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Establishing Novel Ligands for Rett Syndrome Receptors from Medicinal Plants

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Abstract: **The Rett Syndrome is a peculiar, sporadic, atrophic disorder, almost entirely confined to females. Severe dementia, loss of hand skills, apraxia, ataxia, autistic features and irregular breathing with hyperventilation are the major symptoms. There is no cure but medication is provided as speech therapy, occupational therapy, physical therapy, feeding assistance for Rett Syndrome. RS is caused by mutations in the gene MeCP2 and in less than 10% of RS cases, mutations in the genes CDKL5 or FOXG1 have also been found to resemble it. This study was carried out to establish the novel ligands for Rett Syndrome receptors from medicinal plants. Steps involved in the study are choosing the ligands, Homology modeling of the gene receptors, docking of the receptors with ligands and ADME. As per the virtual screening results and ADME studies we find the compound Asiatic acid can be successfully considered as novel drug lead for Rett syndrome.**

Keywords: **Rett syndrome, X-linked, MeCP2 gene, CDKL5 gene, FOXG1 gene, modeling, docking, ADME**

I. INTRODUCTION

The Rett syndrome (RS) is a peculiar, sporadic, atrophic disorder, generally been considered an X-linked disorder in which affected females represent a new mutation, with male lethality with linkage studies suggesting a critical region at Xq28 [1, 2].

Genes involved:

MeCP2 gene (methyl CpG binding protein 2):

MeCP2 is a transcriptional repressor, since it codes for a protein that controls the expression of other genes. Its mutation seems to be more common in X chromosome of sperm cells, explaining why RETT is rare in boys. The gene is found near the end of long arm of X chromosome at Xq28. Mutations in this gene alter the MeCP2 protein or result in the production of less protein which appears to disrupt the normal function of neurons and other brain cells and impair their ability to communicate with one another [3, 4].

CDKL5 gene (cyclin-dependent kinase-like 5):

CDKL5 gene, also known as serine/threonine kinase 9, is essential for normal brain development. It is involved in formation, growth and movement of nerve cells as well as cell division and plays a role in the transmission of chemical signals at the connections between neurons [4].

FOXG1 gene:

FOXG1 gene provides instructions for making a protein known as Fork Head Box G1. The protein of this gene plays an important role in brain development, particularly in a region of embryonic brain known as telencephalon and the telencephalon ultimately develops into severe critical structures, including the largest part of brain(cerebrum), which controls the most voluntary activity, language sensory perception, learning and memory [4].

Table 1: Herbs with their phytocompound for treating Rett syndrome used in this work

Sl. No.	Plant Name	Corresponding phytocompound
1	MedhyaRasayana (Nootropic herbs)	Medicoside, Asiaticoside, Asiatic acid, Microphyllic acid
2	Ashwagandha (*Withaniasomnifera*)	Isopelletierine, Anaferine, Sitoindoside
3	Chitrak (*Plumbagozeylancia)*	Asparticacid
4	Patol (*Tricosanthediocia*)	Sitoindoside
5	Nimba(*Azadirachtaindica*)	Nimbidin, Quercetin, Nimbolinin, Nimbin,

		Nimbidiol, Sitosterol
6	Musta (*Cyperusrotundus*)	Anaferine
7	Yashtimadhuk (*Glycyrrhizaglabra*)	Glycyrrhizine, Glycyrrhetenic acid
8	Onion (*Allium cepa*)	Quercetin
9	Saffron (*Crocus sativus*)	Safranal
10	Shankhpushpi(*Convolvulus pluricaulis*)	Kaempferol-3-glucoside

II. METHODOLOGY

The proteins corresponding to the genes for the Rett syndrome were downloaded from Genbank database, their 3d structure was modeled using modeler and the models were using Ramachandran Plot. The 3d structure of the compounds in Table 1 were downloaded from pubchem database. These compounds were docked with the Rett syndrome receptors. ADME studies was done with the best docked compounds.

III. RESULTS

Target selection

The gene receptors for Rett syndrome are downloaded from Genbank (Table 2).

