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A Computational *In silico* Approach for Identification of Indian Herbs for the Treatment of Alzheimer's Disease.

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ABSTRACT

Alzheimer's disease (AD) is a neuronal disorder associated with dementia and EphB2 gene play a crucial role in the NMDA signaling pathway helping to restore cognitive functions. Therefore, attempt has been made to study the pharmacogenomics of the gene to identify the individual variations by the functional effect of Single Nucleotide Polymorphisms (SNPs). Out of the 4997 SNPs, 9 SNPs were found to be damaging in nature with deleterious amino acid substitutions. Computer Aided Drug Designing (CADD) was instrumental to find out the effective constituent among the plant sources, *Withania somnifera, Centella asiatica, Catharanthus roseus, Curcuma longa* and *Bacopa monnieri with* Anti-Alzheimer Activity. *The corresponding 21 plant constituents analyzed were Withanolide E, Withanolide A, Withanoside IV, Withaferine A, Anaferine, Beta-Sitosterol, Quercetin, Asiatic acid, Asiaticoside, Campesterol, Madecassoside, Madecassic acid, Vincristine, Vincamine, Vinblastine, Catharanthine, Kaempferol, Curcumin, Ascorbic acid, Linalool and Bacoside A. M*olecular property, bioactivity parameter as well as Protein-Ligand Dynamic interaction were analyzed to determine the drug likeness of the selected constituents. Bench marking were done using an antioxidant *Butylated hydroxytoluene (BHT)*. The *in silico* analysis performed indicate the selection of natural compounds like Withanoside IV, Asiaticoside, Madecassoside, Vincristine, Vinblastine and Bacoside A to show significant binding interaction with 1B4F protein.

Keywords: Anti-Alzheimer Activity, Protein-ligand interactions, Computer aided drug designing, Single Nucleotide Polymorphism, docking

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INTRODUCTION

Alzheimer's disease (AD) is a brain disorder which begins gradually by affecting the brain cells which are responsible for controlling thoughts of a person. The person may find it difficult to recall the recent incidents which arise in their day to day life. The protein 'plaques' and 'tangles' developed in the brain might cause serious damage to the brain cells. Interestingly, Alzheimer's disease can deplete certain important chemicals in the brain [1]. AD is characterized by progressive dysfunction of memory and higher cognitive functions associated with memory loss and language deficit which are often accompanied by behavioral and psychological symptoms such as depression, stress, anxiety and mood disturbances [2]. Alzheimer's disease are complex because there is an increased probability of neuronal degeneration, abnormal neurofibrillary tangles, toxic β-amyloid (AB) plaques, decline of neurochemicals which are essential for neuronal transmission and neuro-inflammation, synaptic loss, particularly, the deficiency of acetylcholine (ACh) and the degeneration of cholinergic neurons in the cortex and hippocampus as well as nucleus basalis of Meynert [3,4,5,6,7]. The β-amyloid cytotoxicity to neuronal cells has been identified as one of the major features in Alzheimer's disease and these oligomers might damage the *neuronal NMDA-type glutamate receptors* [8]. The *NMDA-type glutamate receptors is regulated by the receptor tyrosine kinase EphB2 (PDB ID:1B4F) [9]. Ephrin receptors and ephrins mediate numerous developmental processes particularly in the* nervous system [10].

Single-nucleotide polymorphisms (SNPs) account for the *more common form of human genetic variation.* Among these, the non-synonymous SNPs (nsSNPs) cause *changes in the amino acid residues which can lead to diseases.* Computer Aided Drug Designing (CADD) techniques was used for the study of preliminary parameters like molecular properties and bioactivity scores. Herbal drug discovery is playing a pivotal role in combating Alzheimer's disease [11, 12]. Some of the herbals found in the state of Kerala, India with Anti-Alzheimeric activity were Withania somnifera, *Centella asiatica, Catharanthus roseus, Curcuma longa and* Bacopa monnieri.

