International Journal Of Public Mental Health And Neurosciences
ISSN No: 2394-4668
(An Official publication of Sarvasumana Association)

Establishing a Remedy for Phenylketonuria Disease from Medicinal Herbs

Gireesh Hegde[1], Anuradha M[1] and Preenon Bagchi[1,2]
[1]Padmashree Institute of Management and Sciences, Bengaluru, India
[2]Sarvasumana Association, Bengaluru, India

Abstract: **The phenylketonuria disease is an inherited disorder that increases the levels of a substance called phenylalanine in the blood and seizures, tremors or trembling and shaking, stunted growth, hyperactivity, skin conditions such as eczema, a musty order of their breath, skin or urine. Phenylketonuria is caused by mutations in the gene PAH, IGF1, etc. This study was carried out to establish the remedy for the phenylketonuria disease from medicinal herbs. As per virtual screening & ADME studies compounds tyrosine and curcumin can be successfully considered as novel drug leads for phenylketonuria disorder.**

Keywords: **Phenylketonuria, PAH gene, IGF1 gene, modeling, docking, ADME**

I. INTRODUCTION

Phenylketonuria which is commonly known as PKU, is an inherited disorder that increases the levels of a substance called phenylalanine in the blood. Phenylalanine is a building block of proteins (an amino acid) that is obtained through the diet and it is found in all proteins and in some artificial sweeteners. If PKU is not timely treated, phenylalanine can build up to harmful levels (toxins) in the body, causing intellectual disability and other serious health problems.

The signs and symptoms of PKU may vary from mild to severe. Without treatment, children develop permanent intellectual disability. Seizures, delayed development, behavioral problems, including psychiatric disorders are also common. Untreated individuals have a musty or mouse-like odor as a side effect of excess phenylalanine in the body [1-4].

Genes involved and their function:

PAH gene (phenylalanine hydroxylase):

The PAH gene provides instructions for making an enzyme called phenylalanine hydroxylase which is responsible for the first step in processing phenylalanine, which is a building block of proteins (an amino acid) obtained through the diet. Phenylalanine is found in all proteins and in some artificial sweeteners [5, 6].

IGF1 gene (insulin like growth factor 1)

The protein encoded by this gene is similar to insulin in function and structure and is a member of a family of proteins involved in mediating growth and development & its encoded protein is processed from a precursor, bound by a specific receptor, and secreted. Defects in this gene are a cause of insulin-like growth factor I deficiency and alternative splicing results in multiple transcript variants encoding different isoforms that may undergo similar processing to generate mature protein. This is isolated from plasma, are structurally and functionally related to insulin but have a much higher growth-promoting activity [7, 8, 9].

Herbs and their active components:

1. Wood betony: The chemical components present in this plant are Tannins, Betulinic acid, oleonilic acid, rosamarinic acid, rutin, urosolic acid, stachydrine, glycosides etc.
2. Nettle: The chemical components present in this plant are histamine, formic acid, acetylcholine, serotonin, vitamins etc.
3. Plantago ovate: The chemical components present in this plant are xylose, arabinose, alanine, valine, glutamic acid, glycine, cysteine, lysine, leucine, tyrosine, xylose etc.
4. Turmeric: The chemical components present in this plant are curcumin, camphene etc.
5. Dandelion: The chemical components present in this plant are taraxacin,laevulin,resin,inulin etc.

II. METHODOLOGY

The proteins corresponding to the genes for the PKU (phenylketonuria) were downloaded from Genbank database, their 3d structure was modeled using modeler [10] and the models were using Ramachandran Plot [11]. The 3d structure of the compounds above were downloaded from pubchem database. These compounds were docked with the PKU (phenylketonuria) receptors using PATCHDOCK server [12]. ADME studies was done with the best docked compounds [13, 14].

III. RESULTS & DISCUSSION

Homology modeling:

PKU (phenylketonuria) gene receptors were retrieved from GENBANK database (Table 1).

