ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

IN-SILICO SCREENING FOR THE IDENTIFICATION OF SLC6A4 DRUG TARGETS AND ANTI-RHEUMATIC ACTIVITY OF PHYTOLIGANDS

S. Sribal S* [1]. Pooja Sri [2]
Department of Genetic Engineering, Bharath University, Chennai - 600073, Tamil Nadu, India.
Email: Sribalsridhar1996@gmail.com

Received on: 18-08-2017 *Accepted on: 23-09-2017*

Abstract

Fibromyalgia is a disorder which is characterized by musculoskeletal pain accompanied by fatigue, sleep, memory and mood issues. This disorder is common and much more affect the women than the men. The main cause of fibromyalgia is still now unpredictable one and the symptoms are also gradually accumulates overtime with no previous signal triggering event. The people with this disorder also suppress to have headaches, temporomandibular joint (TMJ) disorders, irritable bowel syndrome, anxiety and depression. In meanwhile there is no cure for fibromyalgia and early genetic studies revealed that the gene SLC6A4 showed an increased frequency among patients of fibromyalgia compared to healthy controls. Hence there is a high level of demand to find a drug which cures this disorder. From the basis of this hypothesis my project is designed to find an anti-fibromyalgia drug target from the medicinal plant *Combretum indicum*. There are 25 phyto-ligand were selected such as, Oleic acid, Palmitic acid, Sito sterol, Rutin, Trigonelline, Quisqualic acid, Pelargonidin 3-glycoside, α-pinene, 1-ethyl 1- phenyl, α-amyrin, Dinitrophenyl, Cyaniding monoglycoside, Linoleic acid, Stearic acid, Arachidic acid, 1, 2, 4-oxidiazolidin-3, 5-dione, Myristic acid, Arachonidic acid, α-Xylofuranosyluracil, Betulinic acid, Methylurosalate, Arjunolic acid, Kaempferol, 23, 24-dihydrocucurbitacin F, α-Santonin. The molecular docking of SLC6A4 with that phyto-ligand will show the lead compound on the basis of minimum binding energy values. And finally the ADMET studies will analyse to find a fibromyalgia drug target.

Keywords: Fibromyalgia, HLA-DRB4 AND SLC6A4, *Combretum indicum*, molecular docking, ADMET studies.

Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is a monomer neurotransmitter. Serotonin is primarily found in blood platelets, gastrointestinal tract and CNS of humans. Researchers thought to be the serotonin molecules are found on

the cell membrane of nerve cells and other cell types in humans and also it act as an endogenous ligand. The raphe nuclei's neurons are the main source of serotonin neurotransmitter. There are 9 raphe nuclei are found among all others the B1-B9 have majority of serotonin containing neurons.

The serotonin neuro transmitter is also known as sodium-dependent serotonin transporter and solute carrier family 6 member 4 proteins. The gene which codes for this protein is SLC6A4. Researchers believed this protein is responsible for many mode related activities. This protein transport and remove the serotonin molecules to synaptic cleft and reuptake it for reuse.

Serotonin transporter performs the primary function in the central nervous system involves the regulation of serotonergic signalling via transport of serotonin molecules from the synaptic cleft back into the pre-synaptic terminal for re-utilization. It plays a key role in mediating regulation of the availability of serotonin to other receptors of serotonergic systems.

From the statistical medical studies of fibromyalgia the protein SLC6A4 plays a vital role and it's also showed the elevated expression level of expression in fibromyalgia patients when compared to the normal patients. Hence this bioinformatic work was done to find out anti-fibromyalgia drug through In-silico Drug designing. The complete detailed information about the SLC6A4 protein is given below;

- Protein symbol: p31645-SLC6A4_HUMAN
- Size: 630 amino acids
- Molecular mass: 70,325 Daltons
- Protein length: 549

Materials and Methodology

Software's Used

Open Babel GUI, Chemsketch, SPDBV viewer, Autodock vina, GROMACS, CYGWIN Terminal, and UCSF Chimera.

Preparation of Protein Target SLC6A4

The 3 dimensional structure of protein target SLC6A4 was downloaded from the protein data bank (PDB). The heteroatoms were removed from the protein SLC6A4. The energy minimization of protein target was done by using SPDB viewer and all the spdbv coordinates from the protein complex. Now the protein target was prepared and ready to do docking with selected phyto-ligands.