Table 2: Rett syndrome receptors with their accession number

Receptor name	**Accession number**
MeCP2	UniProtKB/Swiss-Prot:P51608.1
CDKL5	UniProtKB/Swiss-Prot:076039.1
FOXG1	UniProtKB/Swiss-Prot:P55316.2

The homologous template information for the genes were taken from BLAST and downloaded from RCSB PDB (Table 3)

Table 3(a) template information for MeCP2

Query cover	Ident	Accession
25%	97%	1UB1 A
19%	96%	5BT2 A
19%	95%	3C2I A

Table 3(b) template information for CDKL5

Query cover	Ident	Accession
29%	99%	4BGQ A
28%	48%	4AAA A
28%	46%	4AGU A

Table 3(c) template information for FOXG1

Query cover	Ident	Accession
18%	71%	1D5V A
19%	66%	2HDC A
18%	69%	2HFH A

Homology modeling

Based on the template information, the 3d models of the gene receptors were modeled using MODELLER software [5]. Modeller generates 5 templates for each gene. The models were verified using RAMPAGE Ramachandran plot server [6] (Table 4, Fig. 1).

Table 4(a) Homology modeling results for MeCP2 receptor

	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	453 (93.6%)	21 (4.3%)	10 (2.1%)	selected
Model 2	446 (92.1%)	30 (6.2%)	8 (1.7%)	
Model 3	439 (90.7%)	33 (6.8%)	12 (2.5%)	
Model 4	444 (91.7%)	33 (6.8%)	7 (1.4%)	
Model 5	448 (92.6%)	31 (6.4%)	5 (1.0%)	

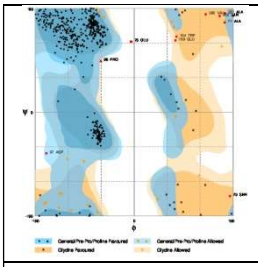
Table 4(b) Homology modeling results for CDKL5 receptor

	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	878 (85.4%)	91 (8.9%)	59 (5.7%)	
Model 2	895 (87.1%)	89 (8.7%)	44 (4.3%)	selected
Model 3	876 (85.2%)	90 (8.8%)	62 (6.0%)	
Model 4	879 (85.5%)	74 (7.2%)	75 (7.3%)	
Model 5	864 (84.0%)	106 (10.3%)	58 (5.6%)	

Table 4(c) Homology modeling results for FOXG1 receptor:

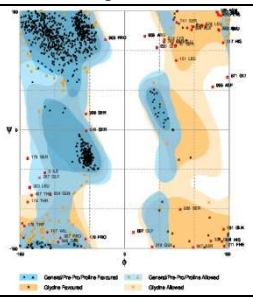
	Number of residues in	Number of residues in	Number of	

	favoured region (~98.0% expected)	allowed region (~2.0% expected)	residues in outlier region	
Model 1	449 (92.2%)	32 (6.6%)	6 (1.2%)	
Model 2	460 (94.5%)	23 (4.7%)	4 (0.8%)	selected
Model 3	459 (94.3%)	21 (4.3%)	7 (1.4%)	
Model 4	443 (91.0%)	38 (7.8%)	6 (1.2%)	
Model 5	456 (93.6%)	24 (4.9%)	7 (1.4%)	

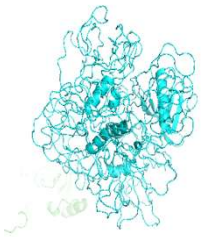


(a) Ramachandran Plot analysis of MeCP2 receptor

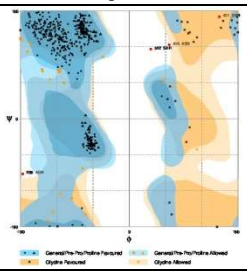
3d structure of MeCP2 receptor (visualization in pymol)



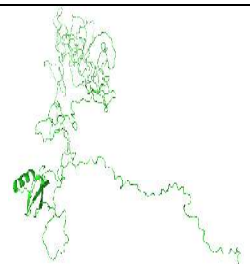
(b) Ramachandran Plot analysis of CDKL5 receptor



3d structure of CDKL5 receptor (visualization in pymol)



(c) Ramachandran Plot analysis of FOXG1 receptor



3d structure of FOXG1receptor (visualization in pymol)

Fig. 1: Ramachandran Plot analysis of Rett syndrome receptors and their visualization in pymol

The selected models were docked with the phytocompounds from Table 1 using PATCHDOCK server [7] (Table 5, Fig. 2).