Table 1: PKU genes with genbank accession number.

Sl.no.	Gene name	Code	Accession number
1.	Phenylalanine hydroxylase	PAH	P00439.1
2.	Insulin-like growth factor 1	IGF1	P05019.1

Using BLAST the homologous templates pertaining to the gene receptors were selected and downloaded from RCSB-PDB database (Table 2).

Table 2 (a): Homologous templates of PAH GENE.

Accession	Query cover	Identity
5DEN A	100%	92%
5FGJ A	100%	92%
5EGQ A	100%	92%

Table 2 (b): Homologous templates of IGF1

Accession	Query cover	Identity
1IMX A	35%	100%
3LRI A	40%	87%
1B9G A	35%	81%

By using the homologous templates, using the software modeler [10], 5 models of each receptor were generated. The above models were verified by Ramachandran Plot server [11] and the best models were selected (Table 3, Fig. 1).

Table 3(a): Homology modeling:

IGF1:

	Number of residues in favoured region (~98.0% expected)	Number of residues in allowed region (~2.0% expected)	Number of residues in outlier region	
Model 1	160 (82.9%)	22 (11.4%)	11 (5.7%)	
Model 2	155 (80.3%)	28 (14.5%)	10 (5.2%)	
Model 3	154 (79.8%)	23 (11.9%)	16 (8.3%)	
Model 4	158 (81.9%)	22 (11.4%)	13 (6.7%)	
Model 5	165 (85.5%)	19 (9.8%)	9 (4.7%)	Selected

PAH:

	Number of residues in favoured region (~98.0% expected	Number of residues in allowed region (~2.0% expected)	Number of residues in outlier region	
Model 1	439 (97.6%)	9 (2.0%)	2 (0.4%)	
Model 2	439 (97.6%)	9 (2.0%)	2 (0.4%)	
Model 3	439 (97.6%)	10 (2.2%)	1 (0.2%)	Selected
Model 4	435 (96.7%)	14 (3.1%)	1 (0.2%)	
Model 5	438 (97.3%)	11 (2.4%)	1 (0.2%)	

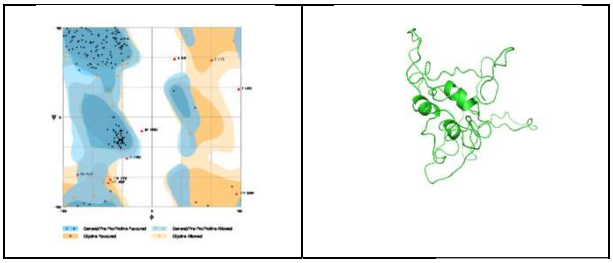


Fig.1(a). Ramachandran plot analysis of the best model (5) of IGF1 receptor

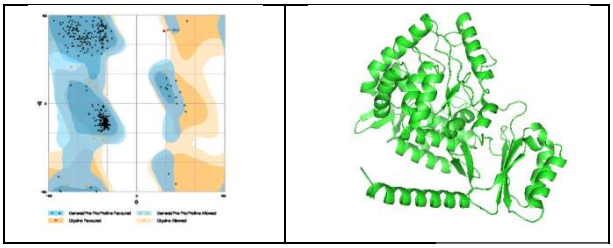


Fig.1(b). Ramachandran plot analysis of the best model (3) of PAH receptor

Docking:

The selected models in Fig. 1 were docked with the phytocompounds from the plants using PATCHDOCK server [12].

The docking scores were noted in table 4.