FASTA SEQUENCE OF SLC6A4

>sp|P31645|SC6A4_HUMAN Sodium-dependent serotonin transporter OS=Homo sapiens GN=SLC6A4 PE=1 SV=1

METTPLNSQKQLSACEDGEDCQENGVLQKVVPTPGDKVESGQISNGYSAVPSPGAGDDTRHSIPATTTTLVA

ELHQGERETWGKKVDFLLSVIGYAVDLGNVWRFPYICYQNGGGAFLLPYTIMAIFGGIPLFYMELALGQYH

RNGCISIWRKICPIFKGIGYAICIIAFYIASYYNTIMAWALYYLISSFTDQLPWTSCKNSWNTGNCTNYFSEDNI

TWTLHSTSPAEEFYTRHVLQIHRSKGLQDLGGISWQLALCIMLIFTVIYFSIWKGVKTSGKVVWVTATFPYIIL

SVLLVRGATLPGAWRGVLFYLKPNWQKLLETGVWIDAAAQIFFSLGPGFGVLLAFASYNKFNNNCYQDAL

VTSVVNCMTSFVSGFVIFTVLGYMAEMRNEDVSEVAKDAGPSLLFITYAEAIANMPASTFFAIIFFLMLITLG

LDSTFAGLEGVITAVLDEFPHVWAKRRERFVLAVVITCFFGSLVTLTFGGAYVVKLLEEYATGPAVLTVALI

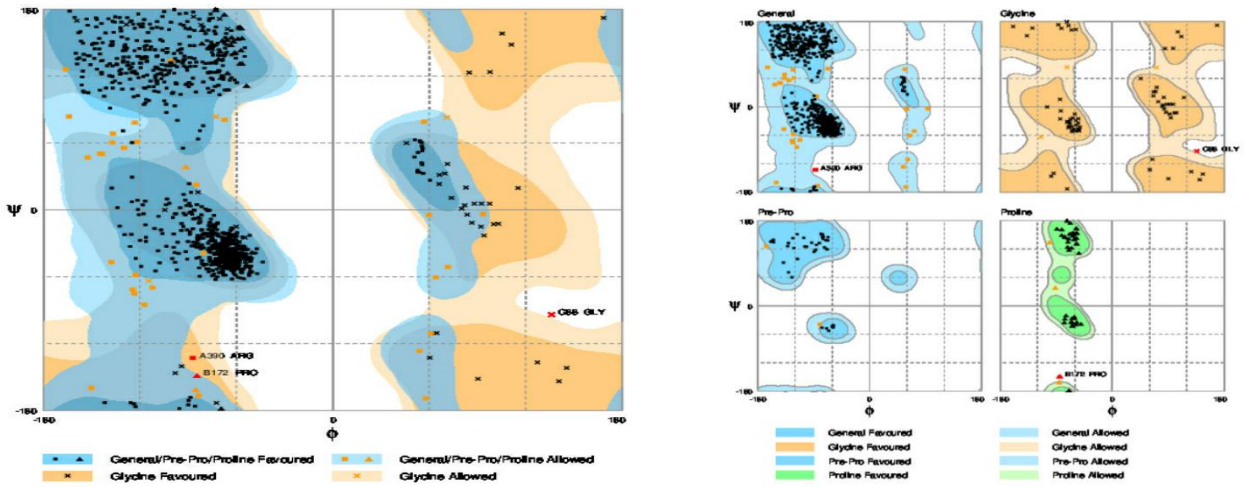
EAVAVSWFYGITQFCRDVKEMLGFSPGWFWRICWVAISPLFLLFIICSFLMSPPQLRLFQYNYPYWSIILGYCI

GTSSFICIPTYIAYRLIITPGTFKERIIKSITPETPTEIPCGDIRLNAV

SLC6A4 RAMACHANDRAN PLOT

PHYSIO-CHEMICAL PROPERTIES OF SLC6A4

- Number of Amino acids: 630



- Molecular weight: 70324.86 Daltons
- Theoretical PI: 5.89
- Amino acid composition of **SLC6A4**

S.NO	AMINO ACIDS	RESIDUES COUNT	COMPOSITION (%)
1.	Ala (A)	46	7.3%
2.	Arg (R)	19	3.0%
3.	Asn (N)	22	3.5%
4.	Asp (D)	18	2.9%
5.	Cys (c)	18	2.9%

6.	Gln (Q)	18	2.9%
7.	Glu (E)	27	4.3%
9.	Gly (G)	50	7.9%
10.	His (H)	07	1.1%
11.	Ile (I)	56	8.9%
12.	Leu (L)	61	9.7%
13.	Lys (K)	21	3.3%
14.	Met (M)	12	1.9%
15.	Phe (F)	43	6.8%
16.	Pro (P)	30	4.8%
17.	Ser (S)	41	6.5%
18.	Thr (T)	46	7.3%
19.	Trp (W)	19	3.0%
20.	Tyr (Y)	31	4.9%
21.	Val (V)	45	7.1%
22.	Pyi (O)	0	0.0%
23.	Sec (O)	0	0.0%
24.	(B)	0	0.0%
25.	(Z)	0	0.0%
26.	(X)	0	0.0%

