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RESEARCH ARTICLE

Insilico approach of interaction studies in *Bacopa monnieri* compounds targeting multi-proteins for Alzheimer's Disease

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ABSTRACT:

Bacopa monnieri Linn (plantaginaceae) is perennial, creeping herb native to the wetlands of southern and eastern India, Australia, Europe and Asia. It is a well-known medicinal plant, is widely used in traditional medicine system in the treatment of epilepsy, asthma, gastrointestinal discomfort, skin disease and has antimicrobial action, antioxidant and anticancer. It is a nootropic and used as memory enhancer and reducing anxiety in the treatment of attention deficit hyperactivity disorder. *Bacopa monnieri* (also known as Brahmi) is used in the treatment of Alzheimer's disease. The objective of this study was to establish the binding energy of *Bacopa monnieri* biological active compounds and drug likeliness by *Insilico* techniques for treatment of Alzheimer's disease with CASP3. The compounds taken were apigenin, rosavin and luteolin. These compounds are docked by using AutoDock 4.2 Software with proteins selected based upon the literature survey and the PDB IDs are 5IAS, 5IAR, 5IAJ, and 5IBC. From the docking results the compounds shows satisfactory dock score values respectively. These compounds are visualized by using Discovery studio 4.1 Visualizer followed by DruLiTo software which satisfies the Lipinski's properties for all the compounds. This result depicts the *Bacopa monneri* derivatives having significant role to design new compounds with these properties for treating Alzheimer's disease.

KEYWORDS: *Bacopa monnieri*; Lipinski's rule; AutoDock 4.2; Discovery Studio Visualizer 4.1.

INTRODUCTION:

Bacopa monnieri is a perennial, creeping herb belonging to the family plantaginaceae, native to the wetlands of southern and Eastern India, Australia, Europe, Africa, Asia, and North and South America. Bacopa is a medicinal herb used in Ayurveda, where it is also known as "Brahmi", after Brahma, the creator God of the Hindu pantheon.[1,2] It is a well-known medicinal plant, is widely used in traditional medicinal in epilepsy, asthma, gastrointestinal discomfort, skin disease and has antimicrobial action, antioxidant and anticancer.[3-5] It is a nootropic and used as memory enhancer and reducing anxiety in the treatment of attention deficit hyperactivity disorder.[6-9] *Bacopa monnieri* has a long history of use in Ayurveda and preclinical research studies have identified biological mechanisms by which it might protect the brain from aging and perhaps Alzheimer's disease.[3] Consequently, the researchers believe that *Bacopa monnieri* is a potential cognitive enhancer and neuroprotectant against Alzheimer's disease.[10] The major constituents of *Bacopa monnieri* are saponins include bacoside A1, A2, A3, bacopasaponins A-G, bacopaside I-VIII, bacopaside N1, N2, X and jujubogenin. Other chemical constituents of the plant are hersaponin, betulic acid, alkaloids-brahmine and herpestine, flavonoids-luteolin-7-

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glucoside, glucuronyl-7-apigenin and glucuronyl-7-luteolin, luteolin-7-O-β-glucopyranoside, a triterpene bacosine and several common phyto sterols. [6]

Alzheimer's disease (AD), also referred to simply as Alzheimer's, is a chronic neurodegenerative disease that usually starts slowly and worsens over time. It is the cause of 60% to 70% of cases of dementia. The most common early symptom is difficulty in remembering recent events (short-term memory loss).

The phytochemical analysis revealed the presence of apigenin, quercetin and luteolin which are termed to be as flavonoids compounds (citrus flavonoids). These flavonoids exert a multiplicity of neuroprotective actions within the brain and the potential to promote memory, learning and cognitive function and other evidence also exists for the beneficial and neuromodulator effects of flavonoid-rich *Bacopa monnieri* extracts, particularly in connection with age-related dementias and Alzheimer's disease.[11]. It has been discovered that APP (amyloid precursor protein) is a precusor molecule whose proteolysis generate amyloid ß (Aß) amino acid peptide. Aß is an amyloidogenic peptide that forms senile plaques found in the brains of AD. Some evidence suggests that senile plaques are formed due to the activation of Caspases particularly caspases CASP 3 and CASP4. Flavonoids are inhibitors of caspases particularly CASP 3 as activation of caspases in AD may be a proximal event that is not just associated with neurodegeneration therefore further affecting disease development and spread through the brain region by induction of apoptosis. [12]

