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AN *IN SILICO* STUDY ON ANTIDIABETIC ACTIVITY OF BIOACTIVE COMPOUNDS IN *LIMONIA ACIDISSIMA*

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ABSTRACT

The present work was designed to trace out probable antidiabetic activities of different phytoconstituents of *Limonia acidissima.* The study was additionally expected to correlate connection between different phytoconstituents with significant 6 receptors assuming significant part in diabetes mellitus by *in silico* procedures. ADMET filters applied to all of the phytoconstituents using ADMETLab. Shortlisted phytoconstituents were further explore through molecular docking. Main six PDB's 1IR3, 1US0, 2FV6, 2OQV, 5NN6 and 2QV4 for Insulin receptor, Aldose Reductase, Protein tyrosine phosphatase 1, Human Dipeptidyl Peptidase IV (DPP4), Alpha amylase and Alpha Reductase respectively appraise in the study. Total 22 molecules screened through ADMETLab. Out of which twelve shows good ADMET profile. Molegro Virtual Docker 6.0 utilized for entire investigation. Out of twelve selected phytoconstituents six shows best docking results in contrast to internal ligands and standard drug Glibenclamide. These compounds include Aurapten, Burgapten, Hederatriol, Marmin Syringaresinol and Yangambin. The entire estimation gives idea that plant likely has antidiabetic potential due to presence of quality of biomolecules.

Keywords: Molegro Virtual Docker, Antidiabetic, Syringaresinol, Diabetes Mellitus, ADMET

INTRODUCTION

Diabetes Mellitus, additionally called as type II diabetes, is one of the pressing concerns of current era and without a doubt the most difficult medical conditions in the 21 century. It is 4th or 5th leading cause of mortality in developed and developing

countries. It seems to be epidemic in many developing countries. Diabetes mellitus is a disorder of different etiologies portrayed by persistent hyperglycemia with disabled digestion of glucose, proteins and lipids **[1]**. *In silico* is an advance technique, whereby utilize the computer for experimentation. It is related to more known normal terms *in vitro* and *in vivo*. The historical backdrop of the *in silico* term is inadequately characterized, with a few specialists asserting their part in its beginning **[2]**. In ongoing situation, computational strategies, for example, molecular docking used to plan novel lead compounds by investigating the connection between the protein and ligand **[3]**.

ADMET and Molecular docking analysis are the tremendous technique **[4]**. Different types of drug targets can be classified by using *in silico* techniques as bioinformatics parameters **[5]**. It is used to predict structural and functional relationship between the compounds **[6]**. Consolidating *in silico* strategies with phytoconstituents operate new freedoms for the cure of diseases. Moreover, matching the digital libraries databases to the natural resources generates new pathway of drug discovery **[7]**.

The plants are being utilized in therapeutics across the globe since days of yore. Indeed, even in current day circumstance man is searching for elective treatments with plant as a hotspot for fresher phytomolecules. It is fascinating to make reference to that huge population of the world actually depends on plant derived products. India is home for a large number of plant species with some popular for their restorative properties some still neglected. *Limonia acidissima* (Rutaceae) utilized for treatment of human and animal disease. Seed, bark, fruits, leaves and roots of the pant utilized for the treatment of various types of disease and disorders. Among them fruit contain most therapeutic active ingredients. *Limonia acidissima* have been reported for antioxidant, anticancer, antidiabetic, antifungal, antibacterial, hepatoprotective, wound healing, antispermatogenic, astringent, dyspepsia, stomachic, antilarvicidal and diuretic potential. various phytoconstituents were isolated from different parts of plant are Lignans, Coumarin, Dimericcoumarin, furano-coumarin, flavonoid, Triterpenoid, monoterpene, carbohydrates, proteins, glycosides, amino acids, saponins, phenols and resins **[8-13]**. As per their nature lignans, polyphenol, and flavonoid shows antidiabetic potential **[14, 15]**. Current molecular docking study was designed to find out antidiabetic potency of these plants on the basis of traditional use and reliability on *in silico* technique results.