Total number of negatively charged residue (Asp + Glu) = 45

Total number of positively charged residue (Arg + Lys) = 40

Atomic composition

Carbon (C): 3278

Hydrogen (H): 4949

Nitrogen (N): 781

Oxygen (O): 879

Sulphur (S): 30

Total number of atoms: 9917

Extinction Coefficient

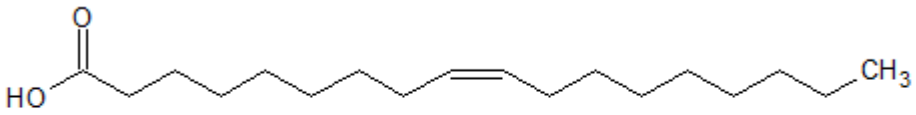
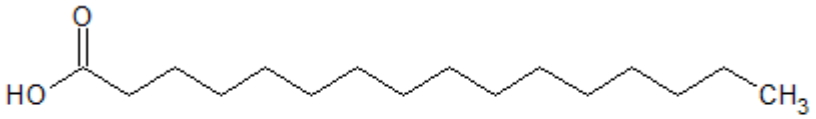
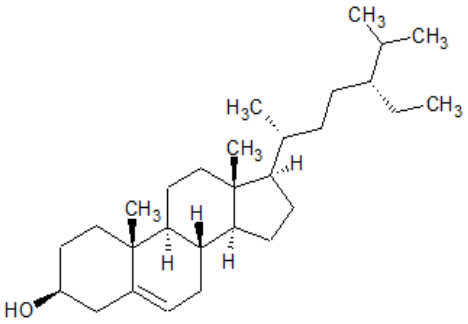
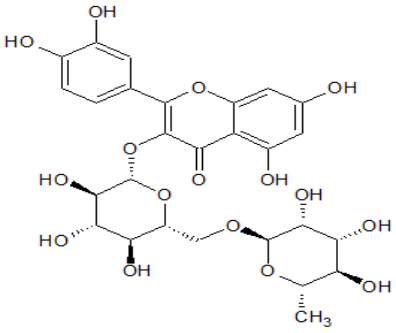
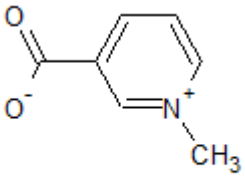
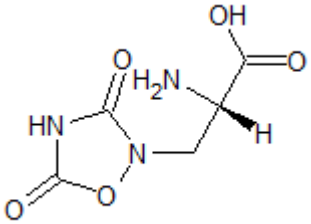
Extinction coefficients are in units of n^{-1} cm^{-1}, at 280nm measured in water.

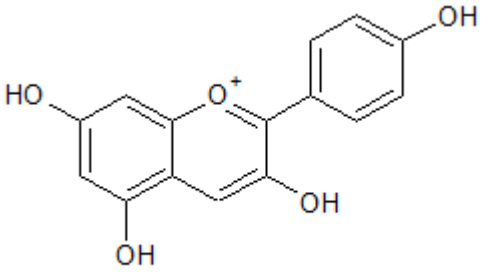
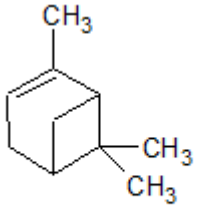
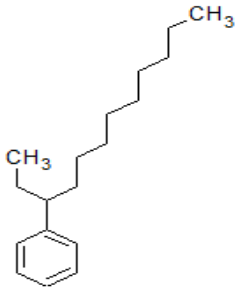
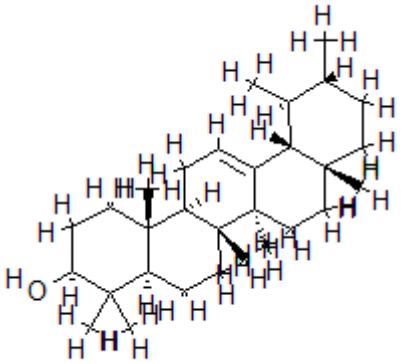
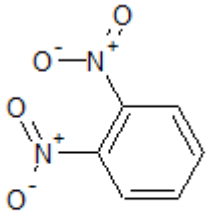
Extinction coefficient: 151815

Absorbance 0.1% (=1g/l) 2.159, assuming all pairs of cys residues from cystines.

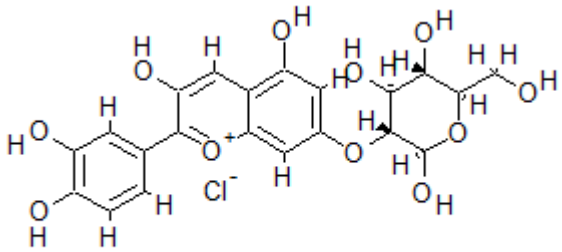
Extinction coefficient: 150690

Absorbance 0.1% (=1g/l) 2.143, assuming all pairs of cys residues are reduced.

