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RESEARCH ARTICLE

IN SILICO ADME AND BIOLOGICAL ACTIVITY ASSESSMENT OF NATURAL PHYTOCONSTITUENTS FOR ANTI-ALZHEIMER POTENTIAL

***[1, 2]Lalit R. Samant, [2]Anuya Rege and [1, 2]Abhay Chowdhary**

[1]Systems Biomedicine Division, Haffkine Institute for Training, Research and Testing, Mumbai, India
[2]Department of Virology and Immunology, Haffkine Institute for Training, Research and Testing, Mumbai, India

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ABSTRACT

Phytoconstituents such as, Wedalolactone Demethylwedalolactone, Valeranone, Jatamansin, Gamma gurjunene, beta-caryophyllene alpha humulin, Oleic acid, Palmitic acid, Linoleic acid, Gallic acid, Ellagic acid, Withafirin A, Withanolide D, Bacoside A, Bacoside B, Asiaticoside, Trans Asarone, Beta/cis asarone Glycyrrhizin, Glycyrrhizic acid and Licoisoflavanone were included in the present *in silico* analysis to evaluate their Anti-Alzheimer's potential. The adsorbtion, distribution, metabolism, excretion (ADME) properties of these phytochemicals were assessed through Lipinski rule of Five. The Bioactivity properties and Phytoconstituents-likeness of the selective phytoconstituents were calculated using Molinspiration and Molsoft tools and their toxic properties were determined by employing the Osiris server. Interestingly, after application of Lipinski's rule of five, our results revealed that all these selected phytoconstituents have fully satisfied the Lipinski's rule of five and thus shall be recommended as safe phytoconstituents for effective treatment of AD though *in vitro* and *in vivo* study required in preclinical and clinical phases.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurological disease of the central nervous system (CNS) that affects aging patients in the world (**Mokhtar *et al*., 2013, Grimm *et al*., 2013**) Alzheimer's disease (AD) is a chronic disorder that slowly destroys neurons and causes serious cognitive disability. AD is associated with senile plaques and neurofibrillary tangles (NFTs). Amyloid-beta (Abeta), a major component of senile plaques, has various pathological effects on cell and organelle function. The extracellular Abeta oligomers may activate caspases through activation of cell surface death receptors. It is the most common form of dementia in the elderly. This condition is characterized by a progressive loss of memory, deterioration of virtually all intellectual functions, increased apathy, decreased speech function, disorientation, and gait irregularities. Most individuals who have advanced disease are 85 years of age and older. Females are slightly more likely than males to develop Alzheimer's disease (**Hajiaghaee *et al*., 2011**). Ayurvedic medicinal plants have shown promise in reversing the Alzheimer's disease pathology in the past and hence they may provide useful leads in the discovery of new drugs for AD therapy (**Rammohan *et al*., 2012, Dastmalchi *et al*, 2007**). Lipinski's rule of five is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski based on the observation that most of the drugs are relatively small and lipophilic molecules. "Rule of 5" Properties: It is set of simple molecular descriptors used by Lipinski in formulating his "Rule of 5" (**Lipinski; 1997**)

Corresponding author: Lalit R. Samant,
Systems Biomedicine Division, Haffkine Institute for Training, Research and Testing, Mumbai, India.

MATERIALS AND METHODS

Phytochemicals namely, Wedalolactone Demethylwedalolactone, Valeranone, Jatamansin, Gamma gurjunene, beta-caryophyllene alpha humulin, Oleic acid, Palmitic acid, Linoleic acid, Gallic acid, Ellagic acid, Withafirin A, Withanolide D, Bacoside A, Bacoside B, Asiaticoside, Trans Asarone, Beta/cis asarone Glycyrrhizin, Glycyrrhizic acid and Licoisoflavanone were included in the present *in silico* analysis to evaluate their Anti-Alzheimer's potential. The Bioactivity properties and drug-likeness of the above mentioned selected phytoconstituents were calculated using Molinspiration, Molsoft tools.

Validation of phytoconstituents was done by

a) Literature review based selection of different plants and their respective phytoconstituents used to treat Alzheimer's disease.
b) Obtaining SMILES format for these phytoconstituents using software's.

c) Applying 'Lipinski's rule of five' for each of these phytoconstituents.

