

## Research Article



## In Silico Approach to Evaluate the Efficacy of Dietary Flavonoids and Their Role in Alzheimer's Disease

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### ABSTRACT

Alzheimer's disease is recognized as fatal neurological disorder that leads to constant degeneration of neurons in later stage of life. Etiology of disease progression is characterized by accumulation of amyloid beta and tau protein that contributes in the formation of senile plaques and tangles. It was also observed that nutritional status of AD patients was low and often not considered in research studies. Here we emphasize on the intake of those dietary items which are good source of natural antioxidants and hence can protect the brain from oxidative and inflammatory damage. We have compared twenty seven secondary metabolites from 5 different classes of Flavonoids found in 506 dietary items (Source: USDA Database for Flavonoid Content of Selected foods, Release 3.1(2013)) along with marketed drugs for Alzheimer's disease to AChE receptor. Our study indicates that Flavan-3-ol family of flavonoids are the best dietary supplements for improving brain health. Theaflavin-3'-gallate is the best binding ligand to AChE receptor. Pharmacophoric studies were conducted to see the potential of respective compounds as drug candidate. Since pharmaceutical industries have always shown interest in compounds from natural sources, a little intervention of these compounds with the synthetic drugs will guarantee lesser side effects with maximum efficacy. Hence the food items like hazelnuts, Green Tea, Black Tea, Pistachio nuts, walnuts, Cocoa mix, Coffee etc. should be incorporated more in the diet of healthy people as well as in AD patients to protect brain from damage and improve the cognitive abilities.

**Keywords:** Alzheimer's disease, dietary flavonoids, natural products, molecular docking, Glide, computational ADME and Pharmacophore.

### INTRODUCTION

Alzheimer's disease (AD) is also called as disease of elderly and mostly affects the older age group (65 years and above). About 5.3 million Americans of all ages are suffering from AD in 2015 and by 2050 the incidences of AD will increase to 14 million and Alzheimer's association of India revealed that around 4 million people of India are suffering from some kind of Dementia<sup>1,2</sup>. AD patho-physiology initiates in the hippocampal region of brain that is involved with memory and learning and later on spreads in the other parts of the brain. AD brain contains low level of acetylcholine due to accumulation of plaques and tangles which effect the synaptic transmission and also starts the inflammatory processes that produce reactive oxygen species<sup>3-5</sup>. Hence along with plaques and tangles, increased oxidative stress is also the established signature of AD pathology<sup>6</sup>. It has been seen that AD patients also suffer from multiple pathology such as depression, hypertension or diabetes which is another reason for their appetite loss. AD patients always have a problem in obtaining healthy and nutritious diet due to the significant barriers produced by the disease like difficulty in chewing, swallowing food and changes in taste patterns<sup>7</sup>. Impaired cognitive abilities makes situation difficult to remember dietary advice. Many of these challenges can only be overcome by a healthy and adequate nutritious diet. With increasing evidences it is proved that nutrition plays an important

role in the progression of Alzheimer's disease<sup>8</sup>. Therefore the role of healthy and balanced diet is very essential for the maintenance of strength and cognitive abilities in AD patients. The increasing epidemiological studies have suggested a diet rich in Vitamin E, folate and Vitamin B12 to reduce the risk and progression of disease<sup>9</sup>.

AChE is an enzyme of tetrameric form bound to neuronal membranes. It is generally found in neuromuscular junctions and brain synapses which hydrolyze acetylcholine into acetate and choline. It is a key component for cholinergic signaling<sup>10</sup>. Studies have shown that in case of Alzheimer's there is continuous breakdown of acetylcholine and its less availability leads to impaired signaling which happens to be a major reason for AD<sup>11</sup>. Many drugs like Rivastigmine, Tacrine, Donepezil etc are AChE inhibitors and are approved by the Food and Drug Administration for the management of AD. These drugs facilitate the hydrolysis of acetylcholine by developing bivalent cations which occupy both the anionic and esteratic binding sites of the enzyme and hence proved to be an effective strategy against AD<sup>12</sup>. But these inhibitors have various side effects such as hepatotoxicity, GI tract disturbance, diarrhea, vomiting, faintness etc. With inadequate therapeutic options for AD, there is a need to find out effective compounds with less or no side effects. U.S Department of Agriculture maintains a National Nutrient Database<sup>13</sup>. It reflects on the major micro/macronutrients and flavonoids present



in our daily dietary food items. Various studies supports the facts that certain flavonoids can protect Alzheimer's disease by disturbing amyloid- $\beta$  peptides assembly and also by reducing tau aggregation.

Therefore to control the pathology of such diseases we should incorporate flavonoids in our daily meals<sup>14,15</sup>.

The study focuses on those secondary metabolites which are present in daily dietary items and possess the potential for replacing these synthetic medicines and work as inhibitors, modulators/agonists and antioxidants with no side effects.

The potential lead molecule obtained from this study can be further evaluated *in vitro* and *in vivo* cell line and animal models.

## MATERIALS AND METHODS

### Ligand and Protein Preparation

Twenty seven dietary flavonoids were identified in 506 food items. The ligands were sketched using 2D-sketcher and later replaced with Maestro workspace. LigPrep2.8 module was used for generating low energy conformations of ligands<sup>16</sup>. Three standard drugs for AChE receptor i.e Rivastigmine, Tacrine and Donepezil were also downloaded and were processed with LigPrep Module, increasing our native dataset of Ligands to 30. Similarly the protein receptor was retrieved from the Protein Data Bank directly in the Protein Preparation Wizard of Maestro9.6 Environment<sup>17,18</sup>. Proteins were analyzed for their missing atoms, unordered side chains and loops. Hydrogen atoms were added and atomic charges were assigned. Extra water molecules were removed and the missing side chains were modeled by the wizard using Prime3.5 program<sup>19</sup>.