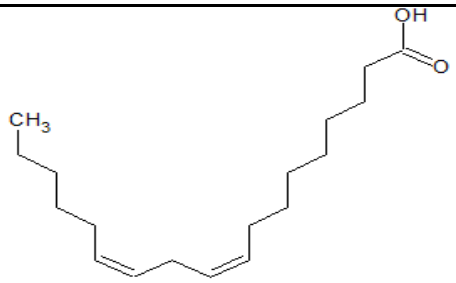
MOLECULES	CHEMICAL STRUCTURE
Oleic acid	
Palmitic acid	
Sito sterol	
Rutin	
Trigonelline	
Quisqualic acid	

Pelargonidin 3-glycoside	
α-pinene	
1-ethyl 1- phenyl decane	
α-amyrin	
Dinitrophenyl	

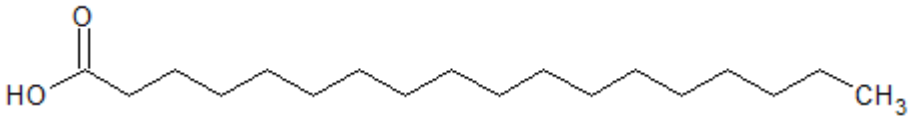
Cyanidin monoglycoside



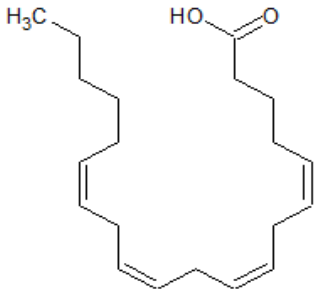
Linoleic acid



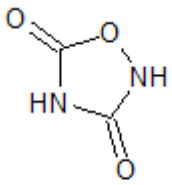
Stearic acid



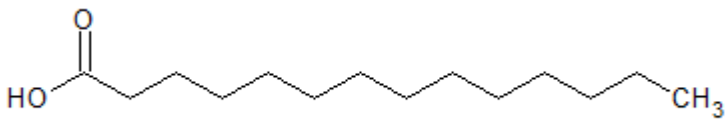
Arachidonic acid



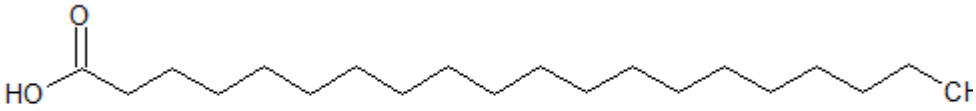
1,2,4-oxidiazolidin-3,5-dione



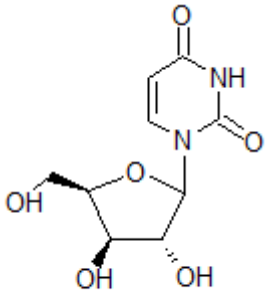
Myristic acid



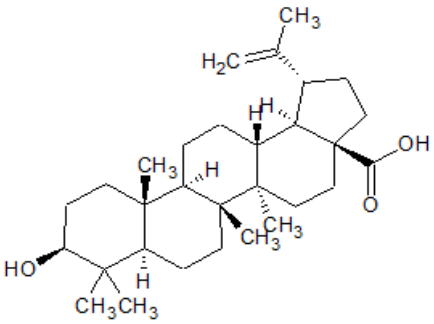
Arachidic acid



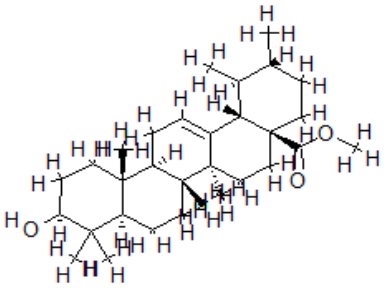
α-Xylofuranosyluracil



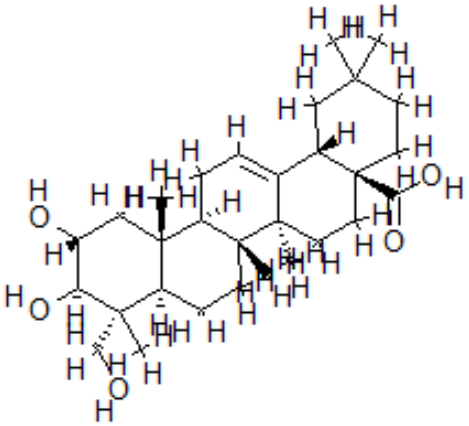
Betulinic acid

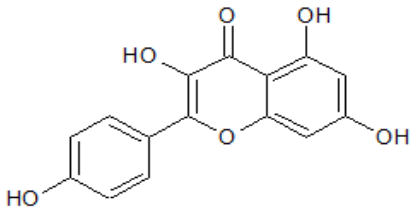
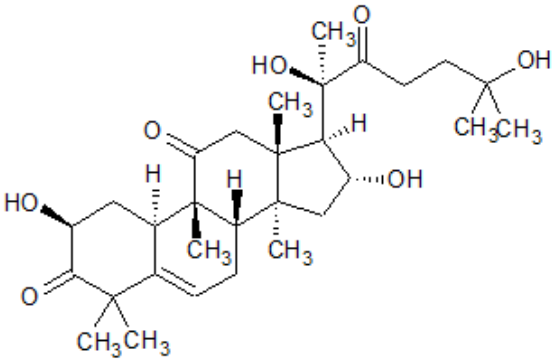
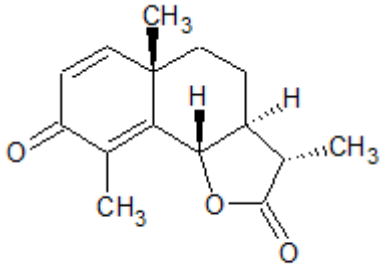


