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Subrata Roy
Department of Biological Science, Seacom Skills University, Kendradangal, Shantiniketan, Birbhum, West Bengal, India

Shoumini Chakravarty
Department of Biological Science, Seacom Skills University, Kendradangal, Shantiniketan, Birbhum, West Bengal, India

Partha Talukdar
Department of Botany, Serampore College, 8, William Carey Sarani, Serampore, West Bengal, India

Soumendra Nath Talapatra
Department of Biological Science, Seacom Skills University, Kendradangal, Shantiniketan, Birbhum, West Bengal, India

Correspondence
Soumendra Nath Talapatra
Department of Biological Science, Seacom Skills University, Kendradangal, Shantiniketan, Birbhum, West Bengal, India

Identification of Bioactive Compounds Present in *Bacopa monnieri* Linn. Against Caspase-3 and Tau Protein Kinase I to Prevent Alzheimer's Disease: An *in silico* Study

Subrata Roy, Shoumini Chakravarty, Partha Talukdar and Soumendra Nath Talapatra

Abstract
Bacopa monnieri Linn. is commonly known as Bramhi sak in Bengali and found in marshy area, traditionally used for the prevention of different neurological disorders especially Alzheimer's disease (AD). An *in silico* study was detected receptor-ligand binding energy and interaction through molecular docking for phytocompounds present in *B. monnieri* on two receptors viz. Caspase-3 or CASP-3 and tau-protein kinase I or TPK I (PDB IDs: 3KJF and 1J1B) as causative agents for Alzheimer's symptoms. The molecular docking was performed by using PyRx tool (Version 0.8) to know favourable binding affinity and energy. The molecular interaction was visualized through molecular graphics laboratory (MGL) tool (Version 1.5.6). The selected phytoconstituents (17 nos.) and 1 no. of synthetic medicine were used for present prediction. Present *in silico* study especially molecular docking revealed that favourable binding energy was observed for four phytoligands such as four phytoligands such as Bacopasaponin G obtained -9.6 Kcal/mol followed by Bacopaside III (-9.2 Kcal/mol), Bacopaside VI (9.2 Kcal/mol) and Silbinin (-9.1 Kcal/mol) respectively while Bacopasaponin N2 obtained -9.1 Kcal/mol followed by Bacopasaponin G (-8.8 Kcal/mol), Bacopaside X (-8.8 Kcal/mol) and Bacopaside VII (-8.7 Kcal/mol) respectively were obtained in comparison with Donepezil (-6.6 and -7.0 Kcal/mol) on CASP-3 and TPK I receptors. The predictions showed Bacopasaponin G and Bacopasaponin N2 individually or combinations could be suitable lead candidate(s) that can prevent AD. In conclusion, the binding was obtained near mouth of the active site, which may be due to suitable inhibition. It suggested in future to validate the present prediction with experimental assay.

Keywords: *Bacopa monnieri*; bioactive compounds; tau protein and caspase-3; molecular docking

1. Introduction
Alzheimer's disease (AD) causes dementia associated problems in which loss of memory or cognition occurs, which hampers the daily life. According to the report documented by Alzheimer's and related disorders society of India in 2010, there are 3.7 million Indians suffering with dementia while the numbers are anticipated to 2-fold by 2030 [1]. For more than a decade, researchers have observed that the protein known as tau protein is an important causative receptor other than the β-amyloid plaques in Alzheimer's symptoms [2]. On the other hand, the protein kinases such as glycogen synthase kinase (GSK)-3² have been discovered as promising drug targets due to the involvement in AD pathways through pathophysiological tau protein phosphorylation or tau hyperphosphorylation [1]. According to Medina [3], and Joshi *et al.* [1], tau protein kinase inhibitor such as GSK-3² in which tau phosphorylation takes place and hyperphosphorylation may lead to neurotoxic effect along with tau-mediated neurodegeneration [4]. Moreover, the biochemical mechanisms of AD has been described by several researchers that senile plaques in AD obtained from the amyloid beta (Aβ) peptide, which accumulated in the form of harmful oligomers and initiate the series of events including NFT (neuro fibrillary tangles) formation, neuronal deterioration resulting in AD dementia [5, 6] and the particular Aβ peptide is produced from the significant type1 integral membrane Amyloid Precursor Protein (APP), through sequential proteolytic cleavage of the β-secretase and γ-secretase enzyme complex [7-9]. Other important protein is caspase-3 (CASP-3), which lead to apoptosis and inhibiting CASP-3 protein may also inhibit the formation of Aβ peptide reported by Johari *et al.* [10].