Table 4(a): Docking results of IGF1 receptor with compounds from
WOOD BETONY

Sl.no.	Receptor	Ligand	Docking score	No. Of interactions	Interacting amino acids
1	IGF1	Betulinic acid	5392	3	LEU-37 CYS-38 LEU-39
2	IGF1	Delphinic acid	2522	1	LYS-75
3	IGF1	Oleonilic acid	5360	4	TYR-135 GLN-136 GLN-161 GLN-133
4	IGF1	Rosamarinic acid	4624	2	LEU-37 ALA-46
5	IGF1	Rutin	5660	6	GLN-161 GLU-166 SER-168 ALA-167
					GLN-133 GLN-136
6	IGF1	Urosolic acid	5278	4	TYR-79 GLY-78 GLY-133 GLN-161
7	IGF1	Stachydrine	2528	2	ARG-179 GLN-178

Docking results of IGF1 receptor with compounds from
NETTLE

Sl.no.	Receptor	Ligand	Docking score	No. Of interactions	Interacting amino acids
1	IGF1	Histamine	2678	0	------
2	IGF1	Formic acid	1232	0	------
3	IGF1	Acetylcholine	3168	1	LYS-176
4	IGF1	Serotonin	3038	2	LEU-40 LYS-176

Docking results of IGF1 receptor with compounds from
PLANTAGO OVATO

Sl.no.	Receptor	Ligand	Docking score	No. Of interactions	Interacting amino acids
1	IGF1	Arabinose	2340	2	TYR-108 ARG-84
2	IGF1	Xylose	2698	2	TYR-108 ARG-84
3	IGF1	Valine	2462	1	ARG-84
4	IGF1	Alanine	2120	0	------
5	IGF1	Glutamic acid	2696	2	ARG-84 SER-83
6	IGF1	Glycine	1710	1	TYR-108
7	IGF1	Cysteine	2250	2	LYS-75 ARG-84
8	IGF1	Lysine	2952	3	ARG-179 ALA-46 ALA-48
9	IGF1	Leucine	2426	1	GLN-178
10	IGF1	Tyrosine	2992	2	ARG-179 CYS-38
11	IGF1	Rhamnose	2538	1	GLN-178

Docking results of IGF1 receptor with compounds from
TURMERIC

Sl.no.	Receptor	Ligand	Docking score	No. Of interact	Interacting amino acids

				ions	
1	IGF1	Curcumin	5342	6	ALA-167 SER-168 GLN-170 LYS-134 LEU-169 ILE-171
2	IGF1	Camphene	2512	1	CYS-38

Docking results of IGF1 receptor with compounds from
DANDELION

Sl.no.	Receptor	Ligand	Docking score	No.of interactions	Interacting amino acids
1	IGF1	Taraxacin	3840	1	GLN-178
2	IGF1	Laevulinic acid	2422	2	ARG-84 TYR-108
3	IGF1	Choline	2216	0	------
4	IGF1	Lecithin	7396	7	PHE-19 CYS-17 CYS-15 LYS-13 LEU-20 VAL-22 LYS-23

Table 4(b): Docking results of PAH receptor with compounds from
WOOD BETONY

Sl.no.	Receptor	Ligand	Docking score	No. Of interactions	Interacting amino acids
1	PAH	Betulinic acid	5758	3	GLY-346 THR-278 SER-349
2	PAH	Delphinic acid	2692	1	ALA-246
3	PAH	Oleonilic acid	5854	2	ARG-270 THR-278
4	PAH	Rosamarinic acid	4916	7	ASP-145 ASP-143 LEU-142 ARG-155 GLU-280 LEU-136 LYS-159
5	PAH	Rutin	5728	8	ARG-270 VAL-379 GLU-381 TYR-277 THR-278 MET-276
					GLU-353 THR-380
6	PAH	Urosolic acid	5830	1	SER-23
7	PAH	Stachydrine	3012	1	TYR-138

Docking results of PAH receptor with compounds from
NETTLE

Sl.no.	Receptor	Ligand	Docking score	No. Of interactions	Interacting amino acids
1	PAH	Histamine	2604	2	ALA-246 GLU-141
2	PAH	Formic acid		1	ASN-28
3	PAH	Acetylcholine	3250	1	TYR-325
4	PAH	Serotonin	3552	2	LEU-142 GLU-280

