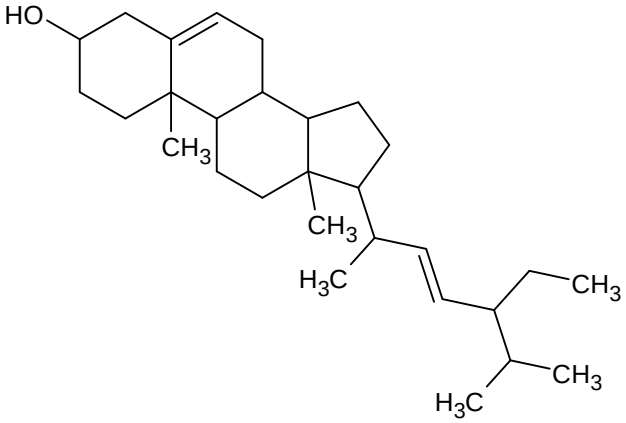
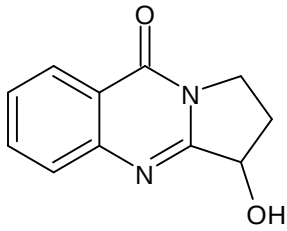
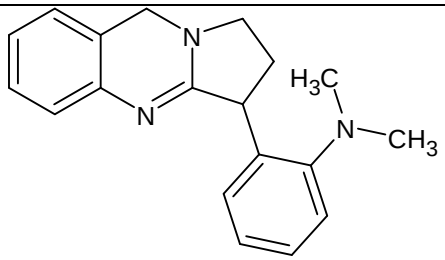
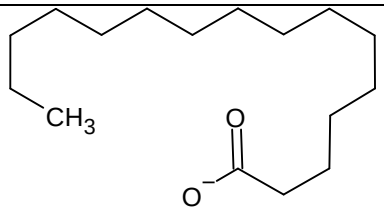
Table.1 The structures and the molecular formula of the phytoconstituents of the J.adhatoda.

COMPOUND NO	COMPOUND NAME	CHEMICAL STRUCTURE	MOLECULAR FORMULA
A1	Amrinone		C10H9N3O
A2	Anisotine		C20H19N3O3
A3	Sulforaphane		C6H11NOS2
A5	Pyrazinamide		C5H5N3O

A6	Squalene		C30H50
A7	Stigmasterol		C29H48O
A8	Vasicinone		C11H10N2O2
A9	Vasicoline		C19H21N3
A10	Hexadecanoic acid		C16H31O2-

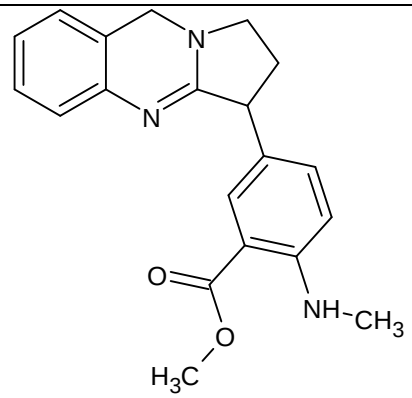
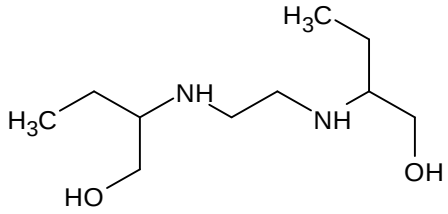
A11	Adhatodine		C20H21N3O2
A12	Ethambutol		C10H24N2O2

Table.2 Physiochemical parameters of the phytoconstituents of the Justica Adhatoda

Compound NO	Physicochemical Properties								
	MW	**Heavy atoms**	**Aromatic heavy atoms**	**H-bond acceptors**	**H-bond donors**	**MR**	**TPSA**	**iLOGP**	**GI absorption**
A1	187.2	14	12	2	2	54.7	71.77	1.14	High
A2	349.38	26	16	4	1	100	73.22	3.21	High
A3	177.29	10	0	2	0	48.4	80.73	2.11	High
A5	123.11	9	6	3	1	30.13	68.87	0.74	High
A6	410.72	30	0	0	0	143.48	0	6.37	Low
A7	412.69	30	0	1	1	132.75	20.23	5.01	Low
A8	202.21	15	10	3	1	56.09	55.12	1.67	High
A9	291.39	22	12	1	0	99.64	18.84	2.94	High
A10	255.42	18	0	2	0	78.86	40.13	3.85	High
A11	335.4	25	12	3	1	106.02	53.93	3.2	High
A12	204.31	14	0	4	4	58.11	64.52	2.46	High