The ADME profile of above mentioned metabolites was predicted using QikProp3.8 program<sup>20</sup>. The properties like molecular weight, octanol/water partition coefficient, brain/blood partition coefficient, Caco-2 cell permeability, HERG K<sup>+</sup> channel's IC<sub>50</sub> values, Human oral absorption, drug-likeness (Lipinski's rule of five) and TPSA were calculated.

### Docking

Extra Precision (XP) program of Glide6.1 module was used to dock secondary metabolites with AChE receptor<sup>21</sup>. Ligands were docked flexibly wherein the conformation generation was limited to variation around asymmetric torsion bonds. Only low energy conformations were kept. The best ligand was chosen according to their Docking Score and Glide Score which are calculated using the standard formula followed by Schrodinger.

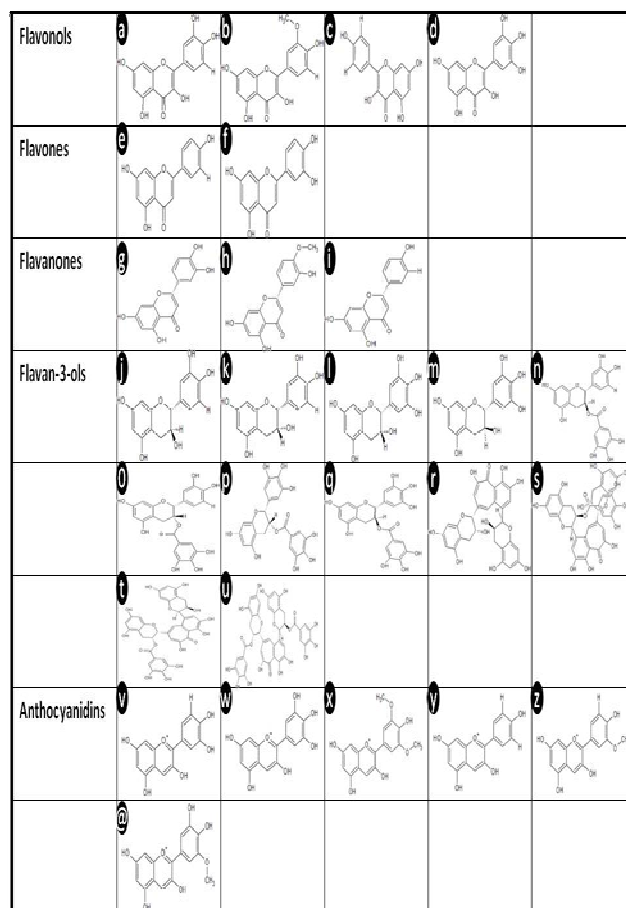
### Pharmacophore Modeling

Three different pharmacophoric hypotheses were generated by using Phase3.8<sup>22</sup> module by using different pharmacophoric features. Later these were screened with AChE focused ZINC Library<sup>23</sup>.

## RESULTS AND DISCUSSION

The ligands were prepared and their different conformations and minimized energies were calculated before docking (Figure 1).

Maximum 4 conformations per ligand were generated. Their ADME and Toxicity predictions were conducted simultaneously (Table 1). Our interest was majorly in compounds crossing Blood Brain Barrier (BBB). Some of the compounds observed to be fit to cross BBB were (+)-Gallocatechin, (-)-Epigallocatechin, (-)-Epicatechin, (+)-Catechin, Naringenin, Hesperetin, Eriodictyol, Quercetin etc.



**Figure 1:** Secondary metabolites used in the current study as reported by USDA Database, Release 3.1 (2013) with their molecular weights (g/mol). **a.** Quercetin (302.236) **b.** Isorhamnetin (316.26) **c.** Kaempferol (286.23) **d.** Myricetin (318.23) **e.** Apigenin (270.23) **f.** Luteolin (286.23) **g.** Eriodictyol (288.25) **h.** Hesperetin (302.27) **i.** Naringenin (272.25) **j.** (+)-Catechin (290.26) **k.** (-)-Epicatechin (290.26) **l.** (-)-Epigallocatechin (458.37) **m.** (+)-Gallocatechin (306.26) **n.** (+)-catechin-3-gallate (442.37) **o.** (-)-Epicatechin-3-gallate (442.37) **p.** (-)-Epigallocatechin-3-gallate (458.37) **q.** (+)-Gallocatechin-3-gallate (458.37) **r.** Theaflavin (564.49) **s.** Theaflavin-3-gallate (716.59) **t.** Theaflavin-3'-gallate (716.60) **u.** Theaflavin-3,3'-digallate (868.70) **v.** Cyanidin (287.24) **w.** Delphinidin (338.70) **x.** Malvidin (331.29) **y.** Pelargonidin (271.24) **z.** Peonidin (301.27) **@** Petunidin (317.27)