Docking results of PAH receptor with compounds from
PLANTAGO OVATO

Sl.no.	Receptor	Ligand	Docking score	No. Of interactions	Interacting amino acids
1	PAH	Arabinose	2484	1	LYS-159
2	PAH	Xylose	2734	3	LYS-159 TYR-138 LEU-142
3	PAH	Valine	2764	2	GLU-141 TYR-138
4	PAH	Alanine	2096	2	LYS-159 GLU-141
5	PAH	Glutamic acid	2744	2	LYS-159 ALA-246
6	PAH	Glycine	1694	2	GLY-247 GLU-286
7	PAH	Cysteine	2254	1	ILE-38
8	PAH	Lysine	3130	2	LEU-136 LYS-159
9	PAH	Leucine	2958	1	GLU-141
10	PAH	Tyrosine	3370	3	GLU-280 GLY-247 LYS-159
11	PAH	Rhamnose	2900	2	LEU-136 ARG-158

Docking results of PAH receptor with compounds from

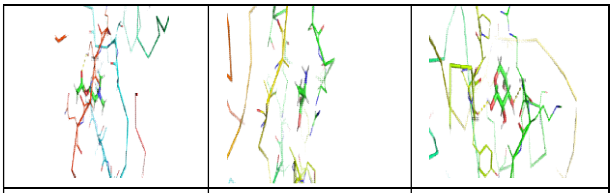
TURMERIC

Sl.no.	Receptor	Ligand	Docking score	No. Of interactions	Interacting amino acids
1	PAH	Curcumin	5370	5	ALA-246 ARG-158 LYS-159 GLU-280 GLU-141
2	PAH	Camphene	3198	0	------

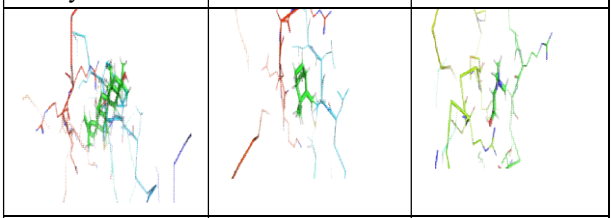
Docking results of PAH receptor with compounds from
DANDELION

Sl.no.	Receptor	Ligand	Docking score	No.of interactions	Interacting amino acids
1	PAH	Taraxacin	4232	2	HIS-264 GLU-286
2	PAH	Laevulinic acid	2622	1	ARG-158
3	PAH	Choline	2640	1	LYS-159
4	PAH	Lecithin	6906	6	SER-349 VAL-379 THR-278 GLU-21 PHE-18 ARG-270