Methylurosalate



Arjunolic acid



Kaempferol	
23,24-dihydrocucurbitacin F	
α-Santonin	

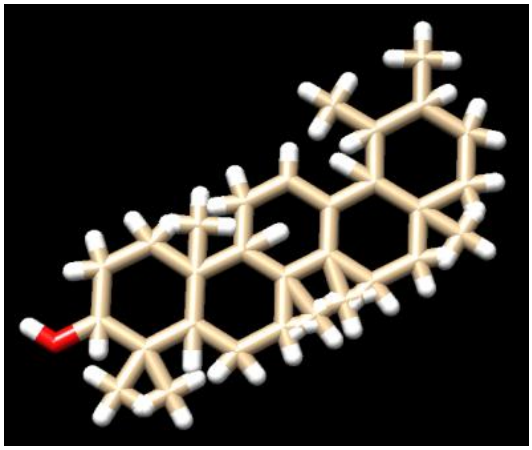
MOLECULAR DOCKING OF PROTEIN TARGET AND PHYTOLIGANDS

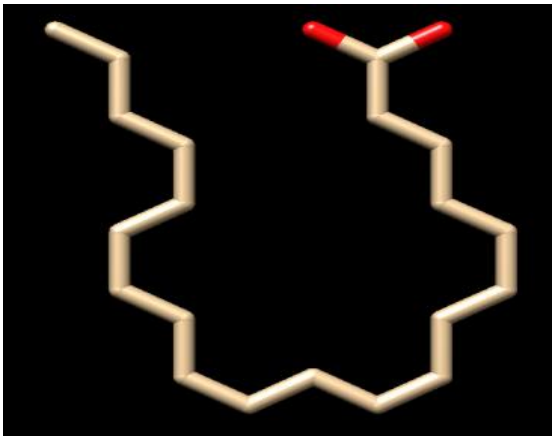
In this bioinformatic studies the molecular docking of phytoligands with protein target SLC6A4 by using Autodock vina 4.0 to find out the binding activity of lead compounds. The hydrogen bonds (polar bonds only), kollman charge and atoms are added and the grid parameter value for the protein target X= -0.376 Y= -28.896 Z= -11.059, then the output file was saved in gpf format. All the docking parameters were applied as per the docking methodology and the Lamarckian genetic algorithm score was applied to save a dpf output file. AMBER and Carlo simulation method is used for predicting the docking score. By entering the commands in Cygwin command box the dlg file was created and the created dlg file was opened by using word pad to find out the RMSD value of phytoligands which are used for docking. The predicted RMSD values had shown the minimum binding energies of all docked phytoligands. From this RMSD score the lead drug compounds are found out from all the selected phytoligands. Finally the phytoligands α-amyrin, Arachidonic acid, arjunolic acid, methylurosalate, and were selected as a lead compounds by their minimum binding energy orientation with the protein target SLC6A4.

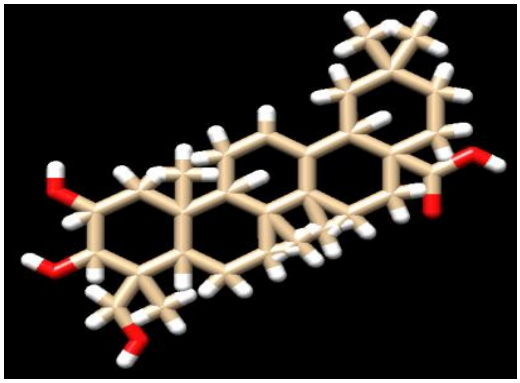
Tabular column no: 1

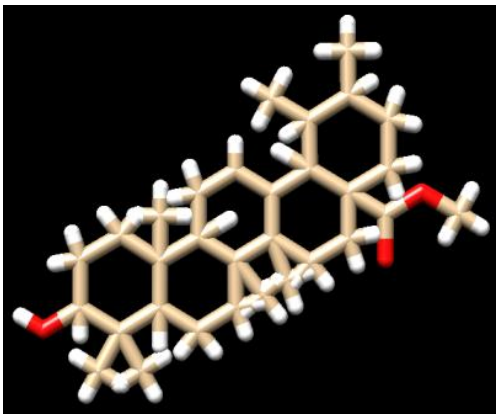
S.NO	MOLECULES	MINIMUM BINDING ENERGY	RUN
1.	Oleic acid	-2.73	8
2.	Palmitic acid	-2.58	4
3.	Sito sterol	-3.98	7
4.	Rutin	-5.38	2
5.	Trigonelline	-3.79	5
6.	Quisqualic acid	-4.98	8
7.	Pelargonidin 3-glycoside	-4.74	6
8.	α-pinene	-2.93	5
9.	1-ethyl 1- phenyl decane	-1.67	5
10.	α-amyrin	-5.92	2
11.	Dinitrophenyl	-4.39	7
12.	Cyanidin monoglycoside	-3.85	6
13.	Linoleic acid	-4.04	1
14.	Stearic acid	-1.75	1
15.	Arachidic acid	-1.46	9
16.	1,2,4-oxidiazolidin-3,5-dione	-3.45	4
17.	Myristic acid	-3.24	9
18.	Arachidonic acid	-4.93	10
19.	α-Xylofuranosyluracil	-3.94	8
20.	Betulinic acid	-4.53	6
21.	Methylurosalate	-6.53	4
22.	Arjunolic acid	-5.87	6
23.	Kaempferol	-4.31	2
24.	23,24-dihydrocucurbitacin F	-4.25	7
25.	α-Santonin	-4.15	2