Software's

Molinspiration tool

Molinspiration supports also internet chemistry community by offering free on-line cheminformatics services for calculation of important molecular properties (for example logP, polar surface area, number of hydrogen bond donors and acceptors), as well as prediction of bioactivity score for the most important phytoconstituents targets. Molinspiration tools are written in Java, therefore are available practically on any computer platform. Molecular properties and bioactivity of the phytoconstituents showing high affinity predicted using Molinspiration tool. This tool allows physico chemical properties to calculate Log P based on group contributions. The values were obtained by fitting calculated logP with experimental logP. PSA is good descriptor characterizing phytoconstituents absorption, including intestinal absorption, bioavailbility, Caco-2 permeability and Blood brain barrier penetration.

Molecular graphics and visualization, Molecular modeling, Docking and Virtual screening, computational biology and Cheminformatics. All molecular property predictors are calculated using fragment-based contributions. It developed an original method for splitting a molecule into a set of linear or non-linear fragments of different length and representation levels and counting the number of occurrences of each chemical pattern found. A Partial Least Squares (PLS) regression model was built and optimized for a particular property using a leave-50%-out cross-validation calculation. The method is very robust and fast (about 5K of compounds per second).

RESULTS AND DISCUSSION

From the data (Table 1), it was observed that among the 22 phytoconstituents were selected, Oleic acid, has high milog P value (7.15) followed by linoleic acid (6.73), palmitic acid and gallic acid(6.65) , Glycyrrhizic acid (6.63) etc. as per the Table 1 and least was for Asiaticoside (-.66). An orally active anti-Alzheimer's phytoconstituents needs not only sufficient metabolic stability to maintain integrity in the intestine and liver but also should across the Blood-Brain

Table 1. Assesment of phytoconstituents for Lipinksy rule of five

S.No.	Plant name	Constituent	Molecular formula:	Molecular weight:	HBA (>10)	HBD (>5)	MLogP (>5)	MLPSA	MLVol	No. of Stereo centers
1	*Eclipta alba* Bhringraj	Wedalolactone	C16 H10 O7	314.04	7	3	2.63	86.22 A^2	289.21 A^3	0
		Demethylwedalolactone	C15 H8 O7	300.03	7	4	2.29	96.30 A^2	267.91 A^3	0
2	*Nardostachys jatamansi* Jatamansi	Valeranone	C15 H26 O	222.20	1	0	4.35	13.60 A^2	286.97 A^3	3
		Jatamansin	C19 H20 O5	328.13	5	0	4.01	47.38 A^2	374.55 A^3	1
		Gamma gurjunene	C15 H24	204.19	0	0	5.13	0.00 A^2	270.66 A^3	4
		BETA-caryophyllene	C15 H24	204.19	0	0	5.4	0.00 A^2	295.65 A^3	2
		Alpha humulin	C15 H24	204.19	0	0	5.47	0.00 A^2	312.62 A^3	0
3	Jyotishmati *Celastrus paniculatus*	Oleic acid	C18 H34 O2	282.26	2	1	7.15	28.89 A^2	348.59 A^3	0
		Palmitic acid	C16 H32 O2	256.24	2	1	6.65	28.89 A^2	305.93 A^3	0
		Linoleic acid	C18 H32 O2	280.24	2	1	6.73	28.89 A^2	353.96 A^3	0
4	*Emblica Officinalis* Amla	Gallic acid	C16 H32 O2	256.24	2	1	6.65	28.89 A^2	305.93 A^3	0
		ellagic acid	C14 H6 O8	302.01	8	4	1.02	105.23 A2	272.21 A^3	0
5	*Withania Somnifera* Ashwagandha	Withafirin A	C28 H38 O6	470.27	6	2	3.21	75.66 A^2	564.08 A^3	11
		Withanolide D	C28 H38 O6	470.27	6	2	3.07	73.94 A^2	569.93 A^3	11
6	*Bacopa monnieri* Brahmi	Bacoside A	C41 H68 O13	768.47	13	8	2.25	173.36 A^2	826.15 A^3	18
		Bacoside B	C41 H68 O13	768.47	13	8	2.25	173.36 A^2	826.15 A^3	18
7	*Centella asiatica* Mandukaparni	Asiaticoside	C48 H78 O19	958.51	19	12	-0.66	251.50 A^2	966.98 A^3	27
8	*Acorus calamus* Vacha	Trans Asarone	C12 H16 O3	208.11	3	0	3.23	23.13 A^2	229.16 A^3	0
		Beta/cis asarone	C12 H16 O3	208.11	3	0	3.23	23.13 A^2	229.16 A^3	0
9	*Glycyrrhizin glabra* Yastimadhu	Glycyrrhizin	C42 H62 O16	822.40	16	8	2.06	206.93 A^2	862.79 A^3	19
		Glycyrrhizic acid	C30 H46 O4	470.34	4	2	6.63	57.38 A	598.63 A^3	9
		Licoisoflavanone	C20 H18 O6	354.11	6	3	3.02	77.46 A^2	359.70 A^3	1