Withania somnifera (ashwagandha, Indian ginseng; family Solanaceae) displays a positive effect on the central nervous systems [13]. Withania somnifera also reverses β-Amyloid induced toxicity in Human Neuronal Cells in HIV-Associated Neurocognitive Disorders [14]. Centella asiatica (Kula Kud, Mandukaparni, Indian pennywort, jalbrahmi; family Umbellifere (Apiceae)) indicate to relieve anxiety and improves cognition [15]. Catharanthus roseus **(***Sadafuli, Sadabahar, Periwinkle;* family *Apocynaceae) appears to inhibit acetylcholinesterase and constituent vincamine can be used in the treatment of primary degenerative and vascular dementia [16, 17]. Curcuma longa (Haridra, Indian Saffron, turmeric; family Zingiberaceae) contain Curcumin having antioxidant, anti-inflammatory, and anti-amyloid activity [18]. Curcumin exert an improvement in memory of patients with AD leading to decreased Beta-amyloid plaques, metal-chelation, delayed degradation of neurons, antioxidant, anti-inflammatory and reduced microglia formation [19].* Bacopa monnieri (Brahmi, Water Hyssop; family Scrophulariaceae) is a potential cognitive enhancer and neuroprotectant against Alzheimer's disease [20].

MATERIALS AND METHOD

The Single-nucleotide polymorphisms (SNPs) of EphB2 gene were investigated to identify the possible mutations and functional effects. The SNPs of t*he corresponding gene EphB2 of the protein 1B4F has been explored and* retrieved from dbSNP database [21]. SNPs are distributed in the coding non-synonymous (nonsense, missense, frameshift), mRNA UTR, 5' and 3' UTR regions. A total of 4997 SNPs were analyzed from the EphB2 gene, out of which 90 SNPs were in the coding non-synonymous region, 41 in the mRNA UTR region, 90 in the coding synonymous region and remaining 4776 SNPs were in the intron region of the gene. The identification of deleterious amino acid substitution in the coding non-synonymous region was predicted by the tools 'Sorting Intolerant from Tolerant (SIFT) based on sequence homology and the physical properties of amino acids [22] and PolyPhen-2 [23]. The protein sequences of 90 nsSNPs were independently given as input to the SIFT program to predict deleterious amino acid substitutions.

Computational screening of compounds based on drug likeness was carried out on constituents such as Withania somnifera, Centella asiatica, Catharanthus roseus, Curcuma longa and Bacopa monnieri found in the state of Kerala, India. Compounds possessing Anti-Alzheimeric activity were identified from their plant sources and their drug likeness and biochemical properties were checked using Lipinski's Rule of Five and Molinspiration respectively. Butylated hydroxytoluene (BHT) was choosen to be the antioxidant standard

reference for this study [24]. Drug-likeness property or Lipinski's Rule of Five formulated by Christopher Lipinski aided to determine molecular properties and structural features to check the predictability of molecules as drug and its pharmacokinetic parametes. The guidelines predicted that the compound should meet the following criteria such as molecular mass less than 500 Da, lipophilicity cLogP not greater than 5, hydrogen bond donors less than 5 and hydrogen bond acceptors less than 10 [25]. Molinspiration, a cheminformatics tool helped for the calculation of important molecular properties like logP, polar surface area, number of hydrogen bond donors and acceptors. The prediction of bioactivity score for important drug targets like GPCR ligand, Ion channel modulator, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor and Enzyme inhibitor were also calculated [26].

The Oligomeric structure of the human EphB2 receptor SAM domain of the protein 1B4F were retrieved from RCSB protein data bank [27]. Water molecules, ligands and other hetero atoms were removed from the protein molecule. Addition of hydrogen atoms to the protein was performed using CHARMm force field. The protein molecules were subjected to stability studies computationally. The primary and secondary structure of proteins was studied [28, 29]. Parameters analyzed were Extinction coefficients, half-life, aliphatic index and Grand average of hydropathicity (GRAVY). The Extinction coefficients indicate how much light a protein absorbs at a certain wavelength. They are measured in units of M^{-1} cm^{-1}, at 280 nm in water. The half-life is the prediction of time it takes for half of the amount of protein in a cell to disappear after its synthesis in the cell. The N-terminal of the sequence considered is M (Met). ProtParam is based on the N-end rule which correlates the half-life of a protein to the identity of its N-terminal residue. The aliphatic index of a protein is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). It helps to influence the thermostability of globular proteins. The GRAVY value for a peptide or protein is calculated as the sum of hydropathy values of all the amino acids, divided by the number of residues in the protein sequence [30]. The in silico technique was employed for the detection of the location of the enzyme in the cell using WoLFPSORT. WoLFPSORT helped in predicting the subcellular localization sites of proteins based on their amino acid sequences [31]. Binding sites and active sites of proteins are often associated with structural pockets and cavities measured using CASTp server which uses the weighted Delaunay triangulation and the alpha complex for shape measurements [32]. The CASTp calculation uses a solvent probe of radius 1.4 angstrom as the default value.