This is an important task to inhibit these two proteins for the therapeutic efficacy during the development of AD. In this context, researchers have emphasized drug development by using natural products through traditional knowledge. The Indian medicinal plant, *Bacopa monnieri* Linn. is commonly known as Bramhi and found in marshy area [11]. The usage of this plant is from traditional knowledge in Ayurvedic medicine for 3000 years [12-13]. From past to recent research, several reports have been documented that the bioactive compounds present in this plant prevent different neurological disorders such as vascular dementia, Alzheimer's disease, cognitive impairments viz. motor functioning, attention, language, memory, executive control, vision, emotion, sensory functions, consciousness, etc. [14-23].

Interestingly, in experimental study the crude extracts show the anti- Alzheimer's activity by this plant species and separation of each phytochemical and their efficacy can be suitable to detect the above-mentioned therapy that may need long duration, huge laboratory expanses, etc. But *in silico* study through computational prediction especially molecular docking supports the identification of lead compound(s) within a short duration. In this context, receptor (macromolecule)-ligand (Small molecule) binding affinity and energy value estimation is a suitable approach for structure-based drug designing and exact phytocompound or combinations of few phytochemicals can easily be predicted [24].

Present *in silico* study was to detect suitable receptor-ligand binding energy and molecular interaction through molecular docking for common bioactive compounds of *Bacopa monnieri* on caspase-3 (CASP-3) and human tau-protein kinase I (TPK I) receptors (PDB IDs: 3KJF and 1J1B).

2. Materials and Methods

2.1 Selection of proteins (receptors)

The crystal three-dimensional (3-D) structure of proteins such as Caspase-3 or CASP-3 (PDB ID: 3KJF) and tau-protein kinase I or TPK I (PDB ID: 1J1B) were downloaded from the website of protein data bank (PDBE). Wang *et al.* [25] and Aoki *et al.* [26] have experimented and deposited the X-ray diffraction crystallographic structures of the target receptor at 2.00 and 2.10Å resolution respectively. The 3-D ribbon structures are exhibited in Fig 1 (A-B) after visualizing in MGL tool developed by The Scripps Research Institute [27].

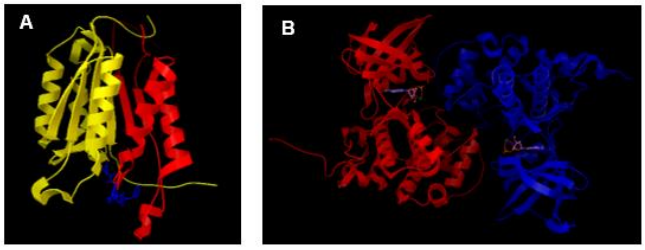


Fig 1: 3-D ribbon structure of three receptors [A = CASP-3 (PDB ID: 3KJF) chain A = yellow colour and chain B = red colour attached with inhibitory molecule B92 at 285 position; B = TPK I (PDB ID: 1J1B) chain A = red colour attached with magnesium ion and ADP (Adenosine-5'-diphosphate) at 431 and 430 position and chain B = blue colour attached with magnesium ion and ADP at 931 and 930 position]