Molsoft software

Molsoft is a California based software company that is a primary source of new breakthrough technologies in:

Barrier (BBB). At the molecular level, the BBB is not homogenous but consists of a number of partially overlapping zones contained in a highly anisotropic lipid

layer (**Sippl 2002**). The conformational mobility of the lipid chains is relatively low at or near the water (blood)/ lipid interface and interface at the center of the bilayer. In addition, the hydrophilic/lipophilic interface at the blood/membrane boundary consists of perturbed and bound water, charged polar lipid head moieties connected to long lipid chains. As a result, a phytoconstituents approaching the BBB is confronted with a thick layer that is capable of non-covalent interactions with the phytoconstituents, similarly to that of receptor but with much looser steric requirements. High lipophilicity frequently leads to compounds with high rapid metabolic turnover (**Waterbeemd *et al*., 2001**) and low solubility and poor absorption. As lipophilicity (LogP) increases, there is an increased probability of binding to hydrophobic protein targets other than the desired one, and therefore, there is more potential for toxicity.

The biological activity of a phytoconstituents was almost entirely due to their Log P and their rate of metabolism was linearly related to LogP. Furthermore, optimal activity is observed at LogP = 2 (**Kellogg and Abraham 2000**). The phytoconstituents used to treat neurological disorders have LogP value mostly between 2 to 4.Subsequently, indicated that LogP is predominantly a measure of phytoconstituents volume or surface area, plus hydrogen bond acceptor potential. Thus, both hydrogen bonding potential and phytoconstituents volume contribute to permeability. Lipophilicity was the first of the descriptors to be identified as important for CNS penetration (**Kujawski *et al*., 2012**), reasoned that highly lipophilic molecules will be partitioned into the lipid interior of membranes and will be retained there. The Polar Surface Area (PSA) and the molecular volume components were the most important descriptors, with PSA strongly predominating (**Van de Waterbeemd and Kansy, 1992). (Palm *et al*., 1999**) developed a dynamic PSA approach whereby the set of available conformationswere used and the contribution of each to the overall PSA was calculated using a Boltzman distribution thereby taking into account conformational flexibility. (**Kelder 1999**) found that the phytoconstituents can be targeted to the CNS with a PSA less than 60–70 $Å^2$. Similar conclusions were made by van de Waterbeemed based on a study of marketed CNS and non-CNS phytoconstituents (**Van de Waterbeemed *et al*., 1998**). Their cutoff for PSA cutoff for CNS penetration is 90 $Å^2$ or below and a molecular weight cutoff of 450. The PSA was in range for all the phytoconstituents.

All the QSAR equations emphasize the importance of hydrogen bonding whether through polarity, PSA, hydrogen bond donor and acceptor counts, or simply counting heteroatoms capable of hydrogen bonding. All of these measurements are correlated, for instance, (O + N) atom count is highly correlated with PSA but measures hydrogen bond acceptors. CNS penetration requires a sum of these heteroatoms of 5 or less (**Österberg and Norinder, 2000**). Compounds with high hydrogen bond forming potential, such as peptides with their amide groups, peptides even as small as di- or tripeptides, have minimal distribution through the BBB (**Pardridge, 1998**). Increasing hydrogen bonding decreases BBB penetration. It should be pointed out that there are other heteroatoms in phytoconstituents that can function as hydrogen bond acceptors (HBA) and total HBA, including (N + O) would probably give a better measure.