The ligand structures of naturally occurring constituents such as Withanolide E, Withanolide A, Withanoside IV, Withaferine A, Anaferine, Beta-Sitosterol, Quercetin, Asiatic acid, Asiaticoside, Campesterol, Madecassoside, Madecassic acid, Vincristine, Vincamine, Vinblastine, Catharanthine, Kaempferol, Curcumin, Ascorbic acid, Linalool and Bacoside A along with Butylated hydroxytoluene (BHT) were generated using MarvinSketch and saved in the PDB format [33]. The ligands and proteins were subjected to energy minimization and finally the docking analysis was performed using the PatchDock server. The Accelrys Discovery studio 3.5 visualizer was used for visualization of the PDB format of the protein [34]. PatchDock is an algorithm for molecular docking. The input for PatchDock is molecules of any type like proteins, DNA, peptides or drugs. The output is a list of potential complexes sorted by shape complementarity criteria [35].

RESULTS AND DISCUSSION

The herbal constituents selected from *Withania somnifera, Centella asiatica, Catharanthus roseus, Curcuma longa and Bacopa monnieri* for the analysis with Anti-Alzheimeric activity were tabulated in Table 1 along with its IUPAC nomenclature. The preliminary *in silico* analysis was performed to check the parameters such as the primary and secondary structure analysis of the protein 1B4F. Primary analysis involved the calculation of Extinction coefficient, estimated half-life, Instability index, Aliphatic index and Grand average of hydropathicity (GRAVY) values. The number of amino acids of the protein was found to be 82 with the molecular weight as 9392.8g. The theoretical pI was found to be 5.21 with the total number of negatively charged residues (Asp + Glu) as 10 and the total number of positively charged residues (Arg + Lys) as 8. The total number of atoms was found to be 1309. The Extinction coefficient was 8480 at Abs 0.1% (=1 g/l) 0.903, assuming all pairs of Cys residues form cystines and Ext. coefficient at 86860 with Abs 0.1% (=1 g/l) 1.422, assuming all Cys residues are reduced. The estimated half-life was 30 hours (mammalian reticulocytes, in vitro), >20 hours (yeast, in vivo) and >10 hours (Escherichia coli, in vivo). The instability index (II) was computed to be 23.49 and this classifies the protein as stable. The Aliphatic index was found to be 77.20 and therefore the protein demonstrates considerable thermostability. Grand average of hydropathicity (GRAVY) was computed to be -0.243 and indicates that the protein was hydrophilic in nature. The protein 1B4F was

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found in large quantity in cytoplasm. The *total binding site inferred using CASTp was found to be 38, with maximum area and volume to be 1912 and 3678.9 respectively and minimum area and volume to be 7.3 and 4.4 respectively* (Figure 1)