2.2 Selection of phytochemicals (Ligands)

The common phytochemicals mainly 5 flavonoids such as Catechin, Galangin, Scopoletin, Silbinin and Memantine and 12 saponins viz. Bacopaside X, Bacopasaponin G, Bacopasaponin F, Bacopaside I, Bacopaside II, Bacopaside A, Bacopaside B, Bacopaside III, Bacopaside VII, Bacopaside N2, Bacopaside VI and Bacopaside C and synthetic medicine as Donepezil were taken from literatures [9-10, 22-23, 28-29]. The Canonical SMILES (simplified molecular-input line-entry system) of these compounds were retrieved from the PubChem database (www.ncbi.nlm.nih.gov/pubchem) and PDB file of each phytochemical was obtained from CORINA online server (www.mn-am.com/online_demos/corina_demo) after incorporating SMILES string in appropriate place. The 3-D structure of all the phytoligands is depicted in Fig 2 and the photograph of twig is exhibited in Fig 3.

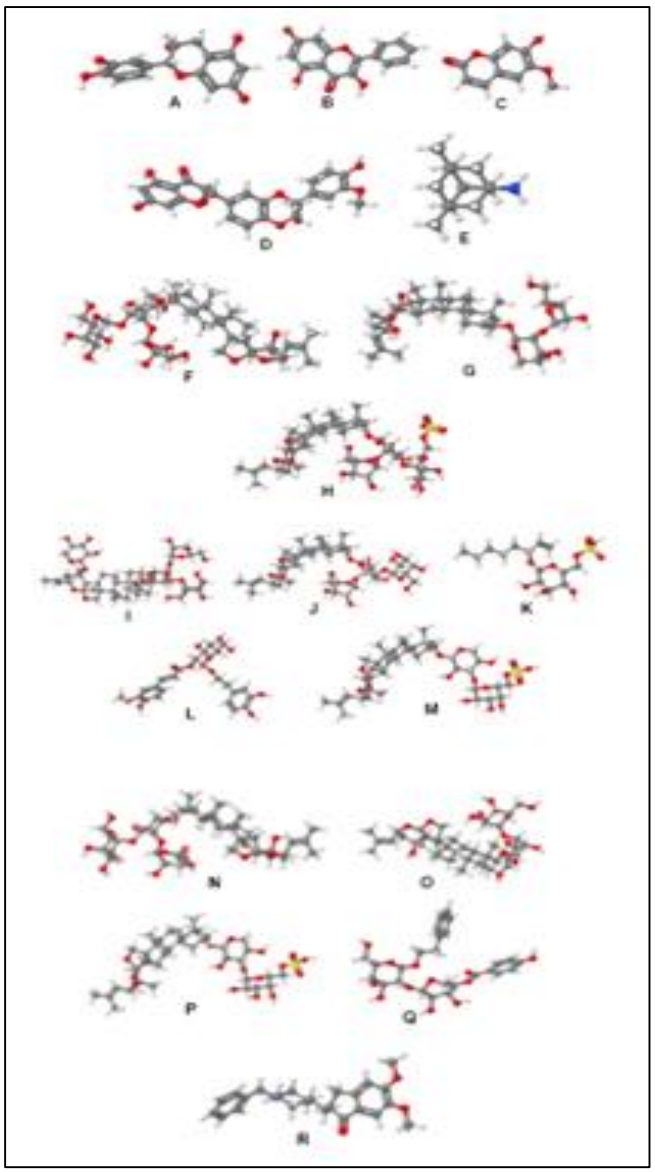


Fig 2: 3-D structure of established phytoligands of *Bacopa monnieri* Linn. (A = Catechin; B = Galangin; C = Scopoletin; D = Silbinin; E = Memantine; F = Bacopaside X; G = Bacopasaponin G; H = Bacopasaponin F; I = Bacopaside I; J = Bacopaside II; K = Bacopaside A; L = Bacopaside B; M = Bacopaside III; N = Bacopaside VII; O = Bacopaside N2; P = Bacopaside VI; Q = Bacopaside C and R = Donepezil)

Fig 3: Photo of Brahmi twig (*Bacopa monnieri* Linn.)