MATERIAL METHODS

Software and hardware

Pubchem, Chemspider, Openbabel Version 3.0, ADMET lab 2.0 (https: //admetmesh.scbdd.com/), Molegro Virtual Docker (MVD) version 6.0, employed in the current study with system specifications Window 10 64 bit with hardware including Intel i3 processor (M330 @2.13 GHz) with 3GB DDR3 RAM.

Ligands preparation

Structure of all phytoconstituents from *Limonia acidissima* retrieved from Pubchem compound database and Chemspider **[16, 17]**. Chemdraw ultra software then used for structure cleanup **[18]**. Smile formats were obtained by Openbabel software **[19]**. Marvin Sketch 18.24 utilized for explicit hydrogen atom in structure of molecule and further molecules were cleaned into 3D **[20]**. The lost or missing bonds, charge along with hybridization state of the ligand, if any, can be corrected using MVD **[21]**.

Selection and preparation of PDB

All 3D structures of selected PDB's were downloaded from Protein Data Bank. Selection of PDB was done by analyzing resolution and survey of literature for a specific target. Total six PDB were selected for respective targets including Insulin Receptor, Aldose Reductase, Protein Tyrosine Phosphatase, Human Dipeptidyl Peptidase IV, Alpha amylase and Alpha glucosidase as 1IR3, 1US0, 2F6V, 2OQV 5NN6 and 2QV4 respectively **[22]**.

QSAR analysis

Zinc15 is a free online tool that helps to trace out imperative parameters required for selection and screening of ligands along with Molinspiration, both are incorporated to the study to do QSAR studies and to investigate potential activators of biological objects **[23]**.

ADMET studies

ADMET studies were carried out using ADMETlab 2.0. Open Babel software used for conversion of ligand's structure in 'smiles' format. Smile formats then uploaded in ADMETlab 2.0.Various molecular aspect of ligand then analyzed like Molecular weight, Log S value , log P value , HIA, PPB, NHA, NHD, TPSA, P-gp substrate. Besides that Lipinski rule, Pfizer Rule, GSK Rule and Golden Triangle rule considered for selection of ligands. Additionally Acute Toxicity Rule, SureChEMBL Rule are also analyzed in detail **[24]**.

Molegro Virtual Docker

MVD is a magnificent tool for prediction of interactions between targets protein and ligands. It is truly outstanding and most recent tool for doing proficient evaluation of docking examination. In contrast with other accessible tools, it gives the most encouraging results of binding tendencies of the ligands. Percentage of results was found 87 with MVD. On the other hand results for FlexX2 (57.9) Surflex (75.3),

Gold (78.2) and Glide (81.8) percent **[25]**. Ligands were imported in MVD. Energy minimization was done afterwards. Selected PDB was imported after removal of water and co factor if (present). Protein preparation was done by repairing the warning present in the structure. Internal ligands are also present with PDB for finding surface for interaction. The surface was detected followed by cavity detection. Cavities are actually grid points where ligand gets fit. After importing ligand and reset view docking was proceeded. Values for hydrogen bond were analyzed and all interactions between target and ligand were labeled. Determination of *in silico* results of ligand was done by calculating moldock score, number of interactions and hydrogen bonding.

RESULTS AND DISCUSSION

ADMET studies

Total 22 phytoconstituents of *Limonia acidissima*, screened by ADMET filter out of which12 followed the ADMET profile parameters including Imperatorin, Xanthotoxin, Lupeol, Bergapten, Marmin, Marmesin, Aurapten, Isopimpinellin, (+)-Syringaresinol,(+)-Yangambin, Hederatriol and Seselin **(Table 1)**. For selection of phytoconstituents various parameter like TPSA, solubility, SA score, PAINS, Human Intestinal Absorption, Pgp-substrate, Plasma Protein Binding, Carcinogencity, Genotoxic, carcinigenocity Rule considered in detail.