MOLECULAR VISUALIZATION OF PROTEIN-LIGAND COMPLEX (5I6X PDB ID OF PROTEINTARGET)









α amyrin	arachidonic acid	arjunolic acid	Methylurosalate

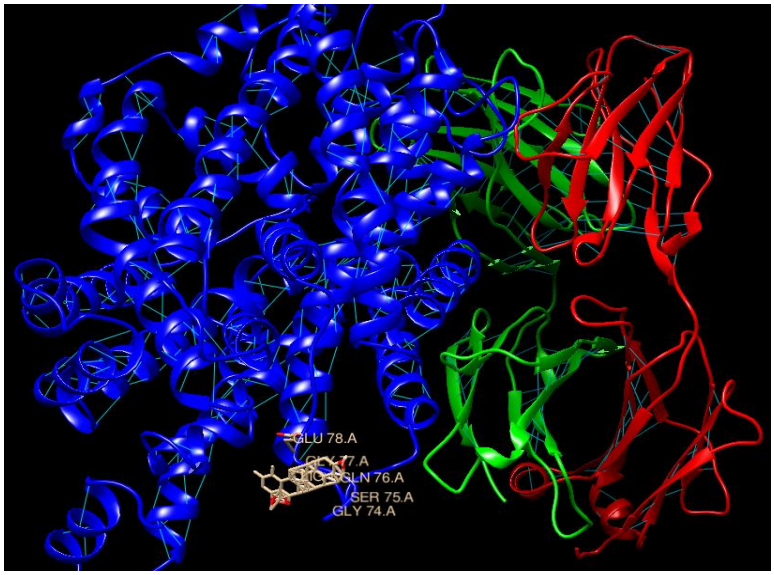


FIG. NO: 2 Methylurosalate bind with 5i6x protein target

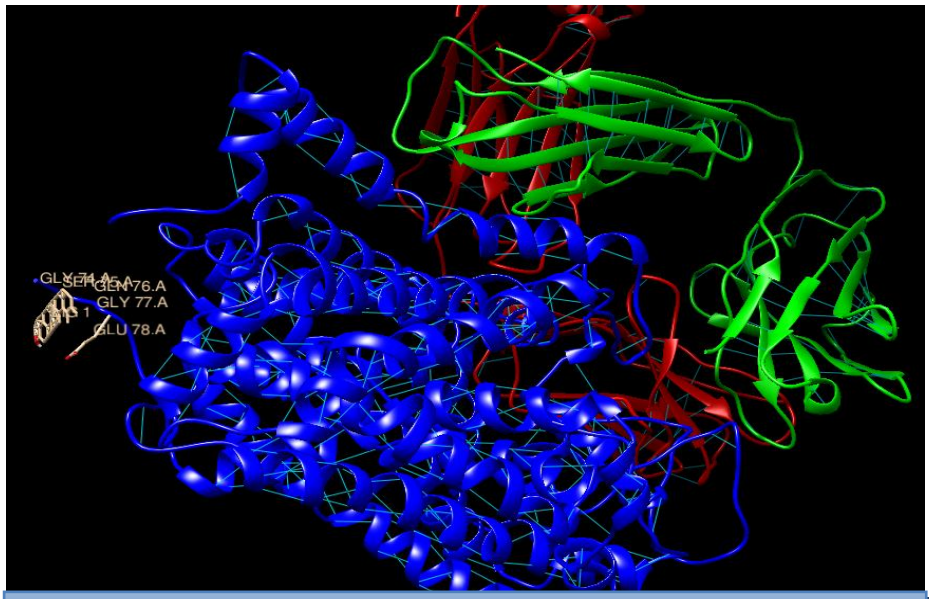


FIG. NO: 3 α-Amyrin bind with 5i6x protein target

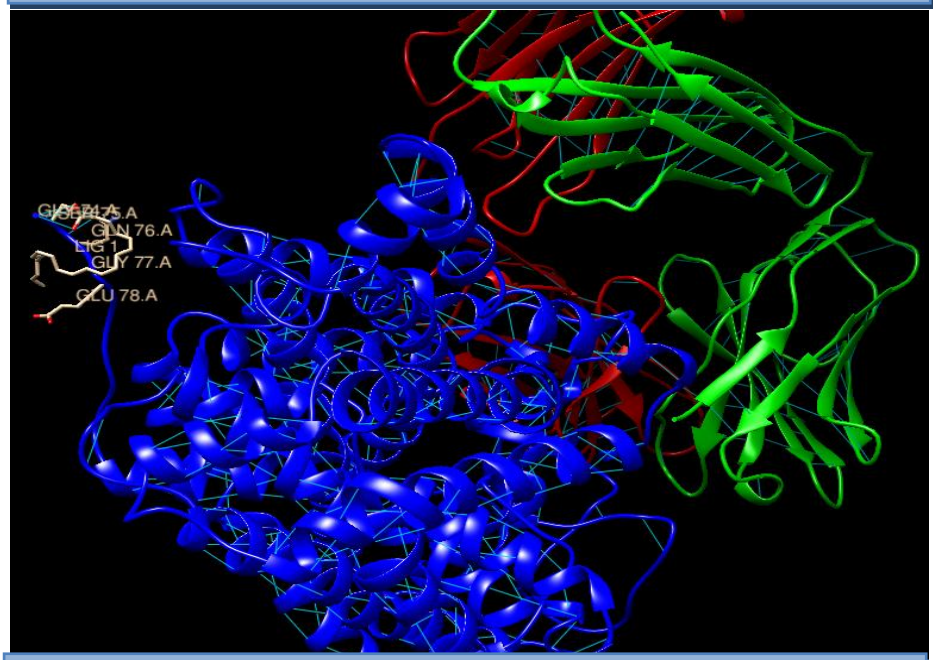


FIG. NO: 4 Arachidonic acid bind with 5i6x protein target

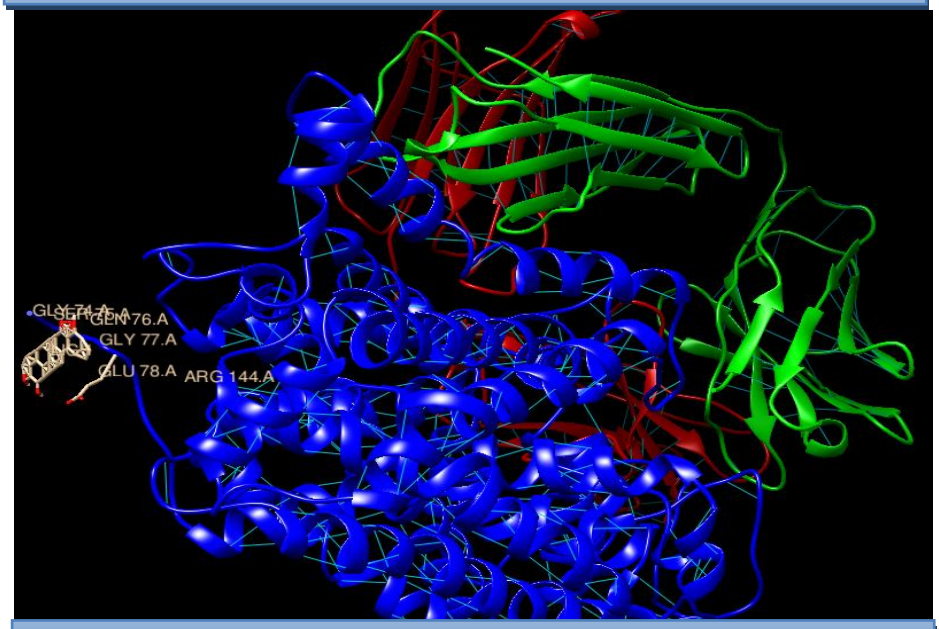


FIG. NO: 5 Arjunolic acid bind with 5i6x protein target

ADMET ANALYSIS

S.NO	α-AMYRIN	ADMET RESULTS
1.	Molecular weight	426.72
2.	No. Of hydrogen bond donors	1
3.	No. Of hydrogen bond acceptors	1
4.	TPSA	20.23
5.	No. Of rotatable bonds	0
6.	Monoisotopic mass	426.386166 Da
7.	Molar refractivity	131.86 ± 0.4 cm^3
8.	Molar volume	421.6 ± 5.0 cm^3
9.	Parachor	1054.5 ± 6.0 cm^3
10.	Index of refraction	1.537 ± 0.03
11.	Surface tension	39.1 ± 5.0 dyne/cm
12.	Density	1.01 0.1 g/cm^3
13.	Polarizability	52.27 ± 0.510 ^-24
14.	Oral bioavailability	Between 30% and 70%
15.	Stability	pH >2
16.	AMES test	0.01 (RI – 0.57) Reliability: moderate
17.	MRDD	1.67 mg/kg/day/
18.	LD 50 (mg/kg)	Mouse/intraperitoneal = 260 Mouse/ oral = 520 Mouse/intravenous = 15 Mouse subcutaneous = 65 Rat/intraperitoneal = 28

		Rat/oral = 6.5
19.	Health effects	Probability effect on Blood 0.96
20.	Log P value	9.28
21.	Toxicity	Cat. 3 and Cat.4 (79% probability)
22.	Absorption rate	Absorption rate Ka = 0.047 min^-¹
23.	Endocrine disruption	No binding to estrogen receptor alpha