Table.3 Druglikeliness of the phytoconstituents of the Justica Adhatoda

Molecule	Druglikeness					
	Lipinski violations	**Ghose violations**	**Veber violations**	**Egan violations**	**Muegge violations**	**Bioavailability score**
A1	0	0	0	0	1	0.55
A2	0	0	0	0	0	0.55
A3	0	0	0	0	1	0.55
A5	0	4	0	0	1	0.55
A6	1	3	1	1	2	0.55
A7	1	3	0	1	2	0.55
A8	0	0	0	0	0	0.55
A9	0	0	0	0	0	0.55
A10	1	0	1	0	1	0.85
A11	0	0	0	0	0	0.55
A12	0	0	0	0	0	0.55

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Table.4 Toxicity data of the phytoconstituents of the Justica Adhatoda

Compound No	Predicted Toxicity Class	Predicted LD50 (mg/kg)	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
A1	4	580	Active	Active	Inactive	Inactive	Inactive
A2	4	1100	Inactive	Active	Inactive	Active	Inactive
A3	4	1000	Inactive	Inactive	Inactive	Inactive	Inactive
A5	4	1800	Active	Inactive	Inactive	Inactive	Inactive
A6	5	5000	Inactive	Inactive	Inactive	Inactive	Inactive
A7	4	890	Inactive	Inactive	Active	Inactive	Inactive
A8	4	1100	Inactive	Inactive	Inactive	Inactive	Inactive
A9	3	290	Inactive	Inactive	Inactive	Inactive	Inactive
A10	4	900	Inactive	Inactive	Inactive	Inactive	Inactive
A11	3	290	Inactive	Active	Inactive	Inactive	Inactive
A12	4	998	Active	Inactive	Inactive	Inactive	Inactive

Table.5 Binding Energy with RMSD value of the phytoconstituents of the Justica Adhatoda

Compound No	Protein Binding Energy									
	1ALU		1GZO		1IRK		5TSW		4JSV	
	Binding Energy	RMSD	Binding Energy	RMSD	Binding Energy	RMSD	Binding Energy	RMSD	Binding Energy	RMSD
A1	-5.4	25.017	-7.3	5.331	-6.4	5.712	-6.3	32.793	-7.1	3.434
A2	-6.9	7.184	-7.4	21.853	-7.9	23.54	-8.1	19.407	-8.4	21.778
A3	-3.1	4.608	-3.6	24.258	-4	22.526	-3.7	26.902	-3.7	85.242
A5	-4.3	4.047	-5.3	27.387	-5	2.365	-5.4	3.874	-5.4	37.925
A6	-5.1	27.193	-4.8	30.247	-5.6	1.813	-6.5	22.646	-6.6	7.912

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A7	-6.3	27.98	-7.6	22.238	-7.9	26.83	-8.4	26.807	-8.7	68.772
A8	-6.1	4.91	-7.7	4.565	-7.3	24.892	-7	26.695	-7.9	2.683
A9	-6.1	27.534	-6.60	2.396	-7.9	21.343	-7.7	20.289	-7.7	84.316
A10	-3.7	4.034	-4	24.51	-5.1	6.886	-4.5	37.89	-4.8	9.983
A11	-6.8	7.479	-7.3	42.534	-8.8	2.774	-7.9	31.697	-8.1	47.265
A12	-4.3	23.188	-5.8	5.52	-4.8	25.371	-5.1	5.538	-4.5	3.334