Figure 1: Flowers of *Bacopa monnieri*

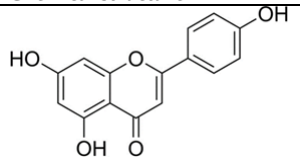
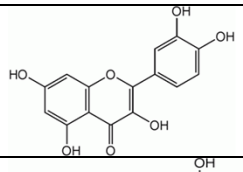
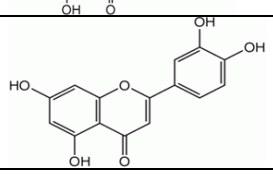
Figure 2: Floral part and leaves of *Bacopa monnieri*

MATERIALS AND METHOD:

Preparation of ligand:

The phytoconstituents derived from *Bacopa monnieri* and selected for this study are a) Apigenin b) Quercetin c) luteolin with their structures in **Table 1.** The 3D structures of the small molecules are retrieved from PubChem chemical databases and saved in .mol format. The ligands are imported to the workspace and preparation is done for docking studies.

Table:1 Compounds and Their Structure

S.No	Constituents	Chemical structure
1.	Apigenin	
2.	Quercetin	
3.	Luteolin	

DruLiTo Software:

DruLiTo is open source software. It can calculate different molecular properties and screen the molecules based on drug likeness rules such as, 'The Lipinski rule of five' (Lipinski 2004), Lipinski's properties such as molecular weight, lop P, number of hydrogen bond acceptor and donors are taken for the plant compounds. All the compounds satisfy the Lipinski's rule of five for drug-likeness and the values are depicted in **Table 2**. [13]

Preparation of protein:

The three-dimensional coordinates of the crystal structures of the proteins (PDB-ID: 5IAJ, 5IAR, 5IAS and 5IBC) were downloaded from the RCSB protein data bank archive and used for docking studies. The protein structures were prepared in order to obtain the correct ionization and tautomeric states of amino acid residues. Further, water molecules were removed and polar hydrogen atoms were added. Then, the kollman united atom partial charges and salvation parameters were assigned. The protein preparation process resulted in a PDBQT file that contained the atomic coordinates of the protein in a format that was necessary to execute Autogrid and Autodock.

Molecular docking analysis:

AutoDock is a tool used for predicting the interactions between the receptor (macromolecule) and the ligand

molecule. Autodock 4.2 suite was used for molecular docking analysis and the docking logs were analyzed using the graphical user interface of ADT. Initially, the grid box was generated for the entire protein molecule, because the protein structure was not complexed with a small molecule. Further, at the end of the docking process (for each of the four protein), a possible ligand binding site was identified and another grid box was generated around that area. Then the final docking results in order to confirm the accuracy of the predicted binding sites. The Lamarckian Genetic Algorithm and empirical free energy scoring function will provide docking results for ligands with approximately 10 conformations based on the cluster root mean square deviation and orientation. The dock score values and hydrogen contact of ligands are tabulated in **Table 3**.

Visualization:

Discovery Studio Visualizer 4.1 is a free, molecular modelling environment, for both small and macromolecule applications. It is developed by accelrys which specializes in scientific software products. It is used regularly in a range of academic and commercial entities, but is most relevant to pharmaceutical and biotechnology industries. The visualization of the docked compounds is tabulated in **Table 4.** [14]

RESULTS AND DISCUSSION:

The 'drug likeness properties' of the phytoconstituents was evaluated according to the 'The Lipinski rule of five' and to develop them as potential lead compound for treatment of Alzheimer's disease. All the compounds pass the drug likeness properties.