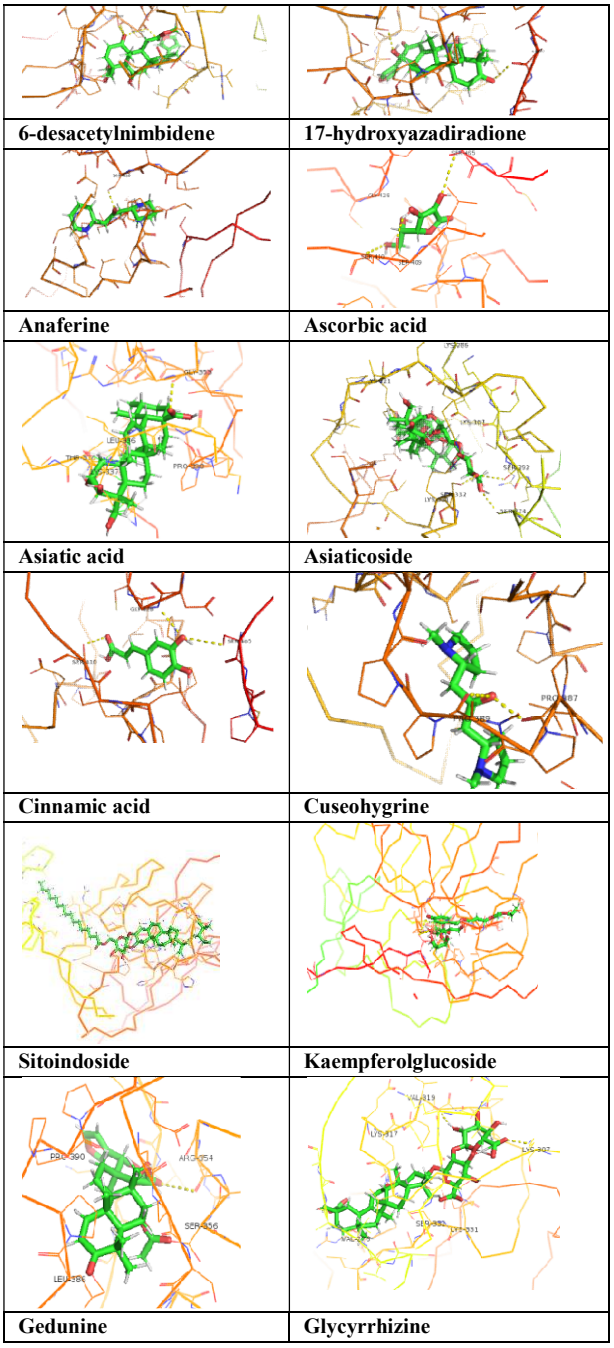
Table 5(a): Docking score of MeCP2 with phytocompounds

SR. NO	Protein	LIGAND	Docking Score (kcal/mol)	Number of interactions	Interacting amino acids
1	MECP2	6-DESACETYLNIMBIDENE	5906	4	LEU- 336 PRO 390 PRO 389 LEU 386
2		17-HYDROXYAZADIRADIONE	5644	3	SER-373 PRO-390 ALA-447
3		ASCORBIC ACID	2852	4	SER-465 SER-410 SER-409 GLY-428
4		ANAFERINE	4114	1	SER-410
5		ASIATICOSIDE	7658	7	LYS-307 SER-396 SER-292 LYS-321 SER-274 SER-332 LYS-286
6		GEDUNINE	5722	4	ARG-354 LEU-386 SER-356 PRO-390
7		MEDICOSIDE	6292	6	SER-373 GLU-374 SER-356 THR-338 PRO-390 LYS-337
8		NIMBIDIOL	4326	1	GLU-392
9		NIMBENE	5952	5	SER-356 THR-338 PRO-389 SER-473 GLY-335
10		SCOPOLAMINE	4464	4	SER-373 PRO-391 GLU-392 GLY-352

11		SITOINDOSIDE	9004		
12		QUERCETIN	5388	6	PRO-390 CYS-339 THR-338 GLY-335 LEU-336 LYS-337
13		NIMBIN	6172	4	LEU-386 THR-338 ARG-354 GLY-353
14		SITOSTEROL	5932	2	GLY-428 SER-410
15		SALANIN	6764	6	SER-373 LEU-386 THR-338 ARG-354 SER-355 SER-356
16		KAEMPFEROL GLUCOSIDE	7924	0	
17		ASIATIC ACID	6202	5	LYS-337 THR-338 GLY-353 PRO-390 LEU-336
18		ISOPELLETIERINE	3050	1	SER-409
19		AZADIRACHTIN	7936		
20		NIMBOLININ	6566	4	GLU-318 LYS-321 SER-395 SER-332
21		GLYCYRRHIZINE	7452	6	SER-332 VAL-276 VAL-319 LYS-307 LYS-331 LYS-317
22		GLYCYRRHETINIC ACID	5954	4	PRO-390 GLY-335 LYS-337 THR-338
23		NIMBIOL	4326	2	GLU-392 SER-473
24		CUSEOHYGRINE	3988	2	PRO-389 PRO-387
25		CINNAMIC ACID	3348	3	SER-410 SER-465 GLY-428
26		MICROPHY	3580	0	