Table.6 Binding Intercations of the phytoconstituents of the Justica Adhatoda

Compound No	**Protein Binding Interactions**				
	1ALU	**1GZO**	**1IRK**	**5TSW**	**4JSV**
	Interactions	**Interactions**	**Interactions**	**Interactions**	**Interactions**
A1	SER A(107), GLU A(42), GLU A(106), LYS A(43), SER A(47), ASP A(160), THR A(43), THR A(163), PHE A(105), ARG A(104)	GLU A(279), GLU A(315), LYS A(277), LEU A(317), VAL A(338), ASP A(275), ARG A(274), VAL A(331), TRP A(334), ILE A(276), GLY A(335), TYR A(316), LEU A(348), THR A(313), PRO A(314)	ASP A(1083), TYR A(1158), THR A(1154), TYR A(1162), ASN A(1137), ASP A(1150), ARG A(1136), MET A(1153), MET A(1139), GLY A(1082), PHE A(1151)	LYS D(65), TYR B(87), ARG B(131), GLN B(47), ILE B(83), LYS B(90), SER B(133), TYR B(141), GLU B(135), ARG D(138), ASP D(140), GLY D(66), GLN D(67)	THR B(1908), GLU B(2419), TYR B(2423), ARG B(1905), ALA B(2420), PRO B(2425), PHE B(2421), ASP B(2424), ARG B(2197), PH B(1911), GLN B(1941), LEU B(1907), LEU B(1904), ARG B(1945), ASP B(1912), THR B(1908)
A2	GLU A(106), THR	ARG A(245), GLY	GLU A(1108), MET	ILE D(136), LEU	GLN A(2200), GLU

	A(163), PHE A(105), THR A(43), ARG A(104), ASP A(160), GLN A(156), SER A(47), TRP A(157), MET A(49), LYS A(46)	A(346), ARG A(347), TYR A(351), PRO A(314), HIS A(355), ASP A(354), PHE A(350), GLN A(353), TYR A(316), LEU A(358), LEU A(348), GLU A(342)	A(1112), PHE A(1144), GLN A(1111), HIS A(1268), ASP A(1143), SER A(1270), THR A(1145), VAL A(1274), HIS A(1057), GLU A(1115)	D(26), LEU D(26), ALA D(134), GLU D(135), ASP D(45), ASN D(46), GLN D(27), GLN D(25), GLN B(27), ALA B(22), GLN B(25), PRO D(139)	A(2196), PRO A(1940), PRO A(1975), PRO A(2229), ILE A(2228), TYR A(1974), PRO A(2141), GLY A(2142), ALA A(2139), TYR A(2144), THR A(2143), GLN A(1970), ALA A(1971), ILE A(1939), LEU A(1936), GLN A(1937)
A3	GLU A(106), LYS A(46), PHE A(105), THR A(43), THR A(163), ARG A(104), ASP A(160)	ARG A(274), VAL A(272), THR A(199), TYR A(273), VAL A(271), THR A(207), LEU A(266), LEU A(204), LEU A(212), THR A(213), ILE A(291), ASP A(293)	ASPA(1150), GLY A(1152), TYR A(1158), ASP A(1083), SER A(1086), ARG A(1136), ASP A(1156), MET A(1153), PHE A(1151)	GLU D(135), LYS D(90), ASP B(140), ARG B(138), SER D(95), ILE D997), THR D(77), THR D(79), ASN D(137), PRO B(139)	LEU C(318), LEU C(48) CYC C(317), ALA C(47), SER C(90), CYC C(133), SER C(175), TRP C(274), VAL C(316), GLN C(225)
A5	GLU A(42), LYS A(46), THR A(43), PHE A(105), THR A(163), ARG A(104), GLU A(106), SER A(108), SER A(107)	ASP A(274), ASP A(275), ASP A(332), TRP A(334), VAL A(331), ILE A(276), ILE A(276), GLY A(335), VAL A(338), TYR A(316), LYS A(277), LEU A(317),	ASP A(1150), ARG A(1136), ASN A(1137)M TYR A(1158), ASP A(1083), MET A(1153), GLY A(1152), PHE A(1150)	GLN C(61), ALA B(96), LEU B(120), TYR A(119), PRO A(117), ILE A(118), TYR C(119), LYS A(98), PRO C(117), LYS C(98), ILE C(118), ILE B(118),	ALA B(2420), THR B(1908), ARG B(1945), PHE B(1911), TYR B(2423), LEU B(1907), PRO B(2425), GLN B(1941), ASP B(2424), ALA B(1944), ARG B(2497), LEU B(1904)