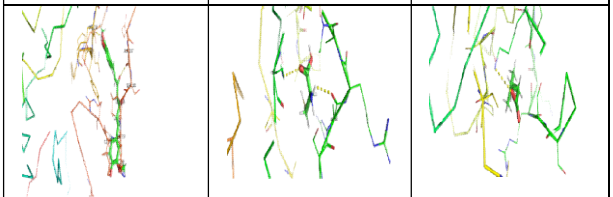
Fig.2 (a):IGF1 docking images



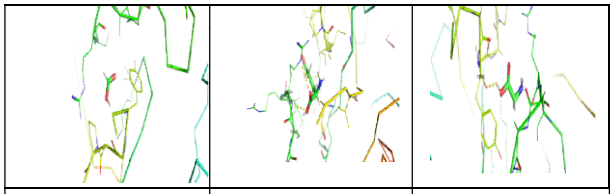
Acetylcholine | Alanine | Arabinose



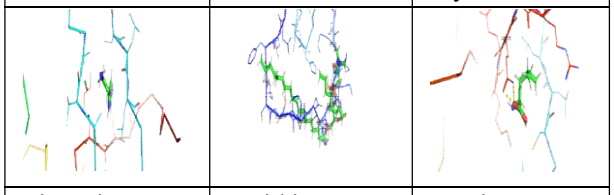
Betulinic acid | Camphene | Choline



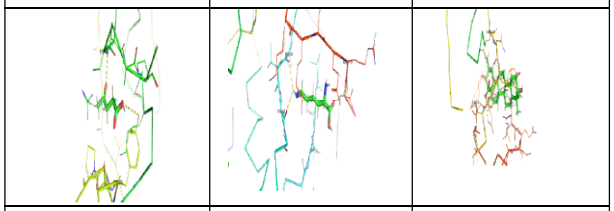
Curcumin | Cysteine | Delphinic acid



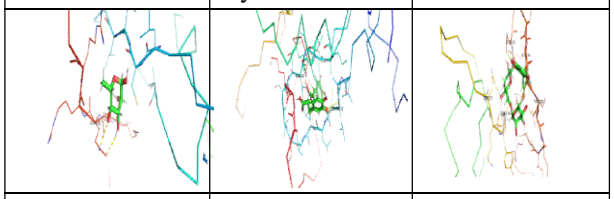
Formic acid | Glutamic acid | Glycine



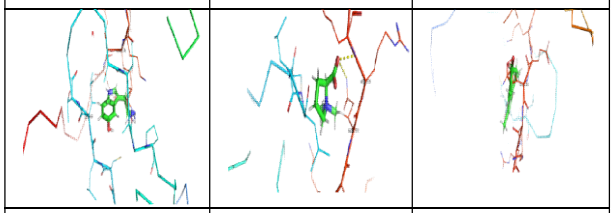
Histamine | Lecithin | Leucine



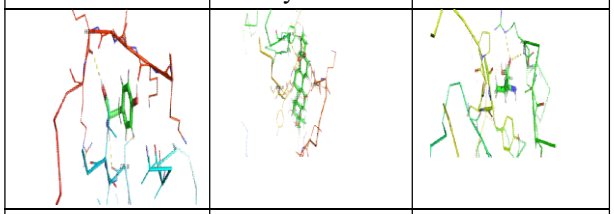
Levulinic acid | Lysine | Oleonilic acid



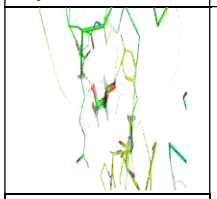
Rhamnose | Rosamarinic acid | Rutin



Serotonin | Stachydrine | Taraxacin

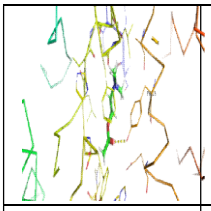
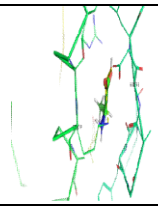
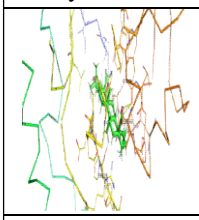
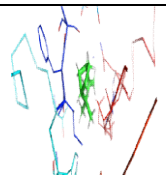
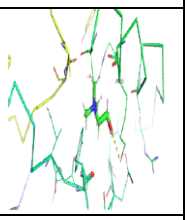
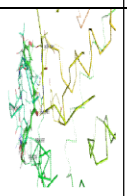
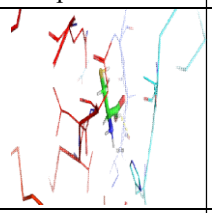
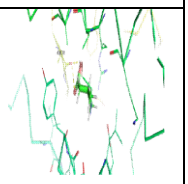
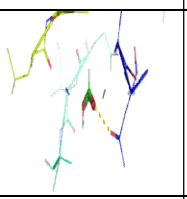
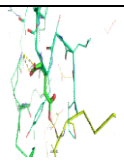
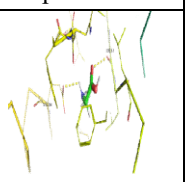
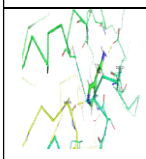
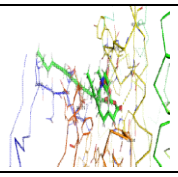
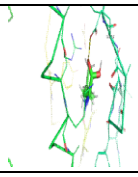
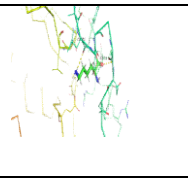
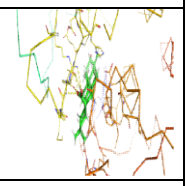
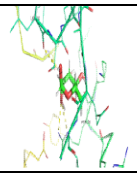
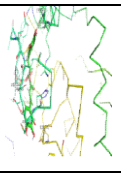
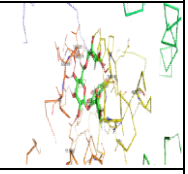