TABLE: 2 LIPINSKI'S RULE

Compounds	Molecular weight (<500 Da)	Log p (<5)	Hydrogen bond acceptor	Hydrogen bond donor
Apigenin	270	2.419	5	3
Quercetin	302	2.010	7	5
Luteolin	286	2.215	6	4

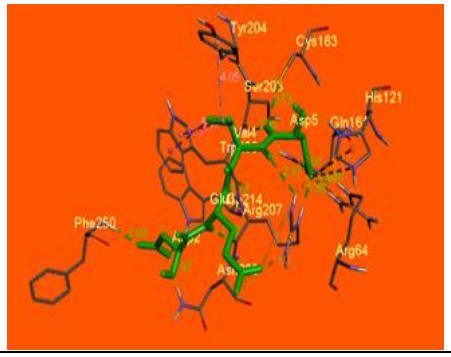
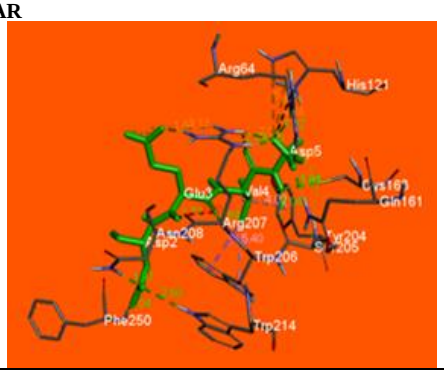
***In-silico* docking results:**

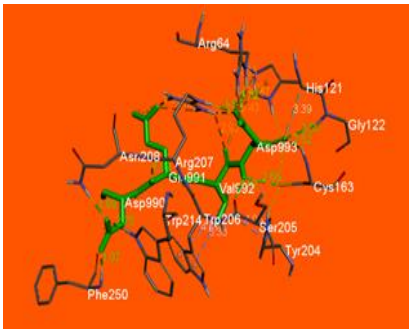
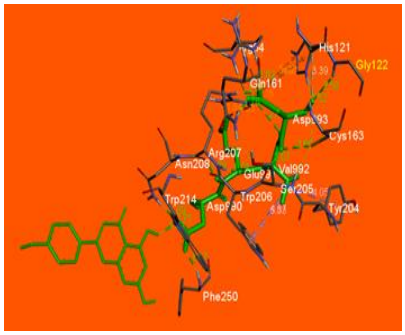
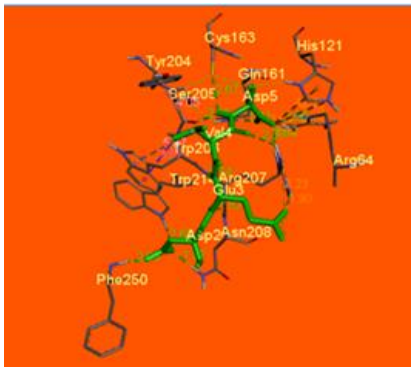
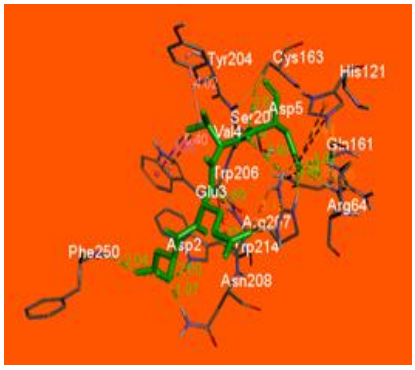
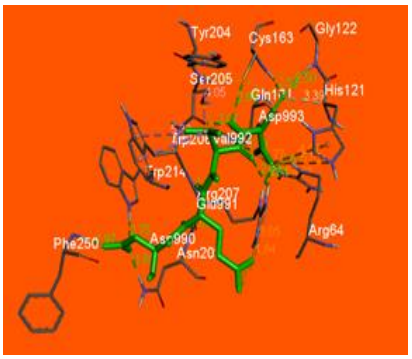
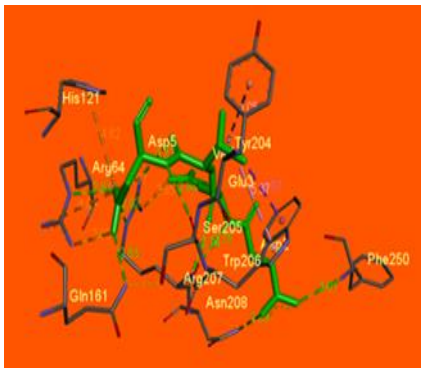
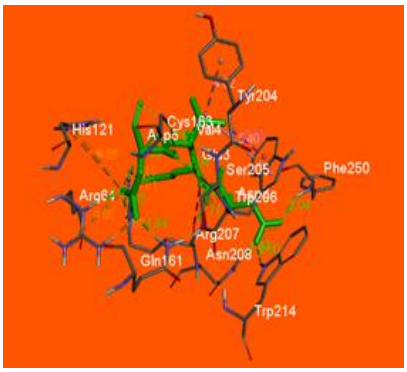
The ability of the phytoconstituents to bind with the targets is given in terms of Dock Scores. The Dock Scores and hydrogen bond contacts are used as the parameters for analyzing the docking results. Amino acid residues such as arginine, asparagine, tyrosine, histidine, and serine have bound to the above-mentioned compounds. The ligand possessing the highest dock score shows a strong affinity towards its target. *In-silico* docking analysis of phytoconstituents from *Bacopa monnieri* based on dock score and hydrogen bond contact is represented in **Table 3.**