**Table 1:** Predicted ADME properties of secondary metabolites by QikProp3.8.

| Flavonoids                     | Molecular Weight (130.0 / 725.0)* | log BB for brain/blood (-3.0 / 1.2) * | Predicted CNS Activity (- to ++) | HERG K+ Channel Blockage: log IC50 (concern below -5) | Caco-2 Permeability (<25 poor, >500 great) | Human Oral Absorption (> 80% is high) | Human Oral Absorption in GI (<25% is poor) |
|--------------------------------|-----------------------------------|---------------------------------------|----------------------------------|---|--|---------------------------------------|--|
| Theaflavin-3,3'-digallate      | 868.714                           | -6.527 (No)                           | --                               | - 5.222   | 0  | Low                                   | 0  |
| Theaflavin-3'-gallate          | 716.608                           | -5.088 (No)                           | --                               | - 5.309   | 0  | Low                                   | 0  |
| Theaflavin-3-gallate           | 716.608                           | -6.513 (No)                           | --                               | - 6.846   | 0  | Low                                   | 0  |
| Theaflavin                     | 564.501                           | -4.288 (No)                           | --                               | - 5.764   | 0  | Low                                   | 0  |
| (+)-Galocatechin-3-gallate     | 458.378                           | -4.282 (No)                           | --                               | - 5.763   | 1  | Low                                   | 1  |
| (-)-Epigallocatechin-3-gallate | 458.378                           | -4.237 (No)                           | --                               | - 5.648   | 1  | Low                                   | 1  |
| (-)-Epicatechin-3-gallate      | 442.378                           | -3.666 (No)                           | --                               | - 5.746   | 3  | Medium                                | 26   |
| (+)-Catechin-3-gallate         | 440.406                           | -3.751 (No)                           | --                               | - 5.889   | 3  | Low                                   | 28   |
| (+)-Galocatechin               | 306.271                           | -2.429 (Yes)                          | --                               | - 4.787   | 19   | Medium                                | 36   |
| (-)-Epigallocatechin           | 306.271                           | -2.380 (Yes)                          | --                               | - 4.688   | 19   | Medium                                | 36   |
| (-)-Epicatechin                | 290.272                           | -1.873 (Yes)                          | --                               | - 4.780   | 55   | Medium                                | 61   |
| (+)-Catechin                   | 290.272                           | -1.914 (Yes)                          | --                               | - 4.883   | 53   | Medium                                | 61   |
| Naringenin                     | 272.257                           | -1.414 (Yes)                          | --                               | - 5.038   | 129  | High                                  | 74   |
| Hesperetin                     | 302.283                           | -1.342 (Yes)                          | --                               | - 5.012   | 194  | High                                  | 79   |
| Eriodictyol                    | 288.256                           | -1.916 (Yes)                          | --                               | - 4.928   | 46   | Medium                                | 63   |
| Luteolin                       | 286.240                           | -1.948 (Yes)                          | --                               | - 5.057   | 42   | High                                  | 62   |
| Apigenin                       | 270.241                           | -1.444 (Yes)                          | --                               | - 5.145   | 116  | High                                  | 74   |
| Myricetin                      | 302.240                           | -2.456 (Yes)                          | --                               | - 4.950   | 15   | Medium                                | 50   |
| Kaempferol                     | 268.268                           | -1.437 (Yes)                          | --                               | - 5.072   | 120  | High                                  | 76   |
| Isorhamnetin                   | 316.267                           | -1.740 (Yes)                          | --                               | - 5.067   | 87   | High                                  | 69   |
| Quercetin                      | 302.240                           | -2.419 (Yes)                          | --                               | - 5.359   | 21   | Medium                                | 54   |
| Petunidin                      | 316.267                           | -1.915 (Yes)                          | --                               | - 4.721   | 50   | High                                  | 61   |
| Peonidin                       | 300.267                           | -1.413 (Yes)                          | --                               | - 4.819   | 140  | High                                  | 73   |
| Pelargonidin                   | 272.257                           | - 1.380 (Yes)                         | --                               | - 5.017   | 151  | High                                  | 73   |

|             |         |                 |    |         |     |        |    |
|-------------|---------|-----------------|----|---------|-----|--------|----|
| Malvidin    | 330.293 | -1.569<br>(Yes) | -- | - 4.789 | 128 | High   | 72 |
| Delphinidin | 302.240 | -2.412<br>(Yes) | -- | - 4.803 | 15  | Medium | 47 |
| Cyanidin    | 286.240 | -1.903<br>(Yes) | -- | - 4.903 | 42  | Medium | 58 |

Ligands were kept flexible and receptor was rigid during docking. All the stereoisomers of flavonoids and drugs were allowed to dock to AChE. The best scoring ligand after docking was Theaflavin-3'-gallate and its two different poses generated best energies (Table 2). The top 15 candidates were from Flavan-3-ol family.

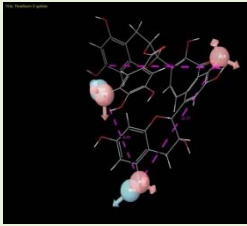
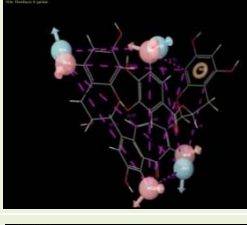