Docking Results

All selected phytoconstituents docked with six selected PDB by MVD. Assessment of binding affinity was done by calculating Moldock score, total number of Interactions between ligand and protein. Besides that length of hydrogen bond also considered. All the results reflect the number of interaction between ligand and protein found in range of 1-18. Below mentioned molecules exhibit a good binding tendency with receptors. A comparative data between internal ligand and phytoconstituents was shown in **Table 2**.

Insulin Receptor (IR)

Moldock score value and number of hydrogen bond for internal ligand (ANP) was found to be-140.98 and 7 respectively. Whereas, standard drug Glibenclamide shows Moldock score -125.221 and number of hydrogen bond 5 with Insulin Receptor. On the other hand following selected phytoconstituents show comparable results for moldock score and hydrogen bond; Aurapten-109.537,(1) Hederatriol -106.253(8) Marmin-109.662(7) Syringar-esinol -116.385 (9) Yangambin -108.949 (3). Syringaresinol shows the maximum 6 number of interaction with 1IR3. Main residue interacted were Ser 1006, Lys 1030 and Met1079. Most common amino acid residue interacted with all molecules is Met

1079 **(Figure 1)**.

Aldose Reductase (AR)

Internal ligand (LDT_320) gives the value of Moldock score -147.82 and 5 H-bonds with Aldose Reductase. Whereas Glibenclamide have value for moldock score and hydrogen bond are -204.55 and 7. In contrast to internal ligand value for moldock score and hydrogen bond were found to be as Aurapten -157.286(7), Marmesin-130.195(10), Marmin-165.423(9), Syringaresinol -154.25(14). Maximum 9 interactions were shown by Syringaresinol. Main Amino acid residues involved Leu 212, Ser 210, Lys 21, Tyr 48, Ser 159, His 110, Trp 110, Asn 160, Cys 298 **(Figure 2)**.

Protein tyrosine phosphatase 1

Internal ligand (SK2-608) gives the moldock score -91.32 and H- bonds 5 with 2F6V. On the other hand value of moldock score of most of phytoconstituents were found to be greater than internal ligand. Moldock score and hydrogen bond found to be Burgapten, Hederatriol, Syringaresinol, Yangambin and Glibenclamide -91.5312(7) -123.078(12) -129.037(0.5) -252.649(10), -114.86(1) respectively. Yangambin forms maximum 18 interactions with 2F6V.Gln 266, Arg 221, Phe 182, Cys 215, Ala 217, Ser 216, Arg 221, Ser 216, Tyr 46, Cys 121, Asn 111, were the main amino acid residues involved in interaction **(Figure 3)**.

Human Dipeptidyl Peptidase IV (DPP4)

Value for moldock score and Hydrogen bonds of internal ligand was found to be -114.86 and 6. Shortest bond length was found 2.69 Å. Whereas value for moldock sore and Hydrogen bonds was found for Aurapten-105.983(4), Hederatriol -113.076(4), Marmin-123.205(6), Syring-aresinol -108.826(7), Yangambin -108.857(6), Glibenclamide (standard drug) -133.91(2). Syringaresinol shows max 10 interaction with the PDB. Residue involved were Arg 358, Phe 357, Tyr 666, Tyr 547, and Tyr 662 **(Figure 4)**.

Alpha glucosidase

Moldock score for the internal ligand of 5NN6 was found to be -119 with 9 hydrogen bonds. Whereas moldock score and number of hydrogen for Hederatriol is 112.393(5), Lupeol-112.895(1), Syring-aresinol -121.917(3), Yangambin -100.644(0) and Glibenclamide -28.0824(4). Lupeol forms shortest bond length of 1.55 Å **(Figure 5)**.