2.3 *In silico* study through molecular docking and interaction

In silico study with special reference to molecular docking was done by using PyRx software (Version 0.8) developed by Trott and Olson [30]. The molecular docking was visualized the output. PDBQT file by using MGL tool [27] and the results of 3-D structure were rendered by using MGL tool. The docking was carried out with 5 flavonoids and 12 saponins on three receptors such as Caspase 3 (PDB ID: 3KJF) and tau-protein kinase I (PDB ID: 1J1C) were analysed to detect suitable binding energy value. The receptor-ligand interaction of these three proteins and phytoconstituents (ligands) were identified to detect the residues involved in each case for the therapeutic efficacy of AD. Table 1 describes the 3-D grid box size values and central position values for docking site on the studied target protein with a grid spacing of 0.375 Å. The present tool predicts docking result by obtaining energy value for each ligand. Finally, all the 18 ligands were analysed to detect binding position and energy value. The resultant 3-D structural complexes of the individual ligand/receptor binding were finally observed in AutoDoc Vina software [27], to determine some specific contacts between the atoms of the test compounds and amino acids of these proteins.

Table 1: Grid size for studied receptor (in Å)

Receptor	Size			Position from center		
	X	Y	Z	X	Y	Z
PDB ID: 3KJF	64.5309	55.6222	49.9444	10.3321	6.9315	2.2869
PDB ID: 1J1B	79.2678	79.6234	109.8353	24.6687	-0.2004	-21.1715

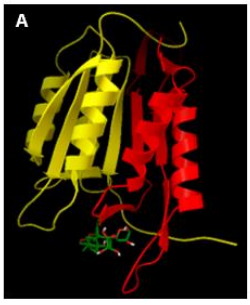
3. Results and Discussion

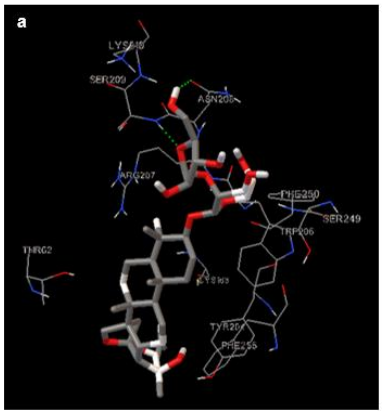
In Table 1, the data of favourable binding energy values, four phytoligands such as Bacopasaponin G obtained -9.6 Kcal/mol followed by Bacopaside III (-9.2 Kcal/mol), Bacopaside VI (9.2 Kcal/mol) and Silbinin (-9.1 Kcal/mol) respectively were obtained on CASP-3 receptor. Other studied phytoligands showed below energy values compared to above-mentioned four phytoligands. These were showed energy values (Kcal/mol) as Bacopaside II (-8.7), Bacopaside I and X (-8.4), Bacopaside VII (-8.3), Bacopaside N2 (-8.2), Bacopaside B (-8.1), Bacopasaponin F (-7.9), Catechin (-7.5), Galangin (-7.4), Bacopaside C (-6.7), Scopoletin (-5.8), Bacopaside A (-5.7) and Meantine (-4.9) respectively on CASP-3 receptor. But the synthetic medicine such as Denepezil was showed below binding energy value (-6.6 Kcal/mol) compared to phytoligands on CASP-3 receptor.