		LIC ACID			

Fig. 2(a) Docking result of MeCP2 with phytocompounds



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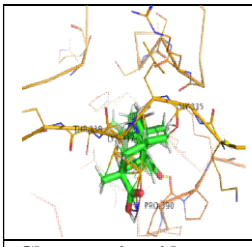	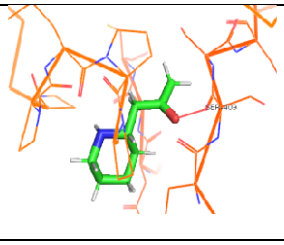
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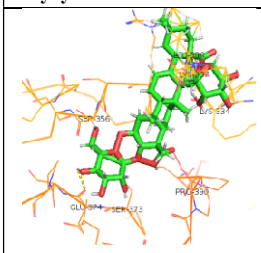	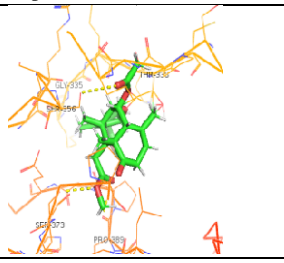
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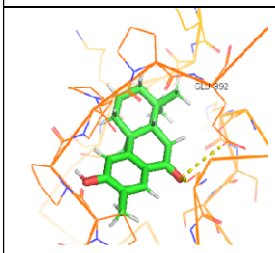	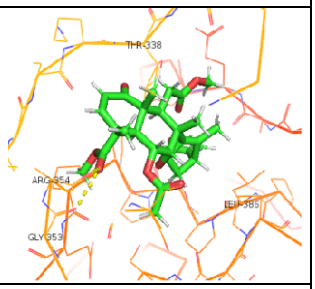
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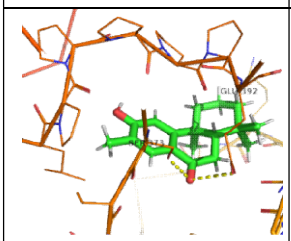	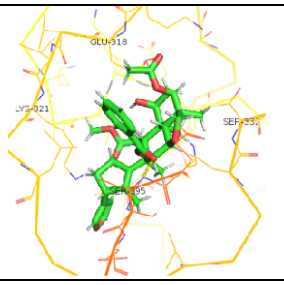
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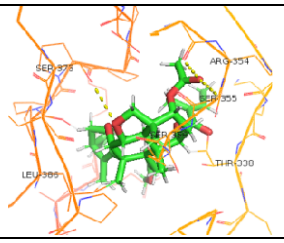
	