Alpha amylase

Internal ligand NAG_497 shows moldock score and hydrogen bonds -65.54(7). On contrary value of moldock score and hydrogen bonds for Aurapten is -112.143(1), Hedertriol-117.725(6), Marmin-101.339(10), Syringaresinol -95.6859 (9) and Glibenclamide (standard drug) -135.219 (3). Syringaresinol and Yangambin shows max interaction 7 **(Figure 6)**. Syringaresinol forms shortest bond length 2.35 Å.

Table 1: ADMET profile of Phytoconstituents of *Limonia acidissima*

Sr. no	Name	LogS	LogP	Pgp-sub	HIA	PPB	MW	nHA	nHD	TPSA	Lipinski	Pfizer	GSK	GoldenTriangle	SureChEMBL
1.	Imperatorin	-4.58	3.879	0.011	0.008	81.85%	270.09	4	0	52.58	Yes	No	Yes	Yes	0
2.	Xanthotoxin	-3.686	2.071	0.003	0.007	82.96%	216.04	4	0	52.58	Yes	Yes	Yes	Yes	0
3.	Lupeol	-6.199	7.291	0.00	0.008	98.80%	426.39	1	1	20.23	Yes	No	No	No	0
4.	Bergapten	-3.393	2.381	0.931	0.006	85.24%	216.04	4	0	52.58	Yes	Yes	Yes	Yes	0
5.	Marmin	-3.019	2.943	0.679	0.034	90.43%	332.16	5	2	79.9	Yes	Yes	Yes	Yes	0
6.	Marmesin	-2.852	2.248	0.005	0.005	87.43%	246.09	4	1	59.67	Yes	Yes	Yes	Yes	0
7.	Aurapten	-5.577	5.868	0.008	0.004	88.57%	298.16	3	0	39.44	Yes	No	No	Yes	0
8.	Isopimpinellin	-3.669	2.132	0.002	0.015	74.69%	246.05	5	0	61.81	Yes	Yes	Yes	Yes	0
9.	(+)-Syringaresinol	-4.725	1.993	0.147	0.014	82.05%	418.16	8	2	95.84	Yes	Yes	No	Yes	0
10.	(+)-Yangambin	-4.133	2.383	0.012	0.004	56.74%	446.19	8	0	73.84	Yes	Yes	No	Yes	0
11.	Hederatriol	-4.61	5.441	0.000	0.019	98.33%	458.38	3	3	60.69	Yes	No	No	Yes	0
12.	Seselin	-4.053	3.08	0.001	0.007	93.88%	228.08	3	0	39.44	Yes	No	Yes	Yes	0

Table 2: Docking results of phytoconstituents of *Limonia acidissima*

Ligand/internal ligand	PDB	MolDock Score	Interaction	HBond
ANP	1IR3	-140.98	-144.151	-7.94
Aurapten	1IR3	-109.537	-101.977	-1.88846
Burgapten	1IR3	-72.4031	-80.3424	-4.96852
Hederatriol	1IR3	-106.253	-110.874	-8.18386
Imperatorin	1IR3	-95.5955	-100.965	-2.5
Isopimpinellin	1IR3	-78.1174	-88.5402	-4.92051
Lupeol	1IR3	-107.923	-100.46	-2.48951
Marmesin	1IR3	-77.4264	-90.477	-5.58609
Marmin	1IR3	-109.662	-121.092	-7.5
Seselin	1IR3	-70.7094	-82.776	-2.5
Syringaresinol	1IR3	-116.385	-130.93	-9.67998
Xanthotoxin	1IR3	-74.1503	-83.4785	-2.32416
Yangambin	1IR3	-108.949	-123.707	-3.13736
Glibenclamide	1IR3	-125.221	-134.111	-5.71798
LDT_320	1US0	-147.82	-167.06	-4.33
Aurapten	1US0	-157.286	-172.326	-7.85586
Burgapten	1US0	-108.628	-116.767	-8.3368
Hederatriol	1US0	-99.2345	-112.794	-11.1965
Imperatorin	1US0	-128.282	-135.338	-4.19766
Isopimpinellin	1US0	-107.853	-118.675	-4.00779
Lupeol	1US0	-130.268	-123.769	-2.5
Marmesin	1US0	-130.195	-142.511	-10.6492
Marmin	1US0	-165.423	-192.13	-9.11642
Seselin	1US0	-114.669	-126.736	-3.56859
Syringaresinol	1US0	-154.25	-176.533	-14.2871
Xanthotoxin	1US0	-105.366	-114.881	-7.13279
Yangambin	1US0	-110.208	-129.278	-4.21542
Glibenclamide	1US0	-204.55	-223.683	-7.5464