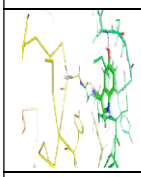
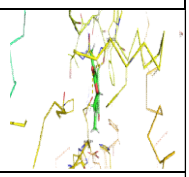
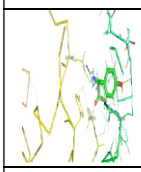
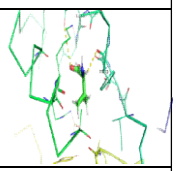
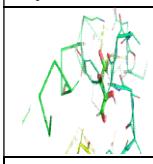
Tyrosine | Urosolic acid | Valine



Xylose

Fig.2 (b): PAH docking images:

		
Acetylcholine	Alanine	Arabinose
		
Betulinic acid	Camphene	Choline
		
Curcumin	Cysteine	Delphinic acid
		
Formic acid	Glutamic acid	Glycine
		
Histamine	Lecithin	Leucine
		
Levulinic acid	Lysine	Oleonilicaicd
		
Rhamnose	Rosamarinic	Rutin

	acid	
		
Serotonin	Stachydrine	Taraxacin
		
Tyrosine	Urosolic acid	Valine
		
xylose		

ADME: The phytocompounds used in this work are subjected to ADME screening using molinspiration server [13, 14]. The results are noted in Table 4.

Table 4: ADME screening

Ligands	miLogP	TPSA	Natoms	MW	volume	nviolations
Acetylcholine	-3.56	26.30	10	146.21	156.67	0
Alanine	-2.69	63.32	6	89.09	84.31	0
Arabinose (Oxane-2,3,4,5-tetrol)	-2.22	90.15	10	150.13	126.96	0
Betulinic acid (Lup-20(29)-en-28-oic acid, 3beta-hydroxy-)	7.04	57.53	33	456.71	472.04	1
Camphene	3.33	0.00	10	136.24	152.37	0
Choline	-4.24	20.23	7	104.17	120.16	0
Curcumin	2.30	93.07	27	368.38	332.18	0
Cysteine	-2.71	63.32	7	121.16	102.22	0

Delphinic acid(Isovaleric acid)	1.21	37.30	7	102.13	106.39	0
Formic acid	-0.51	37.30	3	46.02	39.64	0
Glutamic acid	-3.25	100.62	10	147.13	128.36	0
Glycine	-2.55	63.32	5	75.07	67.73	0
Histamine	-0.85	54.71	8	111.15	109.77	0
Lecithin	2.69	101.97	44	643.89	668.30	1
Leucine	-1.38	63.32	9	131.18	134.50	0
Levulinic acid	-0.35	54.37	8	116.12	108.78	0
Lysine	-3.18	89.34	10	146.19	146.25	0
Oleanoic acid	6.72	57.53	33	456.71	471.14	1
Rhamnose (6-Methyloxane-2,3,4,5-tetro)l	-1.64	90.15	11	164.16	143.55	0
Rosmarinic acid (Rosmarinsaure)	1.63	144.52	26	360.32	303.54	0
Rutin (Vitamin P)	-1.06	269.43	43	610.52	496.07	3
Serotonin	0.57	62.04	13	176.22	165.93	0
Stachydrine	-5.31	40.13	10	143.19	142.62	0
Taraxacin	2.56	43.38	18	242.27	220.04	0
Tyrosine	-1.71	83.55	13	181.19	163.98	0
Urosolic acid(Carissic acid)	6.79	57.53	33	456.71	471.49	1
Valine	-1.91	63.32	8	117.15	117.70	0
Xylose (Ribose, D-)	-2.22	97.98	10	150.13	130.97	0