Quercetin	**Salanin**

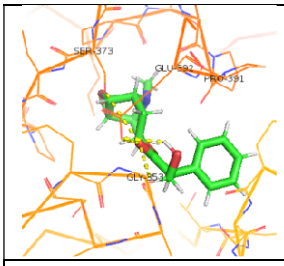
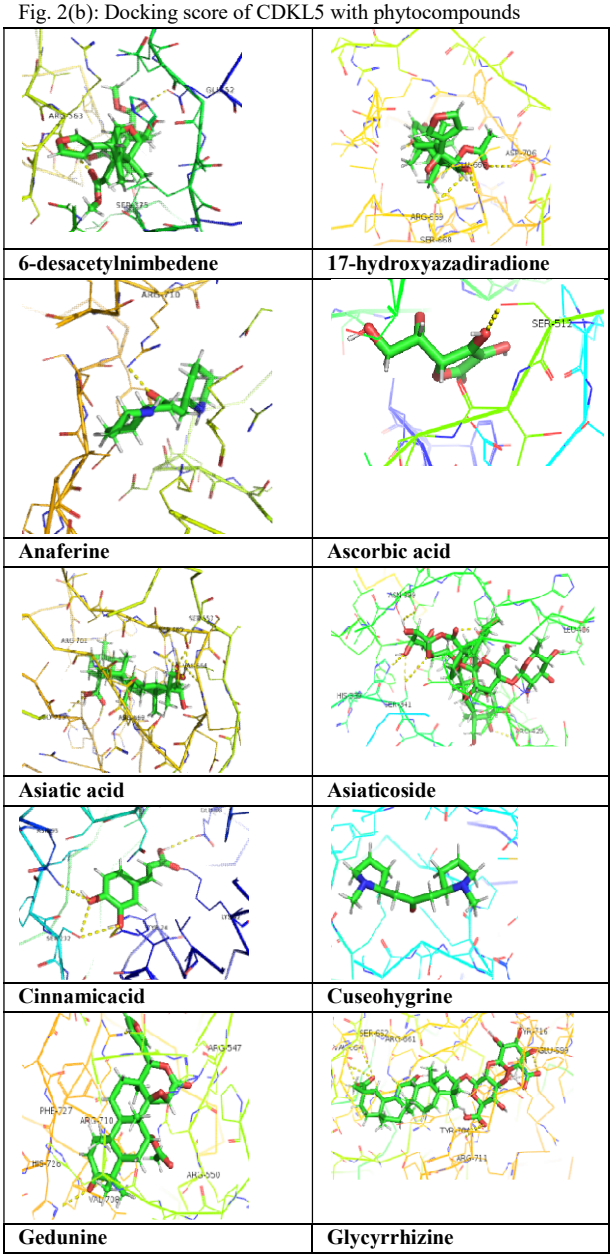
	
Scopolamine	**Sitosterol**

Table 5(b) Docking score of CDKL5 with phytocompounds

SR. NO	Protein	LIGAND	Docking score (kcal/mol)	NO. OF INTERACTIONS	INTERCATING AMINO ACIDS
1	CDKL5	ASCORBIC ACID	2978	1	SER-512
2		17-HYDROXY AZADIRADIONE	6358	4	ASP-706 GLU-666 SER-668 ARG-669
3		CUSEOHYGRINE	4594	0	
4		ISOPELLETIERINE	3406	1	TYR-24
5		GLYCYRRHETINIC ACID	6220	7	ASN-368 SER-436 LEU-642 ARG-483 LYS-487 HIS-338 ARG-340
6		GEDUNINE	6162	7	ARG-547 VAL-708 ARG-710 ARG-547 HIS-728 PHE-727 ARG-550
7		NIMBIDIOL	4620	0	
8		NIMBENENE	6270	5	SER-536 ASN-549 HIS-728 THR-533 PRO-532
9		GLYCYRRHIZINE	8928	7	ARG-661 TYR-716 GLU-699 TYR-704

					ARG-711 SER-662 VAL-664
10		SCOPOLAMINE	4734	4	ARG-661 ALA-660 GLU-670 VAL-659
11		ASIATICOSIDE	8050	5	LEU-406 PRO-425 SER-341 HIS-339 ASN-399
12		MEDICOSIDE	7036	8	ARG-661 GLU-699 TYR-704 ARG-669 TYR-716
					VAL-659 PRO-697 SER-714
13		KAEMPFERGLUCOSIDE	8402	0	
14		SALANIN	6898	4	SER-426 LYS-344 LYS-424 ASN-420
15		SITOSTEROL	6524	2	GLU-569 PHE-727
16		MICROPHYLIC ACID	10950	0	
17		NIMBIOL	4620	1	TYR-239
18		6-DESACETYLNIMBIDENE	6304	3	GLU-359 SER-375 ARG-563
19		QUERCETIN	5580	5	ARG-710 ASN-549 LYS-572 ARG-669 GLU-569
20		NIMBIN	7040	4	SER-438 SER-375 ASN-370 ARG-563
21		NIMBOLININ	7036	2	GLU-416 LYS-432
22		AZADIRACHTIN	8094	0	
23		SITOINDOSIDE	9522	0	
24		ANAFERINE	4530	1	ARG-710
25		CINNAMIC ACID	3388	5	GLU-60 ASN-95 SER-232
					TYR-24 LYS-42
26		ASIATIC ACID	6112	6	GLY-713 ARG-701 ARG-669 SER-557 SER-662 VAL-664