Table 2: Binding energy values for phytoligands on CASP3 (PDB ID: 3KJF) receptor

Sl. No.	Ligands	Binding energy (Kcal/mol)
1.	Bacopasaponin G	-9.6
2.	Bacopaside III	-9.2
3.	Bacopaside VI	-9.2
4.	Silbinin	-9.1
5.	Bacopaside II	-8.7
6.	Bacopaside I	-8.4
7.	Bacopaside X	-8.4
8.	Bacopaside VII	-8.3
9.	Bacopaside N2	-8.2
10.	Bacopaside B	-8.1
11.	Bacopasaponin F	-7.9
12.	Catechin	-7.5
13.	Galangin	-7.4
14.	Bacopaside C	-6.7
15.	Scopoletin	-5.8
16.	Bacopaside A	-5.7
17.	Meantine	-4.9
1.	Donepezil	-6.6

In case of receptor-ligand binding study on CASP-3, the contact residues LYS210, PHE250, ARG207, THR62, CYS163, SER249, TRP206, PHE256 and TYR204 along with 2 hydrogen bonding and contact residues SER209 and ASN208 for Bacopasaponin G were obtained (Fig 4 A and a). For Bacopaside III, the contact residues ASN208, LYS210, ARG207, PHE250, TRP206, THR62, MET61, PHE256 and TYR204, and along with 2 hydrogen bonding and contact residues SER209 and SER249 were obtained (Fig 4 B and b). For Bacopaside VI, the contact residues GLU248, TRP206, ASN208, PHE256, TYR204, THR62, and MET61 along with 3 hydrogen bonding and contact residues PHE250, SER209 and SER249 were obtained (Fig 4 C and c). For Silbinin, the contact residues TRP206, ARG64, HIS121 and CYS163 along with 2 hydrogen bonding and contact residues ARG2017 and GLN161 were obtained (Fig 4 D and d). In case of interaction study, the contact residues TRP2016, PHE256, CYS163, ARG207 and PHE252 were obtained without any hydrogen bonding for the synthetic drug Donepezil (Fig 4E and e).