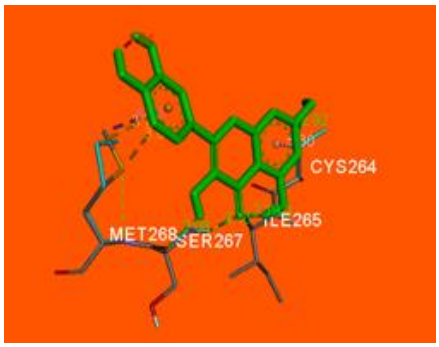
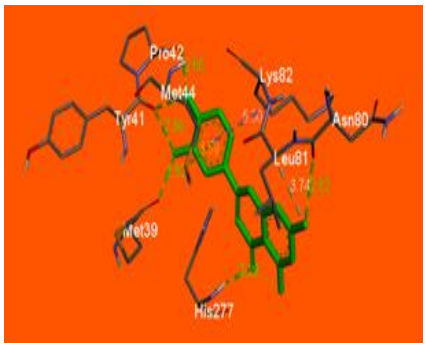
Table: 3 Molecular Docking Analysis

Compounds		Ligand			
		5IAJ	5IAR	5IAS	5IBC
Apigenin	Binding energies	-6.66	-6.41	-7.28	-6.49
	Inhibition constant (µM)	13.2	20.17	4.59	17.61
	Hydrogen bond contact	LYS224	SER267, ILE265	HIS121, SER120, GLN161, ARG64	GLU248, GLN217 ASP990
Quercetin	Binding energies	-5.9	-5.9	-5.6	-5.3
	Inhibition constant (µM)	47.09	47.68	78.4	129.58
	Hydrogen bond contact	ARG238, TYR226, GLN225, ARG238	ILE265	SER267	TYR226, ARG238, GLN225, ARG238
Luteolin	Binding energies	-6.34	-7.35	-8	-6.89
	Inhibition constant (µM)	22.43	4.12	1.36	8.83
	Hydrogen bond contact	VAL85, ARG75	ILE265, SER267	ARG238, ARG24, THR245	TYR226, ARG238

Table: 4 Visualization Of The Docked Compounds

Compounds	Targets	
Apigenin	**5IAJ** 	**5IAR** 

	5IAS	**5IBC**
		
Quercetin	**5IAJ**	**5IAR**
		
	5IAS	**5IBC**
		
Luteolin	**5IAJ**	**5IAR**
		

	5IAS	5IBC
		

The compounds showed good binding energy thus these compounds can be effectively used for the treating Alzheimer's disease.

CONCLUSION:

From the above docking results, we conclude that the flavonoid compounds in *Bacopa monnieri* medicinal plant shows good binding energies and inhibition constants with targets. These compounds can be effectively used for treating Alzheimer's disease. In future studies, we are planning to predict and do *In-vivo* and *In-vitro* studies with *Bacopa monnieri* compounds and above mentioned targets such as 5IAJ,5IAR,5IAS,5IBC.

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