Fig. 2(b): Docking score of CDKL5 with phytocompounds



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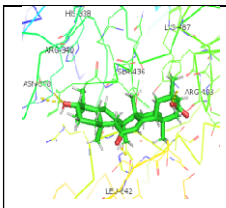	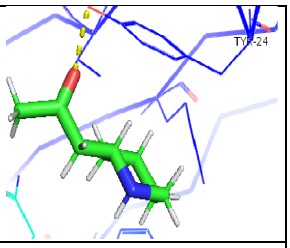
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	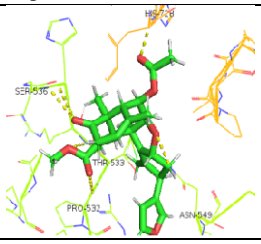
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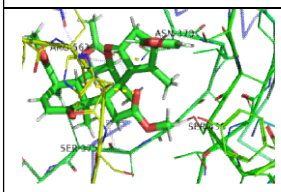	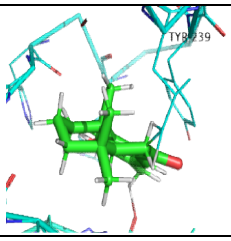
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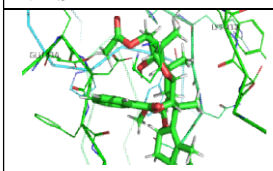	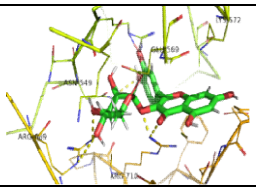
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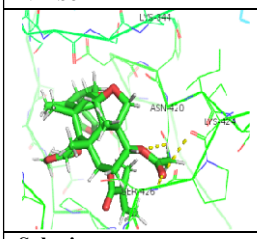	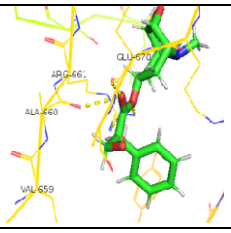
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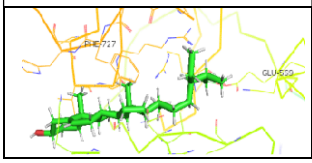	**Sitosterol**

Table 5(c) Docking score of FOXG1 with phytocompounds

SR. NO	Protein	LIGAND	Docking score (kcal/mol)	NO. of interactions	INTERACTING AMINO ACIDS
1	FOXG1	CINNAMIC ACID	2950	1	ASN-353
2		17-HYDROXYAZADIRADIONE	6064	3	THR-414 HIS-423 ASP-248
3		ANAFERINE	4070	2	GLN-413 SER-406
4		6-DESACETYLNIMBIDENE	6178	4	THR-414 ARG-201 THR-427 ASP-248
5		GLYCYRRHETINIC ACID	6194	4	THR-414 SER-406 HIS-420 VAL-421
6		NIMBIDIOL	4308	2	SER-406 LYS-200
7		NIMBIN	6512	4	ARG-201 TYR-254 SER-406 THR-427
8		NIMBIOL	4308	2	SER-406 LYS-204
9		NIMBENENE	6132	5	HIS-245 ASP-247 TYR-254 THR-427 ARG-201
10		MEDICOSIDE	6794	5	PHE-418 ASP-248 SER-406 HIS-420 THR-427
11		GEDUNINE	6102	2	ASP-248 HIS-420
12		QUERCETIN	5262	4	SER-406 GLN-413 ARG-201 HIS-245
13		GLYCYRRHIZINE	8276	5	ASP-247 SER-406 THR-414 PRO-422 HIS-420
14		AZADIRACHTIN	7908	0	
15		SALANIN	6778	4	THR-414 ARG-201 HIS-420 SER-406
16		MICROPHYLLIC	9996	0	