Table 1: The Plant constituents with Anti-Alzheimeric activity

Herbal constituents	IUPAC nomenclature
Withanolide E	(5β,6β,17α,22R)-14,17,20-trihydroxy-5,6:22,26-diepoxyergosta-2,24-diene-1,26-dione
Withanolide A	(22R)-6α,7α-Epoxy-5,20,22-trihydroxy-1-oxo-5α-ergosta-2,24-dien-26-oic acid δ-lactone
Withanoside IV	1,3,27-Trihydroxy with a-5,24-dienolide(1α,3β)form;3-O-[β-D-glucopyranosyl-(1-6)]-β-D-glucopyranoside
Withaferine A	(4β,5β,6β,22R)-5,6-Epoxy-4,22,27-trihydroxy-1-oxoergosta-2,24-dien-26-oicacidδ-lactone
Anaferine	1,3-bis[(2R)-piperidin-2-yl]propan-2-one
Beta-Sitosterol	(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol
Quercetin	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one
Asiatic acid	(1S,2R,4aS,6aR,6aS,6bR,8aR,9R,10R,11R,12aR,14bS)-10,11-dihydroxy-9-(hydroxymethyl)-1,2,6a,6b,9,12a-hexamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylic acid
Asiaticoside	[(2S,3R,4S,5S,6R)-6-[[(2R,3R,4R,5S,6R)-3,4-dihydroxy-6-(hydroxymethyl)-5-[(2S,3R,4R,5R,6R)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxyoxan-2-yl]oxymethyl]-3,4,5-trihydroxyoxan-2-yl] (1S,2R,4aS,6aR,6aS,6bR,8aR,9R,10R,11R,12aR,14bS)-10,11-dihydroxy-9-(hydroxymethyl)-1,2,6a,6b,9,12a-hexamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylate
Campesterol	17-(5,6-dimethylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol
Madecassoside	[6-[[3,4-dihydroxy-6-(hydroxymethyl)-5-(3,4,5-trihydroxy-6-methyloxan-2-yl)oxyoxan-2-yl]oxymethyl]-3,4,5-trihydroxyoxan-2-yl] 8,10,11-trihydroxy-9-(hydroxymethyl)-1,2,6a,6b,9,12a-hexamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylate
Madecassic acid	(1S,2R,4aS,6aR,6aS,6bR,8R,8aR,9R,10R,11R,12aR,14bS)-8,10,11-trihydroxy-9-(hydroxymethyl)-1,2,6a,6b,9,12a-hexamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylic acid
Vincristine	methyl (1R,9R,10S,11R,12R,19R)-11-(acetyloxy)-12-ethyl-4-[(13S,15S,17S)-17-ethyl-17-hydroxy-13-(methoxycarbonyl)-1,11-diazatetracyclo[13.3.1.0{4,12}.0{5,10}]nonadeca-4(12),5,7,9-tetraen-13-yl]-8-formyl-10-hydroxy-5-methoxy-8,16-diazapentacyclo[10.6.1.0{1,9}.0{2,7}.0{16,19}]nonadeca-2(7),3,5,13-tetraene-10-carboxylate
Vincamine	Methyl (3α,14β,16α)-14-hydroxy-14,15-dihydroeburnamenine-14-carboxylate
Vinblastine	methyl (1R,9R,10S,11R,12R,19R)-11-(acetyloxy)-12-ethyl-4-[(13S,15S,17S)-17-ethyl-17-hydroxy-13-(methoxycarbonyl)-1,11-diazatetracyclo[13.3.1.0{4,12}.0{5,10}]nonadeca-4(12),5,7,9-tetraen-13-yl]-10-hydroxy-5-methoxy-8-methyl-8,16-diazapentacyclo[10.6.1.0{1,9}.0{2,7}.0{16,19}]nonadeca-2(7),3,5,13-tetraene-10-carboxylate
Catharanthine	methyl (18β)-3,4-didehydroibogamine-18-carboxylate
Kaempferol	3,5,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one
Curcumin	1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
Ascorbic acid	(2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H-furan-5-one
Linalool	3,7-dimethylocta-1,6-dien-3-ol
Bacoside A	3-[3,4-dihydroxy-6-(hydroxymethyl)-5-(3,4,5-trihydroxyoxan-2-yl)oxyoxan-2-yl]oxy-10-(hydroxymethyl)-17-(2-hydroxy-6-methylhept-5-en-2-yl)-4,4,8,14-tetramethyl-1,2,3,5,6,7,9,11,12,13,15,17-dodecahydrocyclopenta[a]phenanthren-16-one
Butylated hydroxytoluene (BHT)	2,6-bis(1,1-dimethylethyl)-4-methylphenol

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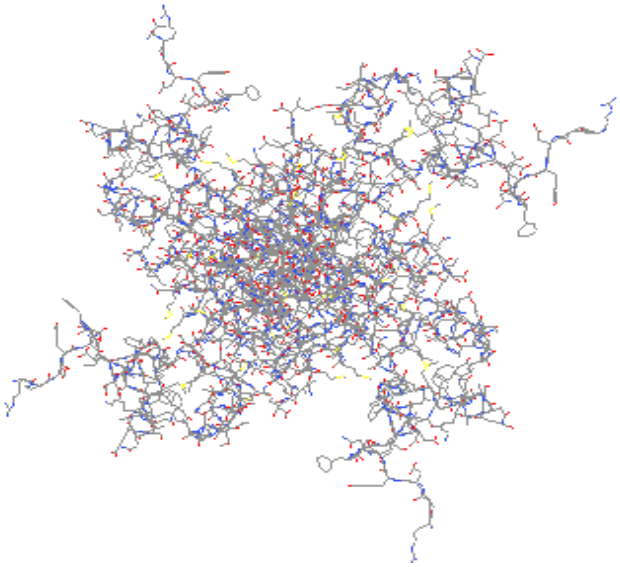

Figure 1: Jmol view of the protein 1B4F

The ligand molecules were generated using MarvinSketch and saved in the PDB format. The ligand molecules were then subjected to Molecular property and Bioactivity activity calculation using cheminformatics tool Molinspiration. The properties calculated were total polar surface area (TPSA), number of atoms, molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, n violations and number of rotatable bonds (Table 2).