S.NO	ARACHIDONIC ACID	ADMET RESULTS
1.	Molecular weight	304.47
2.	No. Of hydrogen bond donors	1
3.	No. Of hydrogen bond acceptors	2
4.	TPSA	37.3
5.	No. Of rotatable bonds	14
6.	Monoisotopic mass	304.24023 Da
7.	Molar refractivity	96.50 ± 0.3 cm^3
8.	Molar volume	327.7 ± 3.0 cm^3
9.	Parachor	798.4 ± 4.0 cm^3
10.	Index of refraction	1500 ± 0.02
11.	Surface tension	35.2 ± 3.0 dyne/cm
12.	Density	0.928 ± 0.06 g/cm^3
13.	Polarizability	38.25 ± 0.510 ^-24 cm^3
14.	AMES test	0.84 (High RI = 0.8)
15.	Oral bioavailability	Between 30% and 70%
16.	Stability	pH < 2
17.	MRDD	0.06 mg/kg/day/
18.	LD 50	Mouse/intraperitoneal = 140 Mouse/ oral = 2700

		Mouse/intravenous = 51 Mouse subcutaneous = 250 Rat/intraperitoneal = 920 Rat/oral = 6700
19.	Toxicity	95% probability belongs to Cat.5 and non-toxic
20.	Log p value	6.75
21.	Absorption	Human jejunum scale (pH =6.5) Absorption Ka – 0.052 min ^-¹
22.	Health affects	Probability of effect on blood = 0.47
23.	Endocrine disruption	No binding to receptor alpha

S.NO	ARJUNOLIC ACID	ADMET RESULTS
1.	Molecular weight	488.7
2.	No. Of hydrogen bond donors	4
3.	No. Of hydrogen bond acceptors	5
4.	TPSA	97.99
5.	No. Of rotatable bonds	2
6.	Monoisotopic mass	488.350175 Da
7.	Molar refractivity	136.62 ± 0.4 cm^3
8.	Molar volume	410.0 ± 5.0 cm^3
9.	Parachor	1109.0 ± 6.0 cm^3
10.	Index of refraction	1.580 ± 0.03
11.	Surface tension	53.5 ± 5.0 dyne/cm
12.	Density	1.19 ± 0.1 g/cm^3
13.	Polarizability	54.16 ± 0.510 ^-24 cm^3
14.	AMES test	Probability of positive AMES test = 0.01

		(Moderate RI = 0.66)
15.	Oral bioavailability	Between 30% and 70 %
16.	Stability	pH(>2)
17.	MRDD	3.76 mg/kg/day
18.	LD 50	Mouse/intraperitoneal = 430 Mouse/ oral = 1200 Mouse/intravenous = 64 Mouse subcutaneous = 1100 Rat/intraperitoneal = 65 Rat/oral = 12
19.	Health affects	79% probability belongs to cat.3, cat.4, cat.5
20.	Toxicity	Belongs to Cat.3 Cat.4 Cat.5
21.	Log P value	6.11
22.	Absorption rate	Human jejunum = 6.59 ×10^-4 Absorption rate Ka = 0.045 min^-¹
23.	Endocrine disruption	No binding to estrogen receptor alpha log RBA < 3

Result and Discussion

Form this work, the molecular docking of protein target and phytoligands, the compounds methylurosalate, alpha amyrin, arjunolic acid and arachidonic acid shows a minimum binding energy with the docked protein target SLC6A4. Hence i concluded by this bioinformatic work the lead compounds are suitable for designing a fibromyalgia multi-drug in pharmaceutical formulation as a generic drug. Furthermore the future study is planned to do in-vitro and in-vivo analysis.

Acknowledgement

It would be my great privilege to express gratitude and respect to all those who have trained me and inspired me during the course of the training period. I express my sincere gratitude to R. PRIYA Research Scholar of the Genetic

Department who is my teacher for having been a constant source of support and encouragement for the completion of the project. I finally would like to thanks my family for their encouragement and support.

Reference

1. Edible medicinal and non-medicinal plants volume 7 flowers by T.K. Lim.
2. A field guide to medicinal plants and herbs: of Eastern and Central North America (Peterson field guides) by Steven foster, James A. Duke, Roger.
3. Medicinal plants of India by Rasheeduz Zafar.
4. Medicinal plants: phytochemistry pharmacology and therapeutics by V.K. Gupta.
5. Medicinal plants cultivation: A scientific approach including processing and financial guidelines by S.S Purohit and S.P. Vyas.
6. Medicinal plants: the healing properties of plants by Helen Mayhew.
7. Indigenous medicinal plants and their practical utility by H.C Lakshman.
8. Atlas of Ayurvedic medicinal plants by Dr. Jasmeet singh.
9. Indian medicinal plants: A Compendium of 500 species (vol. v) by P.K Warrier and V.P.K. Nambiar.

Corresponding Author:

S. Sribal S*,

Email: Sribalsridhar1996@gmail.com