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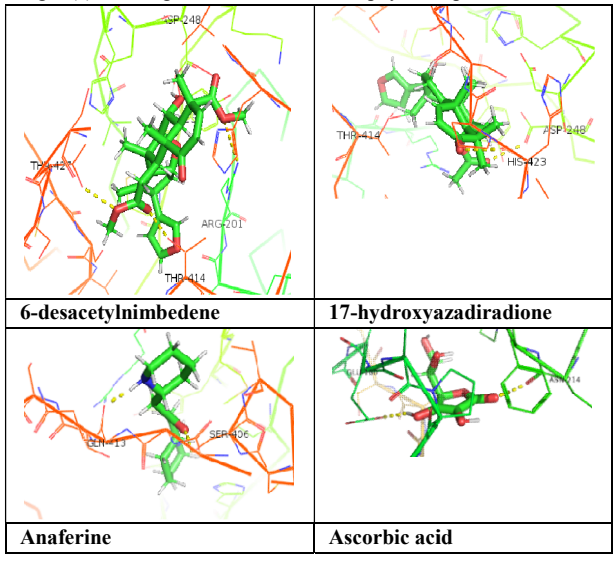
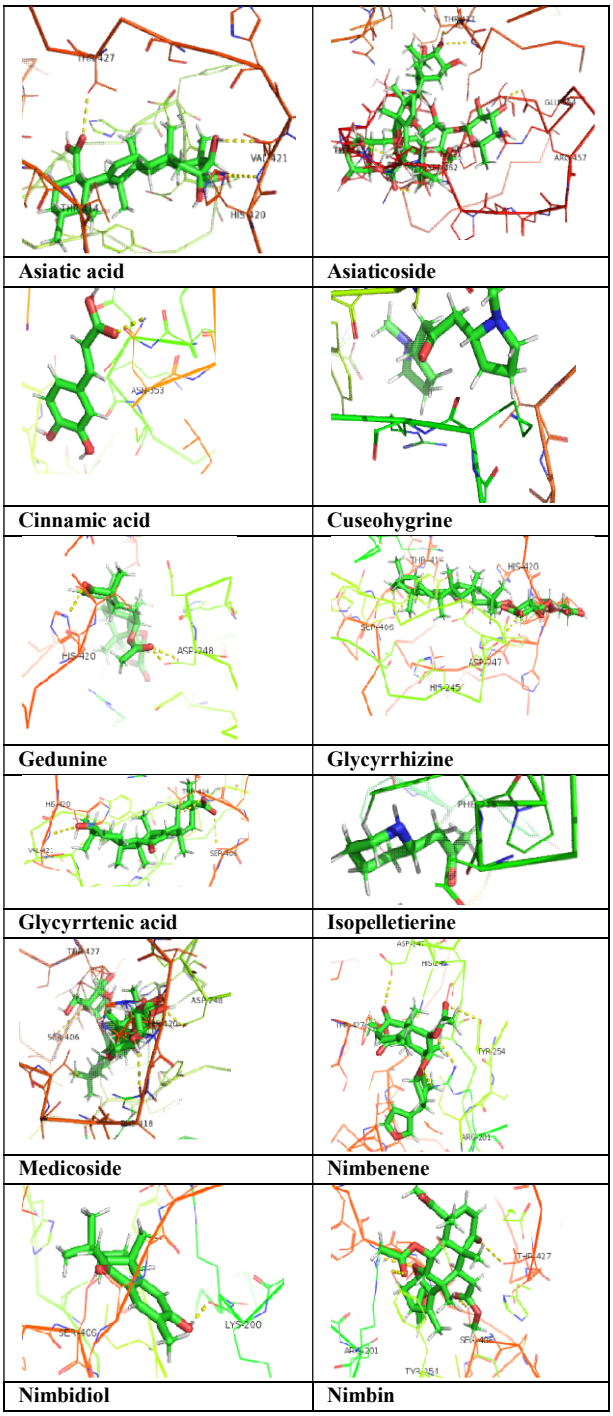
		ACID			
17		SITOSTEROL	6158	4	SER-406 LYS-272 GLY-412 GLN-413
18		ASCORBIC ACID	2700	2	GLU-180 ASN-214
19		NIMBOLININ	6964	5	THR-414 LYS-206 THR-427 ARG-201 VAL-421
20		SITOINDOSIDE	3885	0	
21		CUSEOHYGRINE	4060	0	
22		ISOPELLETIERINE	2896	1	PHE-215
23		KAEMPFEROL	7776	0	
24		SCOPOLAMINE	4582	3	LYS-200 ARG-201 THR-414
25		ASIATIC ACID	5992	4	THR-414 HIS-420 THR-427 VAL-421
26		ASIATICOSIDE	7882	6	GLU-454 ARG-457 THR-427 THR-465 SER-462 PHE-463