From Table 1, the highest rotatable bonds were present in oleic acid (15), palmitic acid and linoleic acid (jyotishmathi) (14), bacoside a, bacoside b and asiaticoside (10), glycyrrhizinic acid (7), trans asarone and cis asarone (4) jatamansin and withafirin a (3) withanolide d (2), glycyrrhizic acid, wedalolactone, valeranone, gamma gurjunene and licoisoflavanone (1) demethylwedalolactone, beta-caryophyllene, alpha humulin does not have any nrotb. Rotatable bond count is now a widely used filter following the finding that greater than ten rotatable bonds correlates with decreased rat oral bioavailability (**Veber DF Johnson *et al*., 2000**).

CNS phytoconstituents have significantly fewer rotatable bonds than other phytoconstituents classes. Most centrally acting compounds have rotatable bondcount of five or less (Leeson and Davis, 2004). Apart from these, it was also observed that Asiaticoside has high volume (966.98 A^3), followed by Glycyrrhizin (862.79 A^3) rest as per mentioned in table 1 and Trans Asarone And cis Asarone has least (229.16 A^3).

Figures 1, 2, 3, 4, 5, 6
showing drug likness model score based on molsoft software prediction. Drug likness score was highest for Licoisoflavanone and lowest for Withanolide D. The subtle modification in Bacoside A and B which violates Lipinski's rule of 5 can make it good drug candidate.

Molinspiration bioactivity

These property was selectively applied to chosen phytoconstituents which were not violating rule of Lipinsky.

Table 2. Bioactivity of the selected Phytoconstituents

Phytoconstituent	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Withafirin A	0.08	0.14	-0.49	0.76	0.15	0.94
Withanolide D	0.05	0.30	-0.50	0.73	0.16	1.07
Licoisoflavanone	0.05	-0.10	-0.03	0.65	0.02	0.50

As per the data presented in the Table 2, it was evident that three phytoconstituents were having property to be druglike and could be targeted against nuclear receptor and may act as enzyme inhibitor also, further computational and statistical studies to support the current findings.