**Table 2:** Docking Energies of 159 different poses of secondary metabolites with AChE Receptor (PDB ID: 1EVE).

| Title                                | Score  | G Score | H Bond | vdW   | Coul  | E model | CvdW  |
|--------------------------------------|--------|---------|--------|-------|-------|---------|-------|
| Theaflavin-3'-gallate                | -14.69 | -14.71  | -5.9   | -60.1 | -15.4 | -126.8  | -75.5 |
| Theaflavin-3'-gallate                | -14.25 | -14.28  | -5.9   | -59.5 | -27.0 | -101.2  | -86.5 |
| Theaflavin-3-gallate                 | -14.00 | -14.02  | -4.3   | -52.9 | -16.1 | -75.5   | -69.0 |
| (+)-Catechin-3-gallate               | -13.87 | -13.89  | -4.1   | -39.1 | -24.0 | -87.8   | -63.1 |
| (-)-Epigallocatechin-3-gallate       | -13.66 | -13.68  | -4.5   | -45.8 | -20.6 | -85.2   | -66.4 |
| (+)-Galocatechin-3-gallate           | -13.63 | -13.65  | -4.5   | -45.7 | -20.3 | -96.4   | -66.0 |
| Theaflavin-3,3'-gallate              | -13.53 | -13.57  | -5.8   | -37.9 | -20.3 | -101.9  | -58.2 |
| Theaflavin-3,3'-gallate              | -13.48 | -13.52  | -5.3   | -71.1 | -15.9 | -90.1   | -87.0 |
| (-)-Epigallocatechin-3-gallate       | -13.47 | -13.49  | -4.2   | -59.2 | -9.1  | -106.8  | -68.3 |
| (+)-Galocatechin-3-gallate           | -13.24 | -13.26  | -4.3   | -53.1 | -13.5 | -107.0  | -66.7 |
| Theaflavin-3-gallate                 | -13.22 | -15.67  | -5.6   | -52.6 | -15.6 | -96.5   | -68.2 |
| Theaflavin-3'-gallate                | -13.12 | -13.14  | -4.9   | -55.8 | -14.6 | -121.7  | -70.3 |
| (-)-Epicatechin-3-gallate            | -13.05 | -13.06  | -4.0   | -54.0 | -11.5 | -105.0  | -65.5 |
| (+)-Catechin-3-gallate               | -13.01 | -13.03  | -3.9   | -54.0 | -11.3 | -100.3  | -65.4 |
| Theaflavin-3,3'-gallate              | -12.91 | -12.95  | -7.0   | -44.4 | -24.0 | -89.2   | -68.5 |
| Donepezil                            | -12.70 | -12.70  | -0.7   | -39.3 | -8.3  | -70.0   | -47.6 |
| Theaflavin                           | -12.49 | -12.49  | -5.1   | -36.8 | -21.2 | -93.3   | -58.1 |
| (+)-Galocatechin-3-gallate           | -12.36 | -12.37  | -3.7   | -47.4 | -14.2 | -95.8   | -61.5 |
| Theaflavin                           | -12.27 | -12.27  | -4.2   | -42.6 | -19.3 | -92.2   | -61.9 |
| (-)-Epicatechin-3-gallate            | -12.26 | -12.28  | -3.4   | -48.6 | -15.4 | -82.5   | -63.9 |
| Theaflavin-3,3'-gallate              | -12.09 | -14.39  | -4.9   | -35.9 | -18.1 | -89.1   | -54.0 |
| Theaflavin-3-gallate                 | -12.04 | -12.07  | -4.5   | -51.9 | -21.7 | -93.4   | -73.6 |
| (+)-Catechin-3-gallate               | -11.98 | -12.00  | -3.2   | -48.1 | -13.9 | -88.5   | -62.1 |
| Theaflavin-3-gallate                 | -11.89 | -11.92  | -4.4   | -43.4 | -25.8 | -91.3   | -69.2 |
| Theaflavin                           | -11.67 | -11.67  | -3.8   | -42.2 | -12.0 | -72.6   | -54.2 |
| Hesperitin                           | -11.53 | -11.53  | -2.4   | -19.4 | -11.6 | -52.0   | -30.9 |
| Theaflavin                           | -11.53 | -11.53  | -4.2   | -43.3 | -18.6 | -83.9   | -61.9 |
| Donepezil                            | -11.50 | -11.50  | -0.7   | -42.8 | -6.1  | -76.5   | -48.9 |
| (-)-Epigallocatechin                 | -11.34 | -11.35  | -3.0   | -27.7 | -15.1 | -45.2   | -42.8 |
| (-)-Epigallocatechin-3-gallate       | -11.31 | -11.33  | -2.8   | -43.3 | -17.3 | -94.5   | -60.7 |
| Theaflavin-3-gallate                 | -11.29 | -13.56  | -4.6   | -27.1 | -23.5 | -87.1   | -50.1 |
| (-)-Epigallocatechin                 | -11.27 | -11.28  | -3.0   | -34.6 | -11.6 | -67.9   | -46.2 |
| Delphinidin                          | -11.26 | -11.28  | -2.2   | -31.0 | -16.9 | -64.1   | -47.9 |
| (+)-Catechin                         | -11.23 | -11.23  | -2.5   | -27.9 | -12.1 | -58.8   | -40.0 |
| Apigenin                             | -11.20 | -11.22  | -1.9   | -18.3 | -13.1 | -52.3   | -31.3 |
| Theaflavin-3,3'-gallate-3,3'-gallate | -11.19 | -11.23  | -5.8   | -49.0 | -20.2 | -73.6   | -69.2 |
| (+)-Catechin-3-gallate               | -11.16 | -11.18  | -3.7   | -35.4 | -8.7  | -77.6   | -44.1 |
| (-)-Epicatechin-3-gallate            | -11.15 | -11.17  | -3.0   | -51.9 | -9.4  | -85.2   | -61.3 |
| (-)-Epicatechin-3-gallate            | -11.14 | -11.16  | -4.0   | -49.2 | -13.2 | -89.6   | -62.4 |
| (+)-Galocatechin-3-gallate           | -11.08 | -11.10  | -3.5   | -53.4 | -10.3 | -94.6   | -63.7 |
| (+)-Galocatechin                     | -11.08 | -11.08  | -2.8   | -33.7 | -9.1  | -59.6   | -42.8 |
| Theaflavin-3,3'-gallate              | -11.08 | -13.55  | -6.1   | -49.1 | -22.8 | -86.5   | -71.9 |
| Theaflavin-3'-gallate                | -11.07 | -11.09  | -5.2   | -58.5 | -20.7 | -109.2  | -79.3 |
| (-)-Epicatechin                      | -11.05 | -11.05  | -2.5   | -32.8 | -9.0  | -48.3   | -41.8 |
| Theaflavin-3,3'-gallate              | -10.98 | -13.27  | -6.1   | -43.3 | -18.1 | -89.5   | -61.4 |
| (+)-Catechin                         | -10.91 | -10.92  | -2.3   | -33.1 | -8.9  | -50.0   | -42.0 |