Table 2: Drug likeness of the herbal constituents with Anti-Alzheimeric activity

Herbal constituents	miLogP	TPSA	natoms	MW	nON	nOHNH	nviolations	nrotb	volume
Withanolide E	3.179	116.59	35	486.61	7	3	0	2	449.16
Withanolide A	4.153	96.36	34	470.61	6	2	0	2	441.81
Withanoside IV	1.216	245.29	55	782.92	15	9	3	9	714.61
Withaferine A	3.856	96.36	34	470.61	6	2	0	3	442.38
Anaferine	1.384	41.125	16	224.35	3	2	0	4	236.41
Beta-Sitosterol	8.62	20.228	30	414.72	1	1	1	6	456.52
Quercetin	1.683	131.35	22	302.24	7	5	0	1	240.08
Asiatic acid	4.697	97.983	35	488.71	5	4	0	2	487.79
Asiaticoside	0.367	315.21	67	959.13	19	12	3	10	875.9
Campesterol	8.305	20.228	29	400.69	1	1	1	5	439.72
Madecassoside	-0.549	335.44	68	975.13	20	13	3	10	883.94
Madecassic acid	3.782	118.21	36	504.71	6	5	1	2	495.84
Vincristine	4.947	171.18	60	824.97	14	3	2	10	747.07
Vincamine	3.181	54.705	26	354.45	5	1	0	3	330.22
Vinblastine	5.563	154.11	59	810.99	13	3	3	10	744.65
Catharanthine	3.987	45.334	25	336.44	4	1	0	3	315.95
Kaempferol	2.172	111.12	21	286.24	6	4	0	1	232.07
Curcumin	2.303	93.066	27	368.39	6	2	0	8	332.18
Ascorbic acid	-1.402	107.22	12	176.12	6	4	0	2	139.71
Linalool	3.213	20.228	11	154.25	1	1	0	4	175.59
Bacoside A	2.535	215.83	54	768.98	13	8	3	10	729.72
Butylated hydroxytoluene (BHT)	5.435	20.228	16	220.36	1	1	1	2	241

TPSA: total polar surface area; natoms: number of atoms; MW:molecular weight; nON: number of hydrogen bond acceptors; nOHNH: number of hydrogen bond donors; nrotb: number of rotatable bonds

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Lipinski Rule of 5 was almost satisfied by the ligands. In case of milogP values, the ligands Beta-Sitosterol, Campesterol and Vinblastine displayed higher values. Molecular weight greater than 500 was showed by Withanoside IV, Asiaticoside, Madecassoside, Vincristine, Vinblastine and Bacoside A while the rest of the ligands selected depicted good permeability across cell membrane. TPSA values greater than 160 $Å^2$ was calculated for Withanoside IV, Asiaticoside, Madecassoside, Vincristine and Bacoside A. *n* violations greater than 0 denotes some compounds to have difficulty in binding to receptors like Withanoside IV, Asiaticoside, Madecassoside, Vinblastine and Bacoside A. Number of rotatable bond is more for Asiaticoside, Madecassoside, Vincristine, Vinblastine and Bacoside A. The calculation of bioactivity scores of ligands were studied using the parameters GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and other enzyme targets (Table 3). The ligands were in agreement with the bioactivity compared to the standard drugs.

Table 3: Bioactivity score of the herbal constituents with Anti-Alzheimeric activity

Herbal constituents	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Withanolide E	-0.07	0.16	-0.5	0.62	0.06	0.89
Withanolide A	0.04	0.32	-0.43	0.71	0.15	0.86
Withanoside IV	-1.25	-2.34	-2.22	-1.71	-0.86	-1.22
Withaferine A	0.08	0.14	-0.49	0.76	0.15	0.94
Anaferine	-0.08	0.17	-0.6	-0.58	-0.14	0.08
Beta-Sitosterol	0.14	0.05	-0.51	0.73	0.07	0.51
Quercetin	-0.06	-0.19	0.28	0.36	-0.25	0.28
Asiatic acid	0.2	-0.19	-0.46	0.91	0.28	0.66
Asiaticoside	-3.38	-4.09	-4.1	-3.63	-2.96	-3.26
Campesterol	0.11	0.01	-0.48	0.71	0.02	0.5
Madecassoside	-3.46	-4.11	-4.18	-3.7	-3.08	-3.3
Madecassic acid	0.25	-0.1	0.45	0.93	0.29	0.75
Vincristine	-2	-3.13	-3.17	-2.94	-1.68	-2.55
Vincamine	0.25	0.06	-0.22	0.06	0.03	0.1
Vinblastine	-1.76	-2.97	-2.99	-2.75	-1.53	-2.42
Catharanthine	0.51	0.29	-0.08	0.09	0.1	0.24
Kaempferol	-0.1	-0.21	0.21	0.32	-0.27	0.26
Curcumin	-0.06	-0.2	-0.26	0.12	-0.14	0.08
Ascorbic acid	-0.53	-0.24	-1.09	-1.01	-0.81	0.2
Linalool	-0.73	0.07	-1.26	0.06	-0.94	0.07
Bacoside A	-1.05	0.07	-1.26	0.06	-0.94	0.07
Butylated hydroxytoluene (BHT)	-0.34	0	-0.48	-0.08	-0.57	-0.07