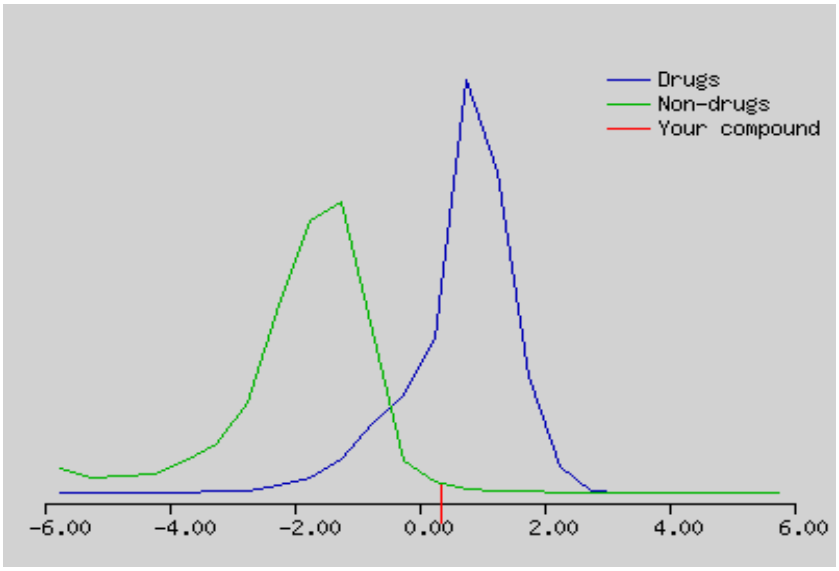

Figure 1. Withafirin A: Druglikeness model score: 0.36

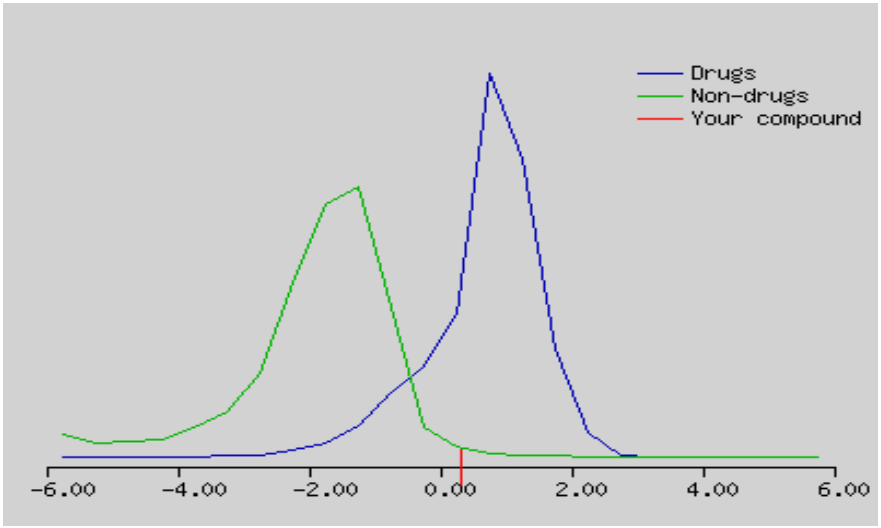

Figure 2. Withanolide D: Druglikeness model score: 0.32

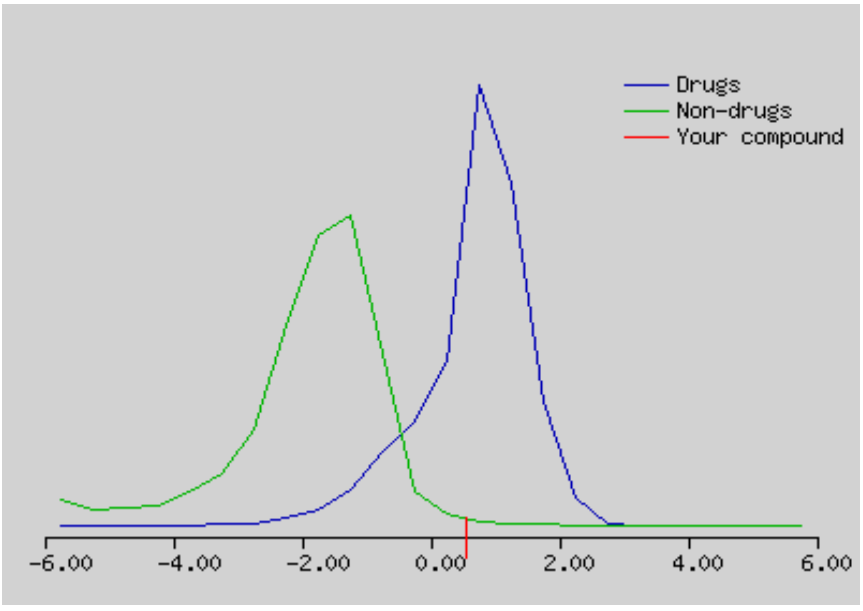

Figure 3. Bacoside A and B : Druglikeness model score: 0.54

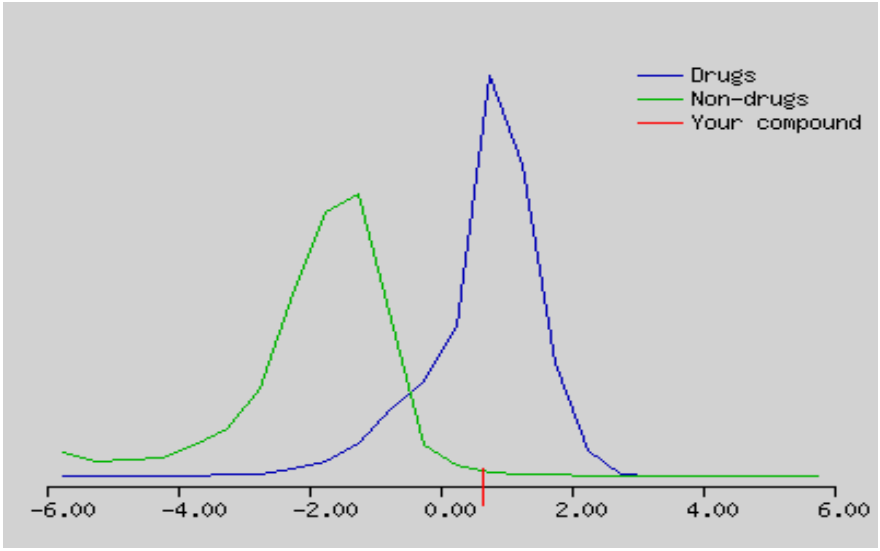