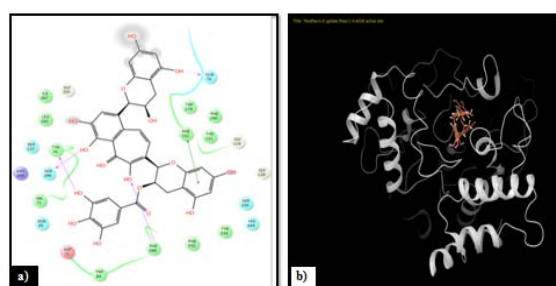
|                                |        |        |      |       |       |        |       |
|--------------------------------|--------|--------|------|-------|-------|--------|-------|
| Petunidin                      | -10.90 | -10.93 | -1.9 | -35.1 | -10.0 | -56.4  | -45.1 |
| Theaflavin-3'-gallate          | -10.85 | -13.19 | -4.9 | -47.2 | -17.9 | -105.9 | -65.1 |
| (-)-Epigallocatechin           | -10.85 | -10.85 | -3.3 | -35.4 | -10.8 | -61.3  | -46.2 |
| Eriodictyol                    | -10.82 | -10.82 | -3.0 | -34.2 | -5.6  | -51.9  | -39.8 |
| Myricetin                      | -10.66 | 10.67  | -3.8 | -38.1 | -11.6 | -73.8  | -49.7 |
| Hesperitin                     | -10.60 | -10.60 | -2.0 | -34.4 | -6.7  | -53.9  | -41.1 |
| (+)-Galocatechin-3-gallate     | -10.60 | -13.06 | -4.1 | -42.8 | -19.9 | -85.5  | -62.7 |
| Cyanidin                       | -10.60 | -10.62 | -1.6 | -34.2 | -14.8 | -67.6  | -49.0 |
| (+)-Galocatechin-3-gallate     | -10.57 | -13.15 | -4.3 | -45.0 | -18.2 | -79.8  | -63.2 |
| (-)-Epigallocatechin-3-gallate | -10.56 | -13.03 | -4.1 | -44.4 | -18.9 | -79.2  | -63.2 |
| (+)-Galocatechin               | -10.51 | -10.51 | -4.0 | -36.3 | -11.8 | -63.3  | -48.1 |
| Galantamine                    | -10.49 | -10.49 | -1.3 | -25.0 | -10.0 | -45.6  | -35.0 |
| (+)-Galocatechin-3-gallate     | -10.43 | -13.02 | -3.8 | -53.0 | -11.1 | -102.8 | -64.1 |
| (+)-Galocatechin               | -10.41 | -10.41 | -2.8 | -36.0 | -11.4 | -69.3  | -47.4 |
| Donepezil                      | -10.37 | -10.37 | -1.5 | -35.7 | -9.6  | -60.5  | -45.3 |
| Isorhamnetin                   | -10.36 | -10.36 | -2.2 | -38.8 | -5.2  | -55.5  | -44.0 |
| (-)-Epicatechin                | -10.34 | -10.34 | -2.7 | -34.4 | -10.0 | -64.6  | -44.4 |
| Theaflavin-3-gallate           | -10.32 | -10.34 | -4.1 | -43.0 | -23.0 | -74.6  | -66.0 |
| (-)-Epigallocatechin-3-gallate | -10.30 | -12.89 | -4.1 | -42.6 | -18.5 | -67.1  | -61.1 |
| (-)-Epigallocatechin-3-gallate | -10.30 | -12.76 | -3.9 | -53.1 | -12.1 | -103.1 | -65.2 |
| Luteolin                       | -10.19 | -10.20 | -3.4 | -33.3 | -14.0 | -53.1  | -47.3 |
| Theaflavin-3-gallate           | -10.11 | -12.39 | -5.7 | -45.7 | -20.5 | -102.2 | -66.3 |
| Theaflavin-3,3'-gallate        | -10.08 | -12.55 | -5.1 | -52.1 | -21.8 | -92.7  | -73.9 |
| Pelargonidin                   | -10.01 | -10.03 | -2.2 | -28.1 | -17.1 | -44.0  | -45.2 |
| Peonidin                       | -10.01 | -10.03 | -2.0 | -35.7 | -11.0 | -36.3  | -46.8 |
| (-)-Epicatechin                | -9.98  | -9.98  | -3.6 | -34.1 | -10.9 | -67.7  | -45.0 |
| (-)-Epigallocatechin           | -9.94  | -9.94  | -2.8 | -31.5 | -7.1  | -42.8  | -38.6 |
| Theaflavin-3-gallate           | -9.90  | -12.35 | -3.4 | -45.6 | -14.0 | -69.1  | -59.6 |