IV. CONCLUSION

As per the virtual screening studies we find the phytocompounds rutin, tyrosine, curcumin and lecithin docks with all the receptor genes of phenylketonuria disease. As per ADME studies compounds rutin and lecithin cannot be considered as drug lead as they have they have 3 and 1 violations in ADME studies respectively. Compounds tyrosine and curcumin successfully clears ADME studies. Hence the compounds tyrosine and curcumin can be successfully considered as novel drug leads for phenylketonuria disease.

References

[1] Bilder DA, Burton BK, Coon H, Leviton L, Ashworth J, Lundy BD, Vespa H, Bakian AV, Longo N, 2013, "Psychiatric symptoms in adults with phenylketonuria" Mol Genet Metab;108:155–60.

[2] Brumm VL, Bilder D, Waisbren SE, 2010, "Psychiatric symptoms and disorders in phenylketonuria" Mol Genet Metab; 99Suppl 1:S59–63.

[3] Brumm VL, Grant ML., 2010, "The role of intelligence in phenylketonuria: a review of research and management" Mol Genet Metab;99Suppl 1:S18–21.

[4] Channon S, Goodman G, Zlotowitz S, Mockler C, Lee PJ. 2007, "Effects of dietary management of phenylketonuria on long-term cognitive outcome". Arch Dis Child; 92:213–8.

[5] Debra S Regier., and Carol L Greene, 2000, "Phenylalanine Hydroxylase Deficiency".

[6] Fonnesbeck CJ, McPheeters ML, Krishnaswami S, Lindegren ML, Reimschisel T.,2013, "Estimating the probability of IQ impairment from blood phenylalanine for phenylketonuria patients: a hierarchical meta-analysis" J Inherit Metab Dis;36:757–66.

[7] Kure S, Hou DC, Ohura T, Iwamoto H, Suzuki S, Sugiyama N, Sakamoto O, Fujii K, Matsubara Y, Narisawa KJ Pediatr,1999, "Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency", 135(3):375-8.

[8] Patrícia Fernanda Schuck., Fernanda Malgarin., José Henrique Cararo., Fabiola Cardoso., Emilio LuizStreck., and Gustavo Costa Ferreira.,2015, " Phenylketonuria Pathophysiology: on the Role of Metabolic Alterations" Aging Dis., 6(5): 390–399.

[9] Waisbren SE, Noel K, Fahrbach K, Cella C, Frame D, Dorenbaum A, Levy H.,2007."Phenylalanine blood levels and clinical outcomes in phenylketonuria: a systematic literature review and meta-analysis". Mol Genet Metab; 92:63–70.

[10] Sali A and Blundell TL, 1993, "Comparative protein modelling by satisfaction of spatial restraints" J. Mol. Biol., 234:779-815.

[11] Laskoswki RA, MacArthur MW, Moss DS & Thorton JM, 1993, "Procheck: a program to check the stereochemical quality of protein structures", J. Appl. Cryst. 26:283-291.

[12] Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ, 2005, "PatchDock and SymmDock: servers for rigid and symmetric docking", Nucl. Acids. Res. 33: W363-367.

[13] Ertl P, Rohde B, Selzer P, 2000, "Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties", J.Med.Chem. 43, 3714-3717.

[14] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ, 1997, "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings", Adv. Drug. Delivery Rev. 23, 4-25.