SK2_608	**2F6V**	**-91.32**	**-122.35**	**-4.92**
Aurapten	**2F6V**	**-51.7446**	**-63.7016**	**-6.88405**
Burgapten	**2F6V**	**-91.5312**	**-100.242**	**-7.1414**
Hederatriol	**2F6V**	**-123.078**	**-126.614**	**-12.3223**
Imperatorin	**2F6V**	**-94.3298**	**-104.365**	**-5.94486**
Isopimpinellin	**2F6V**	**-39.8427**	**-56.8525**	**-9.8019**
Lupeol	**2F6V**	**100.738**	**107.804**	**-2.17584**
Marmesin	**2F6V**	**-90.5078**	**-97.2469**	**-2.5**
Marmin	**2F6V**	**6.7882**	**-23.9962**	**-11.1292**
Seselin	**2F6V**	**-56.7494**	**-68.816**	**-4.4639**
Syringaresinol	**2F6V**	**129.037**	**105.077**	**0.587128**
Xanthotoxin	**2F6V**	**-75.0599**	**-84.3361**	**-10.6966**
Yangambin	**2F6V**	**252.649**	**230.897**	**-10.2771**
Glibenclamide	**2F6V**	**150.417**	**128.863**	**-5.92574**
MA9_901	**2OQV**	**-114.86**	**-140.79**	**-1.95**
Aurapten	**2OQV**	**-105.983**	**-116.134**	**-4.46115**
Burgapten	**2OQV**	**-93.2188**	**-101.723**	**-4.40056**
Hederatriol	**2OQV**	**-113.076**	**-119.222**	**-4.84382**
Imperatorin	**2OQV**	**-92.2384**	**-91.145**	**-5.36752**
Isopimpinellin	**2OQV**	**-77.1985**	**-87.4837**	**-7.61452**
Lupeol	**2OQV**	**-107.034**	**-100.955**	**-4.1296**
Marmesin	**2OQV**	**-85.4979**	**-93.1446**	**-5.13462**
Marmin	**2OQV**	**-123.205**	**-146.127**	**-6.67061**
Seselin	**2OQV**	**-89.2707**	**-101.337**	**-4.08608**
Syringaresinol	**2OQV**	**-108.826**	**-128.063**	**-7.41973**
Xanthotoxin	**2OQV**	**-91.3689**	**-100.655**	**-3.85359**
Yangambin	**2OQV**	**-108.857**	**-132.118**	**-6.40143**
Glibenclamide	**2OQV**	**-133.91**	**-121.778**	**-2.6043**
NAG NAG BMA	**5NN6**	**-119**	**-157.12**	**-19.9**
Burgapten	**5NN6**	**-78.8527**	**-87.1624**	**-2.08452**
Hederatriol	**5NN6**	**-112.393**	**-115.509**	**-5.15451**
Isopimpinellin	**5NN6**	**-66.5109**	**-76.729**	**-2.14435**
Lupeol	**5NN6**	**-112.895**	**-113.052**	**-1.82578**
Marmesin	**5NN6**	**-79.9544**	**-88.6542**	**-1.77678**
Marmin	**5NN6**	**-90.3349**	**-105.403**	**-5**
Seselin	**5NN6**	**-69.0923**	**-81.1587**	**-2.49285**
Syringaresinol	**5NN6**	**-121.917**	**-131.029**	**-3.20616**
Xanthotoxin	**5NN6**	**-77.7896**	**-87.0839**	**0**
Yangambin	**5NN6**	**-100.644**	**-118.639**	**-0.18057**
Glibenclamide	**5NN6**	**-28.0824**	**-41.7339**	**-4.09958**
NAG_497	**2QV4**	**-65.54**	**-75.16**	**-7.84**
Aurapten	**2QV4**	**-112.143**	**-122.248**	**-1.06224**
Burgapten	**2QV4**	**-75.1166**	**-83.1283**	**-3.13985**
Hederatriol	**2QV4**	**-117.725**	**-120.798**	**-6.09232**
Imperatorn	**2QV4**	**-97.7434**	**-103.249**	**-2.65561**
Isopimpinellin	**2QV4**	**-82.967**	**-92.9571**	**-2.60898**
Lupeol	**2QV4**	**-121.726**	**-115.431**	**-1.59318**
Marmesin	**2QV4**	**-88.9645**	**-97.4915**	**-5.67342**
Marmin	**2QV4**	**-101.339**	**-122.662**	**-10.2403**
Seselin	**2QV4**	**-82.2401**	**-94.3067**	**-2.48619**
Syringaresinol	**2QV4**	**-95.6859**	**-110.662**	**-9.34193**
Xanthotoxin	**2QV4**	**-85.6053**	**-94.9655**	**-5.08975**
Yangambin	**2QV4**	**45.9002**	**22.1479**	**-7.60702**
Glibenclamide	**2QV4**	**-135.219**	**-124.862**	**-3.96845**