| Galantamine                    | -9.84  | -9.84  | -0.7 | -33.2 | -4.1  | -52.0  | -37.3 |
| (-)-Epicatechin-3-gallate      | -9.82  | -12.29 | -3.4 | -41.8 | -15.9 | -83.3  | -57.7 |
| (-)-Epicatechin-3-gallate      | -9.81  | -12.28 | -3.3 | -50.6 | -8.9  | -97.0  | -59.5 |
| (+)-Galocatechin               | -9.77  | -9.78  | -2.3 | -33.6 | -12.8 | -64.6  | -46.4 |
| (+)-Catechin-3-gallate         | -9.64  | -11.97 | -3.3 | -53.3 | -8.3  | -99.0  | -61.7 |
| Quercetin                      | -9.62  | -9.63  | -2.2 | -36.2 | -12.8 | -56.7  | -49.0 |
| (-)-Epigallocatechin-3-gallate | -9.60  | -12.19 | -3.6 | -53.2 | -8.9  | -101.1 | -62.1 |
| (-)-Epigallocatechin-3-gallate | -9.55  | -12.14 | -4.3 | -39.4 | -16.6 | -63.1  | -56.0 |
| Eriodictyol                    | -9.45  | -9.45  | -2.9 | -34.2 | -11.1 | -52.2  | -45.3 |
| Theaflavin-3,3'-gallate        | -9.42  | -11.71 | -4.9 | -35.6 | -29.7 | -86.4  | -65.3 |
| (-)-Epicatechin                | -9.42  | -9.42  | -1.8 | -35.6 | -5.2  | -57.7  | -40.8 |
| Galantamine                    | -9.37  | -9.37  | -1.4 | -25.3 | -9.0  | -44.6  | -34.4 |
| Malvidin                       | -9.30  | -9.32  | -1.3 | -34.9 | -9.1  | -63.0  | -44.1 |
| Donepezil                      | -9.28  | -9.28  | -0.6 | -38.6 | -3.0  | -67.8  | -41.5 |
| Kaempferol                     | -9.23  | -9.28  | -2.3 | -35.1 | -9.6  | -65.1  | -44.7 |
| (+)-Catechin                   | -9.16  | -9.16  | -2.1 | -30.8 | -8.8  | -54.3  | -39.6 |
| (+)-Catechin                   | -9.14  | -9.14  | -1.6 | -34.6 | -13.4 | -68.6  | -48.0 |
| Naringenin                     | -9.14  | -9.14  | -2.0 | -30.9 | -13.8 | -57.4  | -44.7 |
| Theaflavin-3,3'-gallate        | -9.11  | -11.60 | -6.1 | -38.2 | -23.0 | -69.5  | -61.3 |
| (+)-Catechin-3-gallate         | -9.10  | -11.43 | -2.9 | -43.6 | -11.9 | -88.1  | -55.4 |
| (+)-Galocatechin-3-gallate     | -9.01  | -11.60 | -3.2 | -48.4 | -7.6  | -81.4  | -56.0 |
| Rivastigmine                   | -8.95  | -9.03  | -0.9 | -35.0 | -6.2  | -57.7  | -41.2 |
| Naringenin                     | -8.94  | -8.94  | -2.3 | -32.4 | -11.5 | -57.0  | -43.9 |
| (+)-Catechin-3-gallate         | -8.94  | -11.27 | -3.3 | -47.8 | -9.4  | -66.5  | -57.2 |
| Theaflavin-3,3'-gallate        | -8.90  | -11.39 | -6.4 | -45.0 | -29.1 | -99.6  | -74.1 |
| (-)-Epigallocatechin-3-gallate | -8.89  | -11.35 | -3.1 | -44.4 | -10.5 | -92.0  | -54.9 |
| Theaflavin-3,3'-gallate        | -8.59  | -11.06 | -4.7 | -48.8 | -21.7 | -78.0  | -70.5 |
| (-)-Epicatechin-3-gallate      | -8.58  | -11.17 | -2.8 | -49.4 | -12.1 | -98.1  | -61.4 |
| Rivastigmine                   | -8.58  | -8.66  | -1.5 | -22.6 | -10.2 | -30.9  | -32.7 |
| (-)-Epigallocatechin-3-gallate | -8.58  | -11.17 | -2.8 | -43.0 | -15.1 | -82.0  | -58.1 |