		VAL A(321), ALA A(318). ARG A(274)		TYR B(119)	
A6	GLU A(106), SER A(107), ARG A(104), GLU A(42), LYS A(46), PHE A(105), TRP A(157), ASN A(48), MET A(49), SER A(47), ASP A(160), THR A(43), GLN A(156)	GLU A(366), GLU A(365), ILE A(367), PHE A(369), ALA A(382), LEU A(385), LYS A(386), LYS A(387), GLN A(391), ASP A(388), MET A(364), LEU A(363)	VAL A(1059), ILE A(1148), PHE A(1128), LEU A(1123), GLY A(1149), VAL A(1050), MET A(1051), LYS A(1030), ASP A(1150), PHE A(1151), HIS A(1130), PHE A(1054), GLU A(1047), SER A(1006), ARG A(1131), VAL A(1129)	ARG E(103), GLN F(102), CYS F(101), PRO F(100), GLN D(102), GLN E(102), ARG D(103), ARG F(103), GLY F(68), ALA F(111), LYS F(112), PRO F(70), CYS F(69), SER F(71), THR F(105), GLU F(104), ALA F(109), GLU F(107), LYS E(112)	ARG A(1585), GLU A(1451), HIS A(1454), LYS A(1452), TRP A(1456), GLU A(1485), ALA A(1486), SER A(1584), ASP A(1632), ILE A(1629), GLU A(1631), LYS A(1635), ARG A(1482), LYS A(1511), TYR A(1583)
A7	ASN A(63), PRO A(65), LEU A(64), TYR A(97), VAL A(96), GLU A(93), ASN A(144), LEU A(147), THR A(143), ASP A(140), PRO A(139), THR A(137), THR A(138)	LEU A(215), ARG A(208), LYS A(216), TYR A(178), GLU A(230), GLU A(433), TYR A(177), ARG A(176), VAL A(434), ASN A(233), TYR A(231), ALA A(214)	ARG A(1000), ALA A(1080), HIS A(1081), PHE A(1151), GLY A(1152), ,MET A(1153), ASP A(1150), ASN A(1137), ARG A(1136), TYR A(1158), ASP A(1156), ASP A(1083), SER A(1086), LEU A(1002), GLY A(1082)	THR B(72), HIS B(73), LYS C(112), TYR E(115), VAL B(74), ASN D(137), THR D(77), ARG B(138), ASN B(137), LEU D(75), PRO E(113), LEU B(75), LYS E(65), PRO C(113)	LEU A(1900), GLN A(1937), LEU A(1936), VAL A(2227), PRO A(1975), ALA A(1971), TYR A(1974), GLN A(1970), TYR A(2144), ALA A(2139), GLY A(2142), PRO A(2141), THR A(2143), ARG A(2224), GLU A(2196), MET A(2199), PRO A(1940),

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					ARG A(2197), LEU A(1900), GLN A(2200)
A8	GLU A(106), PHE A(105), GLU A(42), THR A(43), LYS A(46), SER A(47), ARG A(104), ASP A(160), GLN A(156)	ARG A(274), LEU A(317), GLU A(315), VAL A(338), GLY A(335), VAL A(339), ILE A(276), LYS A(277), TYR A(316), TRP A(334), VAL A(331), ALA A(318), VAL A(321), TRY A(327)	ASP A(1156), ARG A(1136), ASN A(1137), TYR A(1158), MET A(1153), ASP A(1083), SER A(1086), GLY A(1082), ASP A(1150) , MET A(1139), PHE A(1151), GLY A(1152)	GLN E(102), PRO F(100), GLU E(116), GLU F(116), LYS F(98), GLU D(116), LYS D(98), SER D(99), GLN D(102), PRO E(100)	ASP A(2297), GLN A(1405), VAL A(2389), ASN A(2385), GLU A(2388), ALA A(2386), ARG A(2317), MET A(2387), TRP A(2313), ALA A(2300), HIS A(1398), TRP A(2304), LYS A(2301), LEU A(1402)
A9	GLN A(124), GLN A(127), ILE A(123), LYS A(120), LEU A(92), GLU A(95), ASN A(144), PRO A(139), PRO A(141), THR A(138)	ASN A(233), ALA A(232), ASP A(284), GLU A(433), LYS A(285), GLU A(230), LYS A(290), TYR A(178), ALA A(214), LYS A(216), ARG A(208), ARG A(176), TYR A(231)	ARG A(1101), GLU A(1108), PHE A(1144), GLN A(1107), GLN A(1111), HIS A(1268), GLU A(1115), MET A(1112)	ILE D(136), GLU D(135), LEU D(26), PRO D(139), ASN D(46), GLN D(27), GLU B(23), GLN B(21), ALA B(22), ASP D(45), GLN B(27), GLN B(25), GLN D(25)	ASN A(1421), ILE A(1417), ALA A(1429), RO A(1426), ALA A(1430), LEU A(1453), HIS A(1454), LEU A(1433), LYS A(1452), TYR A(1583)
A10	GLN A(156), GLU A(106), ARG A(104), ASP A(160), THR A(43), PHE A(105), LYS A(46), GLU A(42),	ALA A(232), ASP A(284), ASN A(233), LYS A(285), GLU A(433), ARG A(208), LYS A(216), ALA A(214), ARG A(176),	ARG A(1000), ALA A(1080), HIS A(1081), PHE A(1151), GLY A(1152), MET A(1153), ASP A(1150), ASN A(1137), ARG A(1136),	ARG A(103), ARG C(103), CYS C(69), LYS C(112), CYC C(101), CYC B(69), GLN B(102), ARG B(103), GLN C(102),	ARG A(1896), GLY A(1897), GLN A(1937), ASN A(1899), LEU A(1936), ASP A(2145), PRO A(2146), TYR A(2144), ALA