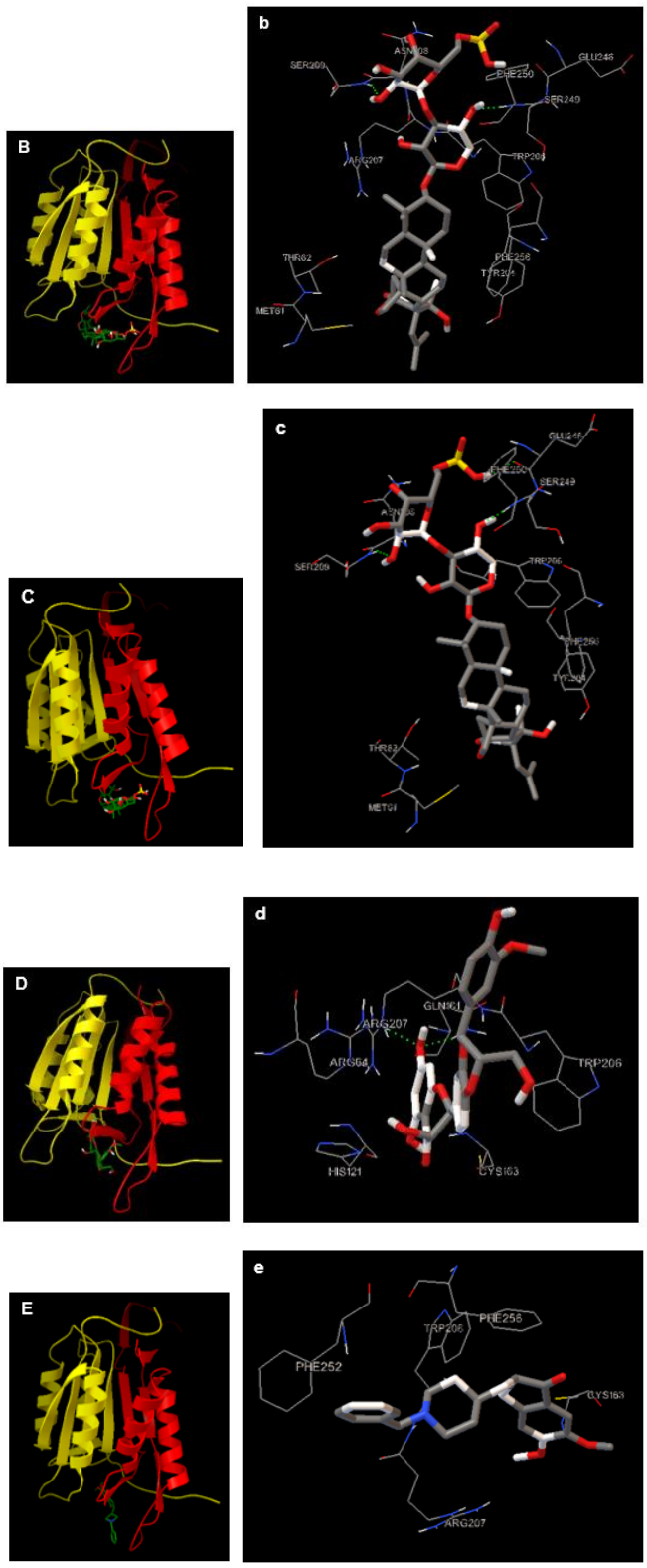


Fig 4: Binding pose and interaction study of favourable energy based phytoligands on CASP3 (A & a = Bacopasaponin G; B & b = Bacopaside III; C & c = Bacopaside VI; D & d = Silbinin and E & e = Donepezil)

In Table 2, the data of favourable binding energy values, four phytoligands such as Bacopasaponin N2 obtained -9.1 Kcal/mol followed by Bacopasaponin G (-8.8 Kcal/mol), Bacopaside X (-8.8 Kcal/mol) and Bacopaside VII (-8.7 Kcal/mol) respectively were obtained on TPK I receptor. Other studied phytoligands showed below energy values compared to above-mentioned four phytoligands. These were showed energy values (Kcal/mol) as Bacopaside II (-8.4), Bacopaside III and VI (-8.3), Bacopaside I, Bacopasaponin F and Silbinin (-8.2), Catechin (-7.9), Bacopaside C (-7.4), Bacopaside B and Galangin (-7.0), Scopoletin (-6.7), Bacopaside A (-6.2) and Meantine (-5.9) respectively on TPK I receptor. But the synthetic medicine such as Denepezil was showed below binding energy value (-7.0 Kcal/mol) compared to phytoligands on TPK I receptor.

Table 3: Binding energy values for phytoligands on TPK I (PDB ID: 1J1B) receptor

Sl. No.	Ligands	Binding energy (Kcal/mol)
1.	Bacopaside N2	-9.1
2.	Bacopasaponin G	-8.8
3.	Bacopaside X	-8.8
4.	Bacopaside VII	-8.7
5.	Bacopaside II	-8.4
6.	Bacopaside III	-8.3
7.	Bacopaside VI	-8.3
8.	Bacopasaponin F	-8.2
9.	Bacopaside I	-8.2
10.	Silbinin	-8.2
11.	Catechin	-7.9
12.	Bacopaside C	-7.4
13.	Galangin	-7.0
14.	Bacopaside B	-7.0
15.	Scopoletin	-6.7
16.	Bacopaside A	-6.2
17.	Meantine	-5.9
1.	Donepezil	-7.0

In case of receptor-ligand binding study on TPK I, the contact residues GLN685, TYR640, GLY563, ASN564, ARG641 and attached to Mg ion and ADP at 961 and 930 position at chain B along with 3 hydrogen bonding and contact residues GLU268, ASP260 and LYS263 for Bacopaside N2 were obtained (Fig 5 A and a). For Bacopasaponin G, the contact residues LYS849, ASP855, ASN861, PHE860, ALA882 and ARG883 along with 1 hydrogen bonding and contact residue THR856 were obtained opposite side of Mg ion and ADP in chain B (Fig 5 B and b). For Bacopaside X, the contact residues LYS349, VAL348, THR356, PRO357, HIS381, PHE360, CYS107 and ASP133 and attached to Mg ion and ADP at 431 and 430 position at chain A along with 5 hydrogen bonding and contact residues ASP355, PRO346, ALA358, LEU359 and LYS197 were obtained (Fig 5 C and c). For Bacopaside VII, the contact residues ARG644, GLN754, TYR640, PRO755, GLU749, TYR721, ASP764, ASP750, SER66 and LYS183 attached to Mg ion and ADP at 431 and 430 position at chain A along with 2 hydrogen bonding and contact residues ASN164 and GLN185 were obtained (Fig 5 D and d). For Donepezil, the contact residues SER555, LEU575, ASP577, SER578, ARG611, GLY547, PRO544 and GLN552 without hydrogen bonding were obtained opposite side of Mg ion and ADP in chain B (Fig 5 E and e).