|                                |       |        |      |       |       |        |       |
|--------------------------------|-------|--------|------|-------|-------|--------|-------|
| Theaflavin-3,3'-gallate        | -8.44 | -11.10 | -4.9 | -45.5 | -25.7 | -72.6  | -71.2 |
| (+)-Galocatechin-3-gallate     | -8.43 | -10.89 | -3.1 | -55.9 | -5.9  | -95.8  | -61.8 |
| (-)-Epicatechin-3-gallate      | -8.40 | -10.99 | -2.7 | -48.8 | -7.8  | -85.3  | -56.6 |
| Theaflavin-3'-gallate          | -8.35 | -10.69 | -5.0 | -50.7 | -11.6 | -99.0  | -62.3 |
| (-)-Epicatechin-3-gallate      | -8.34 | -10.81 | -3.5 | -47.4 | -8.5  | -91.9  | -55.9 |
| (+)-Galocatechin-3-gallate     | -8.32 | -10.79 | -2.7 | -45.5 | -7.7  | -73.6  | -53.1 |
| (+)-Catechin-3-gallate         | -8.27 | -10.78 | -2.3 | -49.6 | -6.3  | -83.1  | -55.9 |
| Theaflavin-3-gallate           | -8.26 | -10.53 | -3.8 | -54.7 | -14.0 | -84.9  | -68.8 |
| Theaflavin-3,3'-gallate        | -8.23 | -10.70 | -4.8 | -38.6 | -22.1 | -79.4  | -60.7 |
| (+)-Catechin-3-gallate         | -8.22 | -10.72 | -3.1 | -50.8 | -8.3  | -88.3  | -59.1 |
| Delphinidin                    | -8.20 | -10.44 | -1.9 | -34.3 | -6.2  | -54.3  | -40.4 |
| Petunidin                      | -8.18 | -10.22 | -1.4 | -34.0 | -3.5  | -54.1  | -37.5 |
| (+)-Catechin-3-gallate         | -8.07 | -10.57 | -2.9 | -35.3 | -9.3  | -69.8  | -44.6 |
| Theaflavin-3-gallate           | -8.01 | -10.29 | -3.3 | -42.3 | -8.0  | -77.5  | -50.3 |
| Theaflavin-3-gallate           | -7.79 | -10.24 | -4.3 | -52.4 | -7.8  | -72.9  | -60.2 |
| Myricetin                      | -7.71 | -10.32 | -3.6 | -35.2 | -9.8  | -46.7  | -45.0 |
| Tacrine                        | -7.64 | -7.64  | -0.3 | -24.6 | -3.2  | -41.3  | -27.8 |
| (+)-Catechin-3-gallate         | -7.52 | -10.03 | -2.0 | -50.5 | -5.9  | -61.6  | -56.4 |
| Theaflavin-3'-gallate          | -7.46 | -9.91  | -4.3 | -58.8 | -16.6 | -93.7  | -75.4 |
| (+)-Galocatechin-3-gallate     | -7.35 | -9.94  | -2.5 | -46.9 | -5.7  | -75.5  | -52.6 |
| (-)-Epicatechin-3-gallate      | -7.27 | -9.86  | -3.4 | -34.6 | -11.3 | -75.9  | -45.9 |
| Theaflavin-3,3'-gallate        | -7.19 | -9.68  | -5.0 | -56.9 | -17.6 | -85.7  | -74.6 |
| Theaflavin-3'-gallate          | -7.19 | -9.64  | -4.7 | -58.9 | -18.2 | -123.7 | -77.1 |
| Petunidin                      | -7.06 | -9.49  | -1.9 | -33.8 | -8.9  | -55.0  | -42.7 |
| (-)-Epigallocatechin-3-gallate | -7.04 | -7.06  | -4.4 | -35.0 | -12.9 | -82.4  | -47.8 |
| Theaflavin-3,3'-gallate        | -6.94 | -9.23  | -4.4 | -63.4 | -22.1 | -109.2 | -85.4 |
| Kaempferol                     | -6.91 | -8.40  | -2.2 | -28.0 | -9.8  | -54.1  | -37.8 |
| (-)-Epicatechin-3-gallate      | -6.84 | -9.43  | -1.7 | -42.7 | -12.9 | -73.9  | -55.7 |
| Theaflavin-3'-gallate          | -6.80 | -9.25  | -3.7 | -61.3 | -11.3 | -114.4 | -72.6 |
| Malvidin                       | -6.71 | -8.95  | -1.2 | -30.5 | -8.3  | -50.6  | -38.8 |
| Cyanidin                       | -6.66 | -9.32  | -1.6 | -33.0 | -13.6 | -64.6  | -46.6 |
| Malvidin                       | -6.54 | -9.20  | -1.1 | -33.9 | -5.4  | -57.4  | -39.3 |
| Theaflavin-3,3'-gallate        | -6.47 | -8.95  | -3.6 | -36.9 | -20.6 | -70.4  | -57.5 |
| Theaflavin-3-gallate           | -6.33 | -8.78  | -1.9 | -44.0 | -16.4 | -77.6  | -60.4 |
| Theaflavin-3'-gallate          | -6.20 | -8.54  | -3.7 | -56.3 | -5.7  | -94.2  | -62.0 |
| Theaflavin-3,3'-gallate        | -6.13 | -8.79  | -5.8 | -41.3 | -26.8 | -82.0  | -68.1 |
| Delphinidin                    | -6.02 | -8.68  | -1.7 | -32.3 | -8.9  | -61.5  | -41.1 |
| Luteolin                       | -5.94 | -8.54  | -1.7 | -25.6 | -1.7  | -46.0  | -27.3 |
| Theaflavin-3,3'-gallate        | -5.92 | -8.58  | -5.2 | -43.2 | -24.2 | -68.1  | -67.4 |
| Cyanidin                       | -5.87 | -8.11  | -1.5 | -30.5 | -3.5  | -37.9  | -34.0 |
| Theaflavin-3'-gallate          | -5.84 | -8.18  | -3.4 | -55.4 | -9.2  | -107.2 | -64.7 |
| (+)-Galocatechin-3-gallate     | -5.47 | -7.93  | -3.1 | -52.4 | -8.9  | -89.6  | -61.3 |
| (-)-Epicatechin-3-gallate      | -5.27 | -7.74  | -2.6 | -51.7 | -7.9  | -77.0  | -59.5 |
| Theaflavin-3'-gallate          | -5.24 | -7.69  | -3.1 | -62.0 | -7.8  | -110.1 | -69.8 |
| Rivastigmine                   | -4.99 | -6.25  | -0.4 | -32.9 | -4.3  | -51.0  | -37.1 |
| (+)-Catechin-3-gallate         | -4.98 | -7.31  | -2.6 | -49.5 | -5.6  | -72.5  | -55.2 |
| Rivastigmine                   | -4.97 | -6.24  | -0.4 | -32.1 | -3.3  | -36.0  | -35.4 |
| Pelargonidin                   | -4.97 | -7.63  | -0.3 | -29.5 | -7.6  | -53.5  | -37.1 |
| Peonidin                       | -4.82 | -7.48  | -1.0 | -31.0 | -10.2 | -60.0  | -41.2 |
| Galantamine                    | -4.78 | -4.78  | -0.4 | -32.3 | -6.9  | -52.3  | -39.2 |
| Theaflavin-3,3'-gallate        | -4.77 | -7.43  | -6.2 | -48.7 | -18.8 | -79.4  | -67.5 |
| Peonidin                       | -4.70 | -6.94  | -1.8 | -32.1 | -8.4  | -54.5  | -40.4 |
| Apigenin                       | -4.66 | -7.27  | -0.5 | -31.1 | -3.9  | -50.5  | -35.0 |
| Pelargonidin                   | -2.73 | -4.97  | -0.9 | -28.3 | -2.6  | -41.8  | -30.8 |
| (-)-Epigallocatechin-3-gallate | -0.86 | -3.32  | -3.7 | -32.3 | -14.5 | -80.4  | -46.8 |