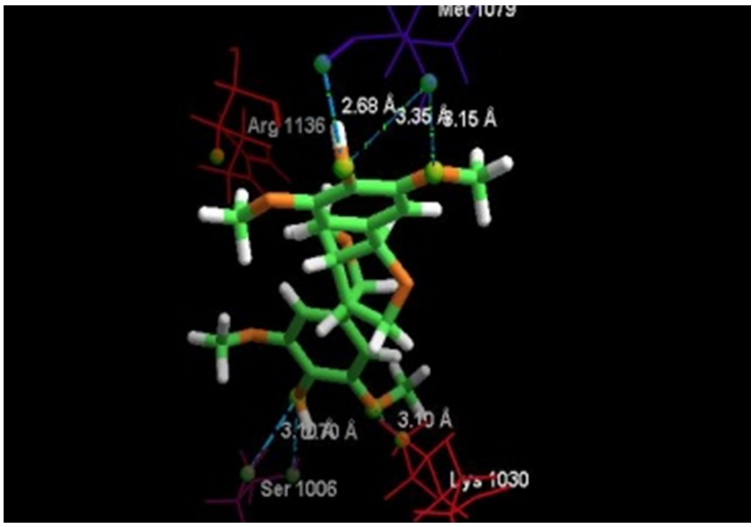

Figure 1: PDB-1IR3, Ligand Syringaresinol

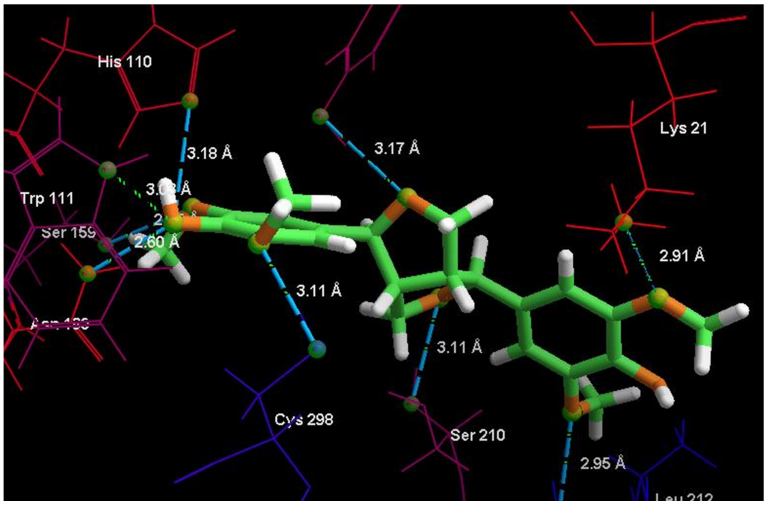

Figure 2: PDB-1US0, Ligand Syringaresinol

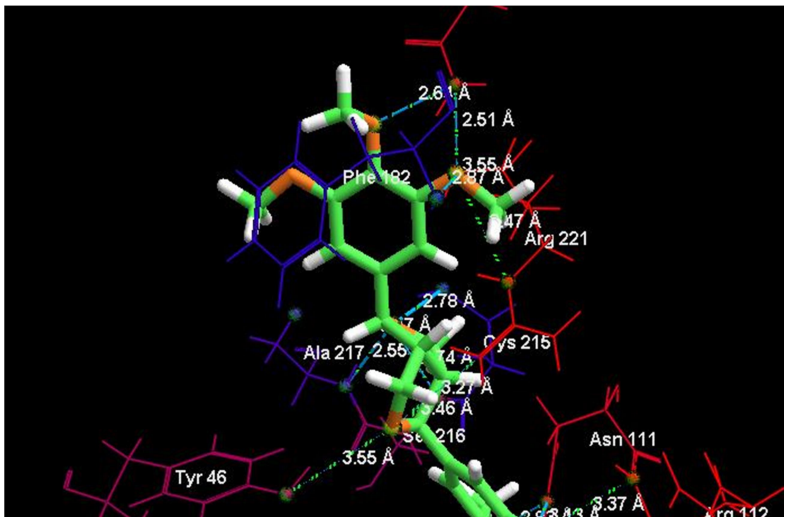

Figure 3: PDB-2F6V, Ligand Yangambin

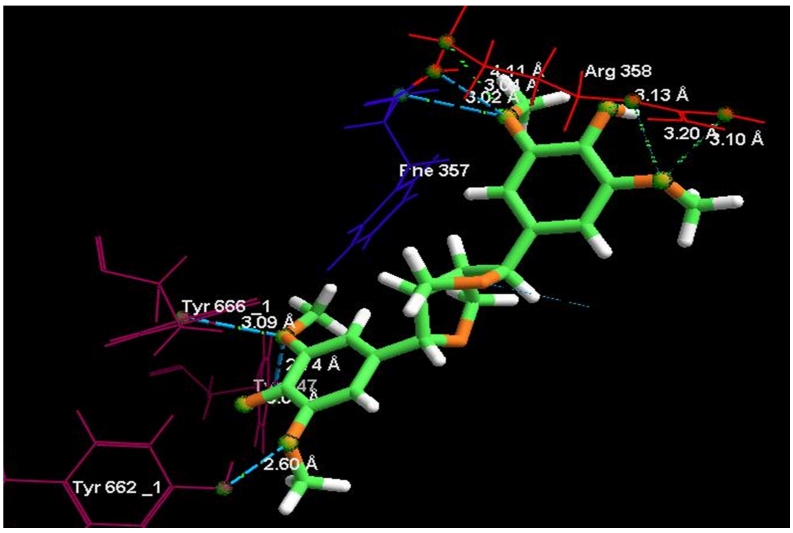

Figure 4: PDB-2OQV, Ligand Syringaresinol

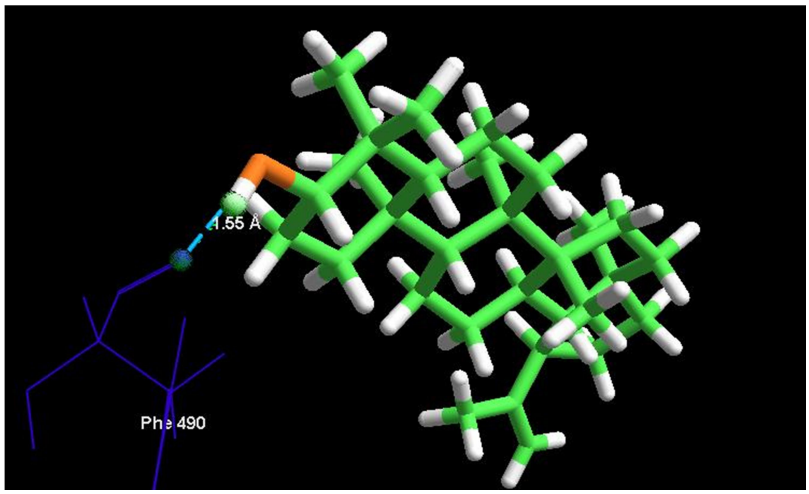

Figure 5: PDB-5NN6, Ligand Lupeol

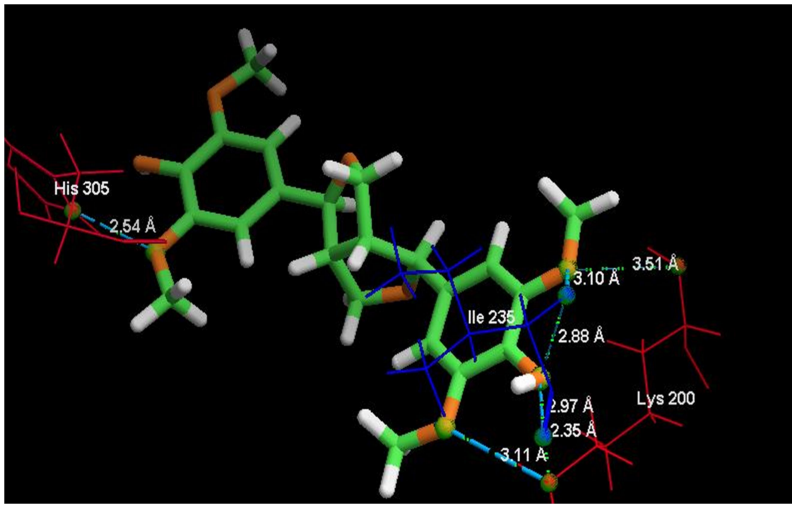

Figure 6: PDB-5NN6, Ligand Syringaresinol

CONCLUSION

In the current study antidiabetic potential of different phytoconstituents of *Limonia acidissima* by molecular docking analysis was evaluated. Before docking analysis, ADMET profile of each phytoconstituents studied in detail. ADMET data obtained from ADMETlab 2.0 give an idea regarding absorption, dissolution, metabolism, excretion and toxicity parameters in detail. These parameters aid to evaluate drug likeness of the selected phytoconstituents. Total 12 out of 22 Constituents of *Limonia acidissima* shows good ADMET profile parameters like Human Intestinal Absorption, SA score, Plasma Protein Binding, PAINS, Carcinogencity, Genotoxic carcinigenocity. Comparative analysis of moldock score shows comparable results with internal ligand and standard drug Glibenclamide. Besides that the value of hydrogen bonds and number of interaction gives remarkable results. From gist of whole study it was concluded that 6 compounds namely Aurapten, Burgapten, Hederatriol, Marmin, Syringaresinol and Yangambin have good affinity towards receptors involved in the study. Interestingly, Syringaresinol shows great affinity towards every receptor. It is also concluded from the study that especially flavanoids, lignans, coumarines, furanocoumarines and triterpenoids seems to be good candidates for antidiabetic poteintial. Thus selected 6 phytoconstituents of *Limonia acidissima* probably use for the management of diabetes mellitus after further analysis and research.

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