Figure 4. Asiaticoside: Druglikeness model score: 0.64

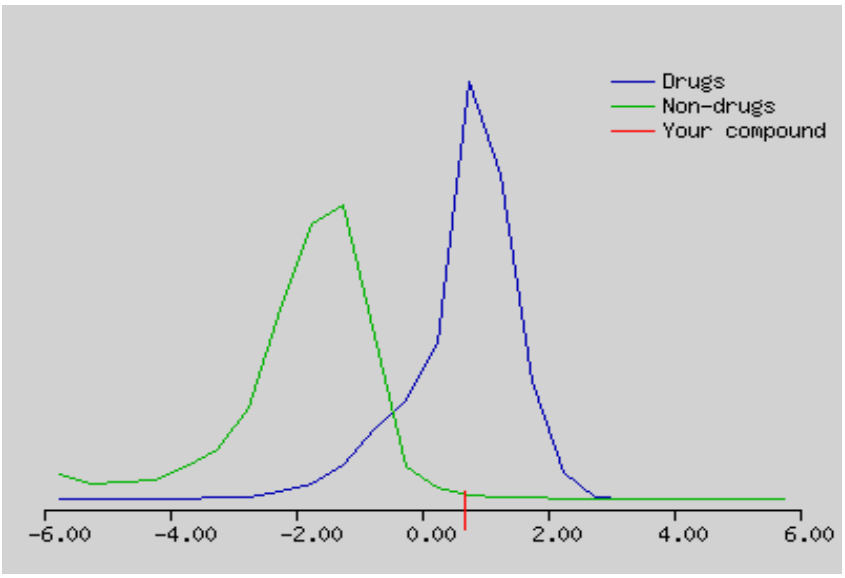

Figure 5. Glycyrrhizin: Druglikeness model score: 0.68

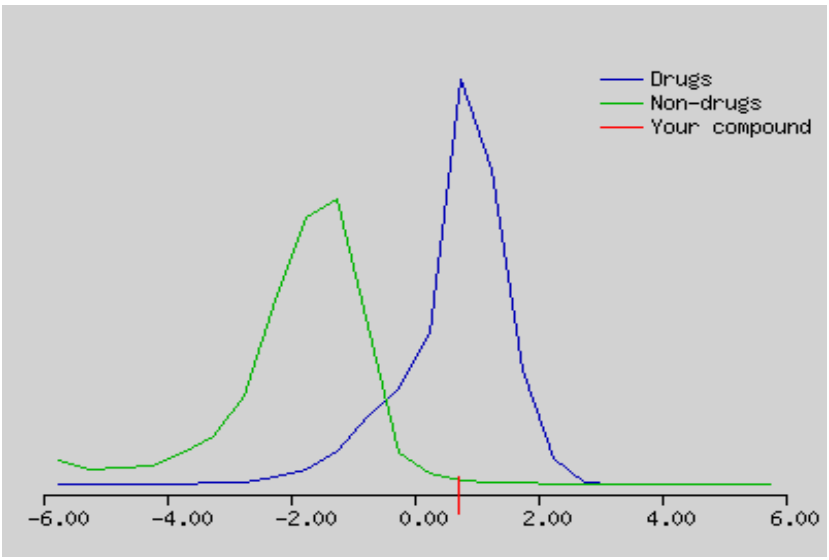

Figure 6. Licoisoflavanon: Druglikeness model score: 0.73

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper

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