Fig. 2(c) Docking score of FOXG1 with phytocompounds



6-desacetylnimbedene | **17-hydroxyazadiradione**

Anaferine | **Ascorbic acid**



Asiatic acid | **Asiaticoside**

Cinnamic acid | **Cuseohygrine**

Gedunine | **Glycyrrhizine**

Glycyrrtenic acid | **Isopelletierine**

Medicoside | **Nimbenene**

Nimbidiol | **Nimbin**

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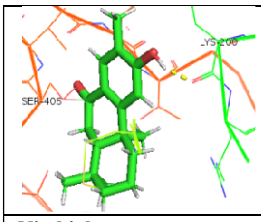
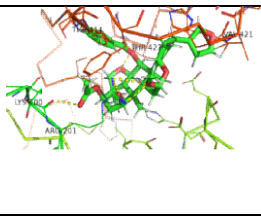
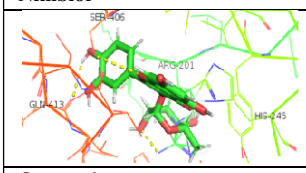
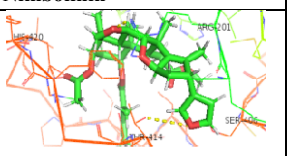
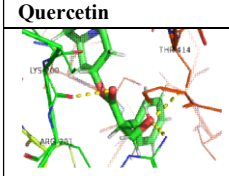
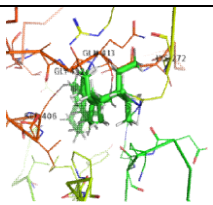
	
Nimbiol	**Nimbolinin**
	
Quercetin	**Salanin**
	
Scopolamine	**Sitosterol**

ADME: ADME studies for the phytocompounds listed in table 4and the results were noted in Table 6 [8, 9].

Table 6: ADME studies of phytocompounds

LIGAND	miLogP	TPSA	natoms	MW	nON	nOHNH	nrotb	Volume	nviolations
6-desacetylnimbidene	2.85	112.28	36	498.57	8	1	6	452.45	0
17-hydroxyazadiradione	3.65	93.81	34	466.57	6	1	3	433.80	0
Anaferine	1.38	41.12	16	224.35	3	2	4	236.41	0
Ascorbicacid	-1.40	107.22	12	176.12	6	4	2	139.71	0
Asiaticacid	4.70	97.98	35	488.71	5	4	2	487.79	0
Asiaticoside	0.37	315.21	67	959.13	19	12	10	875.90	3
Azadirachtin	1.60	215.37	51	722.74	16	3	11	617.90	2
Cinnamicacid	0.94	77.75	13	180.16	4	3	2	154.50	0
Cusecohygrine	0.86	23.55	16	224.35	3	0	4	236.69	0
Gedunine	4.34	95.35	35	482.57	7	0	3	439.15	0
Glycyrrhizine	1.97	267.04	58	822.94	16	8	7	741.93	3
Glycyrrtenicacid	5.62	74.60	34	470.69	4	2	1	473.32	1
Isopelletierine	0.81	29.10	10	141.21	2	1	2	150.58	0
Kaempferolglucoside	-2.36	328.35	53	756.66	20	12	9	620.17	3
Lecithin	2.69	101.97	44	643.89	8	0	32	668.30	1
Medicoside	-0.42	353.91	74	1061.22	22	13	12	958.19	3
Microphylicacid	0.28	372.38	70	1019.14	24	13	25	927.04	3
Nimbenene	3.89	92.06	35	482.57	7	0	6	444.73	0
Nimbidiol	4.92	37.30	20	272.39	2	1	0	272.65	0
Nimbin	3.55	118.36	39	540.61	9	0	8	488.96	1
Nimbiol	4.92	37.30	20	272.39	2	1	0	272.65	0
Nimbolinin	5.66	113.68	45	620.74	9	1	7	568.67	2
Quer	0.6	19	32	448.	11	7	3	363.	2

cetin	4	0.28		38				95	
Salanin	5.40	110.52	43	596.72	9	0	9	551.94	0
Scopolamine	1.05	62.30	22	303.36	5	1	5	277.20	0
Sitoindoside	10.06	105.46	58	815.27	7	3	25	860.38	2
Sitosterol	8.62	20.23	30	414.72	1	1	6	456.52	1

IV. CONCLUSION

As per the virtual screening results we find the phytocompounds Asiaticoside, Glycyrrhizine, Medicoside and Asiatic acid docks with all the 3 receptor genes of Rett syndrome. As per ADME studies compoundAsiaticoside, Glycyrrhizine and Medicoside cannot be considered as drug leads as they all have >0 violations in ADME studies respectively. Compound Asiatic acid successfully clears ADME studies. Hence the compound Asiatic acid is successfully considered as novel drug lead for Rett syndrome.

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