	SER A(107)	GLU A(230), TYR A(178), LYS A(290)	TYR A(1158), ASP A(1156), ASP A(1083), SER A(1086), LEU A(1002), GLY A(1082)	GLN A(102), LYS B(112), PRO B(100), PRO A(100), CYS A(101)	A(1971), ILE A(1939), ASP A(1933), THR A(1934)
A11	GLU A(106), THR A(163), PHE A(105), THR A(43), ARG A(104), ASP A(160), GLN A(156), SER A(47), TRP A(157), MET A(49)	ARG A(208), LYS A(216), ALA A(214), TYR A(178), GLU A(433), GLU A(230), ASP A(284), ALA A(232), LYS A(285), TYR A(231), ASN A(233), TYR A(177), VAL A(434), ARG A(176)	ARG A(1000), LEU A(1078), LEU A(1002), GLY A(1082), TYR A(1158), ASP A(1083), ASN A(1137), ARG A(1136), MET A(1139), PHE A(1151), ASP A(150), MET A(1153), GLY A(1152)	ILE D(136), LEU D(26), GLU D(135), PRO D(139), ASN D(26), GLN D(27), ASP D(45), ALA B(22), GLN B(27), GLN D(25), GLN B(25)	SER A(1582), GLU A(1414), LYS A(1452), ILE A(1417), LEU A(1433), ALA A(1429), PRO A(1426), ALA A(1430), HIS A(1454), SER A(1584), ASN A(1421), TYR A(1583), SER A(1418), GLU A(1581)
A12	LYS A(46), ASP A(160), PHE A(105), GLN A(156), GLU A(106), ARG A(104), GLU A(42), THR A(43), THR A(163)	LEU A(348), LEU A(278), VAL A(339), TYR A(316), GLY A(335), TRP A(334), ILE A(276), LEU A(317), ASP A(332), ASP A(332), ARG A(274), ASP A(275), VAL A(331), VAL A(321), TYR A(327), VAL A(338), ALA A(318), LYS A(277), GLU A(2790	ASN A(1132), GLY A(1152), ASP A(1150), ILE A(1148), MET A(1051), LEU A(1123), VAL A(1059), GLY A(1149), PHE A(1128), HIS A(1130), ASN A(1137)	PRO C(100), TRP C(114), GLN B(102), PRO B(100), GLU C(116), GLN A(102), CYC B(101), GLU B(116), LYS B (98), LYS A(98), TYR B(115), SER A(99), GLU A(116), PRO A(100)	THR D(208), VAL D(207), HIS D(161), GLN D(209), ILE D(154), ASP D(156), GLU D(142), GLU D(163), GLU D(206)

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Fig.1 binding sites of the IL – A5(Pyrazinamide)