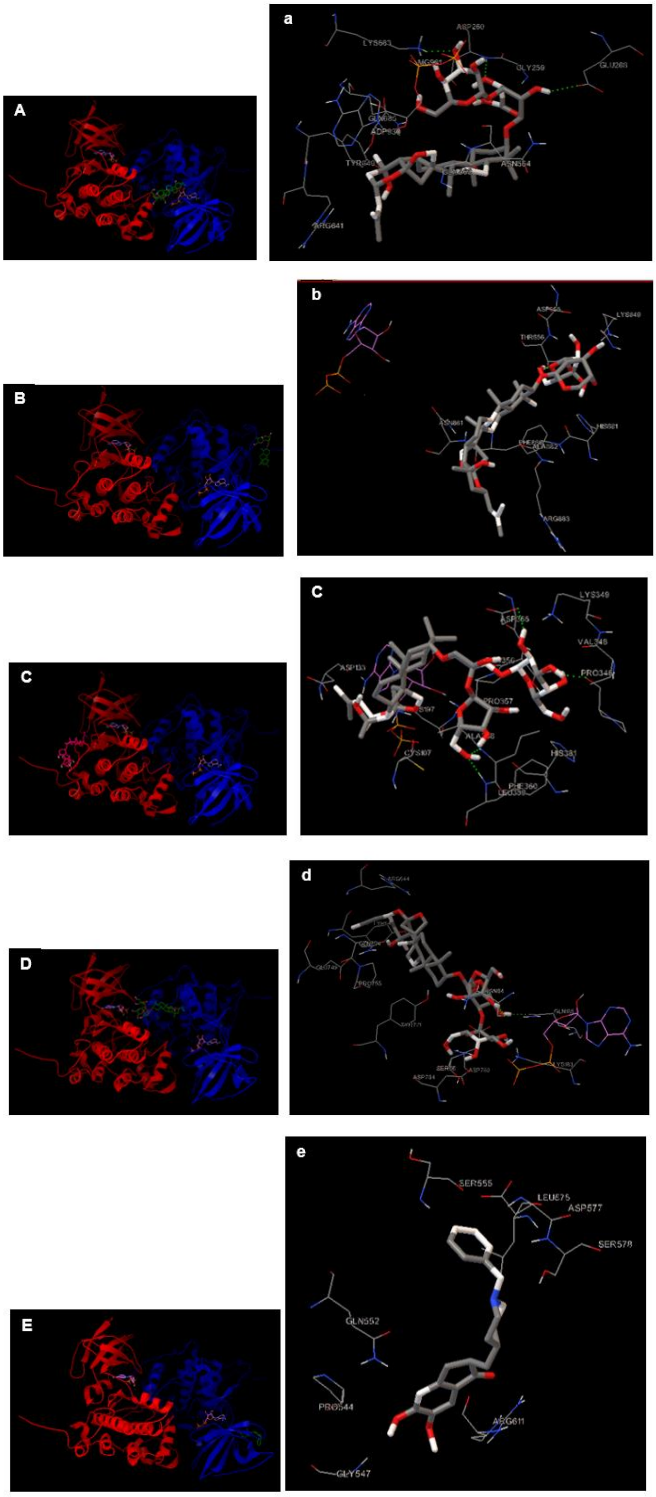


Fig 5: Binding pose and interaction study of favourable energy based phytoligands on TPK I (A & a = Bacopaside N2; B & b = Bacosaponin G; C & c = Bacopaside X; D & d = Bacopaside VII and E & e = Donepezil)