After the preliminary analysis, the protein molecule was subjected to the energy minimization. The minimized protein and ligands were saved in PDB format. The Accelrys Discovery studio 3.5 visualizer was used for visualization of the PDB format of the protein 1B4F. Docking analysis *was performed by PatchDock server* and the docking score were computed as in Table 4. *Docking plays a central function in the selection of a better lead compound. It was seen that ligands Withanoside IV, Asiaticoside, Madecassoside, Vincristine, Vinblastine and Bacoside A displayed the highest binding with the protein 1B4F.*

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Table 4: Docking analysis result using PatchDock server

Compounds	Docking Score
Withanolide E	5528
Withanolide A	5746
Withanoside IV	7844
Withaferine A	5536
Anaferine	4140
Beta-Sitosterol	5948
Quercetin	4650
Asiatic acid	6056
Asiaticoside	8732
Campesterol	5742
Madecassoside	8796
Madecassic acid	6078
Vincristine	7832
Vincamine	5238
Vinblastine	7766
Catharanthine	4090
Kaempferol	4346
Curcumin	5670
Ascorbic acid	2960
Linalool	3552
Bacoside A	7308
Butylated hydroxytoluene (BHT)	4098

The Single-nucleotide polymorphisms (SNPs) of EphB2 gene were investigated to identify the possible mutations and functional effects. The SNPs of t*he corresponding gene EphB2 of the protein 1B4F has been explored and* retrieved from dbSNP database. SNPs are distributed in the coding non-synonymous (nonsense, missense, frameshift), mRNA UTR, 5' and 3' UTR regions. A total of 4997 SNPs were analyzed from the EphB2 gene, out of which 90 SNPs were in the coding non-synonymous region, 41 in the mRNA UTR region, 90 in the coding synonymous region and remaining 4776 SNPs were in the intron region of the gene. The protein sequences of 90 nsSNPs were independently given as input to the SIFT program to predict deleterious amino acid substitutions. During the analysis, 9 SNPs have been predicted as damaging (Table 5).

Table 5: List of nsSNPs that were predicted to have functional significance by SIFT

SNP	Amino acid change	Prediction	Score	Median
rs28936395	D679N	Damaging	0.03	2.79
rs121912582	Q723*	Damaging	-1	-1
rs72653677	V64M	Damaging	0.02	2.8
rs116848191	T484P	Damaging	0.02	2.79
rs142113032	D283H	Damaging	0.02	2.79
rs142890560	A330T	Damaging	0.02	2.79
rs143865228	Q876R	Damaging	0.04	2.78
rs149014913	V414I	Damaging	0.01	2.8
rs185887197	R155H	Damaging	0.01	2.75

The damaging SNPs with deleterious amino acid substitutions could be modified to prevent the individual variations.

CONCLUSION

The *in silico* analysis performed indicate the selection of natural compounds like Withanoside IV, Asiaticoside, Madecassoside, Vincristine, Vinblastine and Bacoside A to show significant binding interaction with 1B4F protein. But drug likeness property is just moderate and therefore further studies are required to improve the interactions with the corresponding protein. The EphB2 gene and 1B4F protein were investigated in this work by evaluating the influence of functional SNPs through computational analysis. SNPs were analyzed from the EphB2 gene, out of which 90 SNPs were in the coding non-synonymous region, 41 in the mRNA UTR region, 90 in the coding synonymous region and remaining 4776 SNPs were in the intron region of the gene. The protein sequences of 90 nsSNPs were independently given as input to the SIFT program to predict deleterious amino acid substitutions. During the analysis, 8 SNPs have been predicted as damaging. The SNPs which were damaging could be modified to prevent the individual variations. Further investigations are essential to modify the naturally occurring ligands for better drug likeness characteristics and to enhance the binding properties with the proteins.

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