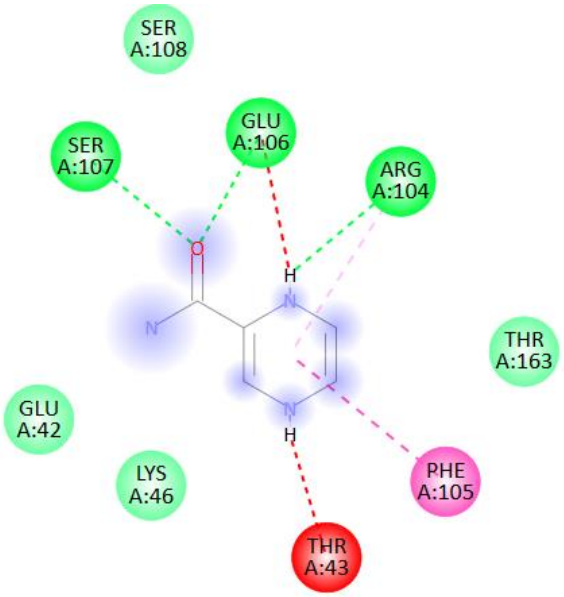

Fig.2 binding sites of the TNF - A6 (Squalene)

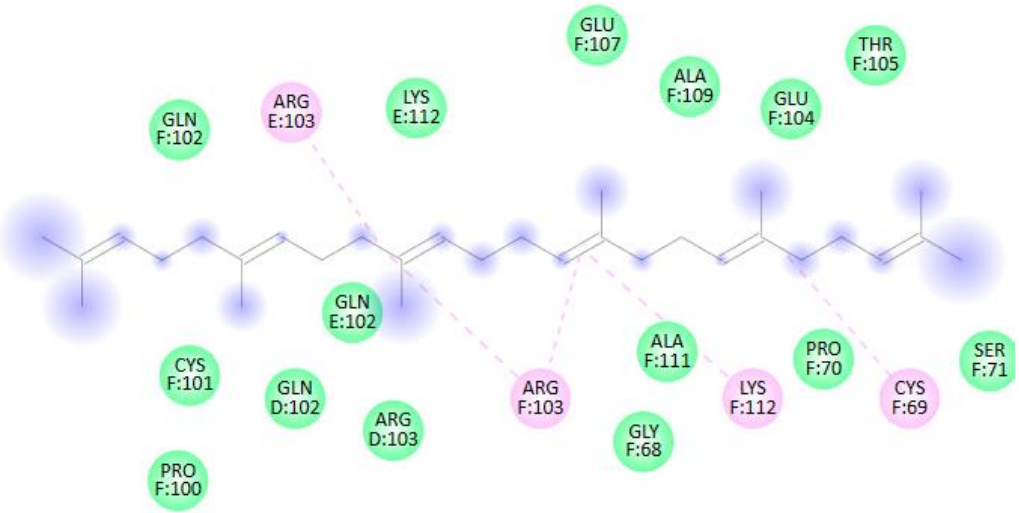

Fig.3 binding sites of the Mtor – A8 (Vasicinone)

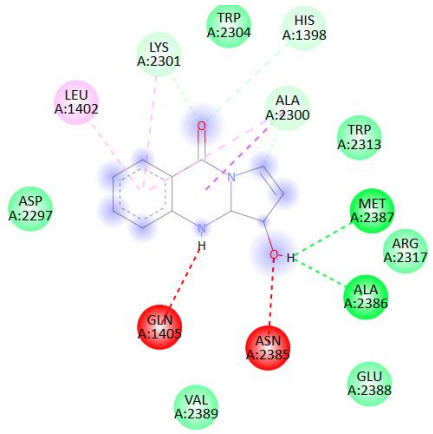

Fig.4 binding sites of the IRS- A5 (Pyrazinamide)

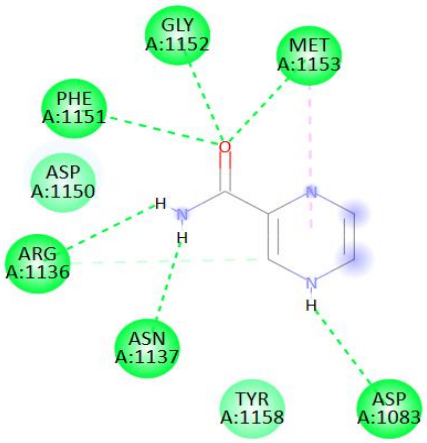

Fig.5 binding sites of the AKT- A8 (Vasicinone)

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