According to Chaudhari *et al.* [23], it was established that Donepezil has potent anticholinesterase activity and protect the cholinergic neurons. On the other hand, Nobili *et al.* [31] reported that cerebral blood flow did not show significant decrease after Donepezil treatment. The present predictive study was done with Donepezil to compare with phytoligands of *B. monnieri* as anti-Alzheimer's activity. Moreover, tau-mediated neurodegeneration is occurred by the increase of tau protein hyperphosphorylation and formed neurofibrillary tangles in the cytoplasm of neurons [4, 23] and CASP 3 protein is formed by Aβ peptide through biochemical mechanism during the development of AD [10].

The present *in silico* study revealed that four phytoligands such as, Bacopasaponin G obtained -9.6 Kcal/mol followed by Bacopaside III and VI (-9.2 Kcal/mol) and Silbinin (-9.1 Kcal/mol) and synthetic ligand (Donepezil, -6.6 Kcal/mol) favourable energy values on CASP 3 receptor. All these phytoligands and synthetic ligand were showed binding pose opposite to the active site in chain B on CASP 3, which may be due to the inhibitory effect. In case of other protein (Tau-protein kinase I), favourable binding energy value (Kcal/mol) were obtained for four phytoligands such as Bacopasaponin N2 (-9.1 Kcal/mol) followed by Bacopasaponin G (-8.8), Bacopaside X (-8.8 Kcal/mol) and Bacopaside VII (-8.7 Kcal/mol) and synthetic ligand (Donepezil -7.0 Kcal/mol) respectively. Among these phytoligands and synthetic ligand, Bacopasaponin N2 was found binding near mouth of the active site of chain B while Donepezil was also found binding in chain B but opposite to the active site on Tau-protein kinase I receptor.

In several experimental studies on mice and human, it was observed that crude extract of *B. monnieri* in different solvents are potential for the anti-amyloidogenic and anti-cholinergic activities [18, 21-23, 32-35]. In the present prediction, the favourable binding energy value of Bacopasaponin G and Bacopasaponin N2 compared to Donepezil on CASP 3 and TPK I receptors. It was observed in other *in silico* study with Astaxanthin derivatives that active site binding with contact residues such as ASN689, ASN686, LYS585P700, ASP700, LYS585, ILE562, ARG96, ARG180, GLU97, ARG96, LYS585, GLN89, GLN795, ASP700 and LYS585 and hydrogen bonding contact residues viz. ASN689, ASN686 and ASP700 of Tau protein kinase I receptor [36] but in present study, the contact residues such as GLN685, TYR640, GLY563, ASN564 and ARG641 and hydrogen bonding contact residues viz. GLU268, ASP260 and LYS263 were obtained on Tau protein near mouth of the active site. According to Joshi *et al.* [1], Tau protein kinase inhibition by phytoligands is suitable for the therapeutic efficacy of AD and in the present molecular docking the receptors were used as TPK I and CASP-3, which is cleaved tau protein during the development of AD.

4. Conclusion

In *in silico* study, the docking binding energy value prediction revealed that the among 17 natural compounds selected from *B. monnieri*, two saponins viz. Bacopasaponin G and Bacopasaponin N2 compared to Donepezil on CASP-3 and TPK I receptors showed favourable binding energy value. Both the phytocompounds obtained inhibitory effects on the studied receptors. These small molecules, Bacopasaponin G and Bacopasaponin N2 individually or combinations may be lead compound(s) for AD therapy. However, it is suggested further *in vitro* and *in vivo* assay for toxicology, pharmacology and inhibition study for anti-Alzheimer's drug candidate to validate the present *in silico* study.

5. Acknowledgement

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Conflict of interest

No conflict of intererest

6. References

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