**Table 3:** Pharmacophoric hypothesis with their screening results (Phase 3.7).

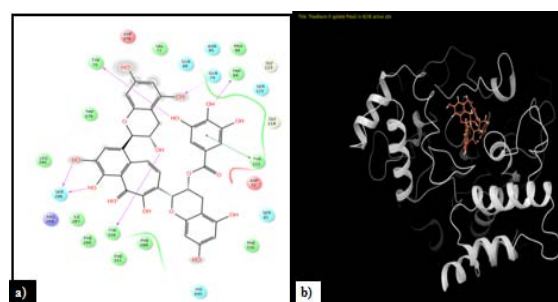
| S.No | Pharmacophoric Hypothesis                        | Image   | Hits in AChE focused Zinc Library (min features/total features)  | Marketed Drugs (min features/total features)   |
|------|--|---|--|--|
| 1    | AAADD (Common Pharmacophores of Pose1 and Pose2) |  | 5/5 - No hits<br>4/5 - 2 hits<br>3/5 - 12 hits   | 5/5 - No hits<br>4/5 - No hits<br>3/5 - 4 hits   |
| 2    | AAAADDDR (Pharmacophore of Pose1)                |  | 8/8 - No hits<br>7/8 - No hits<br>6/5 - No hits<br>5/8 - 7 hits<br>4/8 - 7 hits  | 8/8 - No hits<br>7/8 - No hits<br>6/5 - No hits<br>5/8 - No hits<br>4/8 - No hits                                      |
| 3    | AAAAAADDDDR (Pharmacophore of Pose2)             |  | 12/12 - Nohits<br>11/12 - Nohits<br>10/12 - Nohits<br>9/12 - Nohits<br>8/12 - Nohits<br>7/12 - Nohits<br>6/12 - 4 hits | 12/12 - Nohits<br>11/12 - Nohits<br>10/12 - Nohits<br>9/12 - Nohits<br>8/12 - Nohits<br>7/12 - Nohits<br>6/12 - Nohits |

Top 2 poses were retained for post docking analysis. Ligand receptor interactions and binding patterns were studied. Residues involved in Ligand-Receptor interaction of Pose1 of Theaflavin-3'-gallate and AChE were observed. Gln74 Ser286 Tyr70 and Phe288 side chains made hydrogen bonds with Theaflavin-3'-gallate. Phe331 was involved in pi-pi interaction with the ring (Pharmacophoric feature R31) (Figure 2a).



**Figure 2:** (a) Ligand Receptor Interaction diagram of Pose 1 of Theaflavin-3'-gallate and AChE (b) Docking of Pose1 with AChE.

Similarly in Pose 2 Theaflavin-3'-gallate made H-bonds with backbone of residues Tyr70, Trp84, Tyr334 (Figure 3a). Side chains of residue Gln74 were involved in H-bonds. Tyr121 had pi-pi interaction with ring (Pharmacophoric feature R32). The energy of docking was satisfactorily lower and the bonding was observed to be stable.



**Figure 3:** (a) Ligand Receptor Interaction diagram of Pose 2 of Theaflavin-3'-gallate and AChE (b) Docking of Pose2 with AChE.

The best two poses were used to deduce the chemical groups responsible for bonding Theaflavin-3'-gallate with AChE. This information was used to build pharmacophores. Three different pharmacophores were developed and screening was done with AChE focused ZINC Libraries. Pharmacophoric hypothesis AAADD gave good results when the 60% of pharmacophoric features were retained for screening (Table 3).

The Scientists and Pharmaceutical Companies in the field of drug discovery and development have always appreciated the importance of plant secondary metabolite based medicines. More than half of the new drugs approved by FDA are derived directly or modified from the natural sources. The use of natural products as drug resources will enhance in future as the safety of synthetic drugs has become a distressing issue for almost all the Pharmaceutical Companies. Flavonoids exert array of neuroprotective actions in the brain that includes the

suppression of inflammation, protection of neuronal cells from toxins and enhance the memory and cognitive functions. Flavonoids inhibit the apoptotic process and promotes synaptic plasticity by interacting with significant protein and lipid kinase signaling pathways. The significant properties of flavonoids to work as an important precursor molecule generate the interest to develop drugs for neurodegenerative diseases<sup>24</sup>.

Theaflavin-3'-gallate was observed to be the best binding partner out of all the 27 dietary metabolites and 3 marketed drugs against AChE. According to Jankun, Theaflavin-3'-gallate is a potent inhibitor of PA11 receptor and hence a good compound for treating multiple pathologies like cancer, obesity, diabetes and Alzheimer's disease<sup>25</sup>. Our study also proposes the eligibility of Theaflavin-3'-gallate as potential inhibitor for AChE.

Theaflavin-3'-gallate is found mostly in blueberry, blackcurrant, raspberries etc. Intervention of Green Tea, Black Tea and blackcurrant are encouraged to incorporate in daily diet to improve cognitive abilities<sup>26</sup>. Till now many researches confirmed about the restoration and enhancement of dementia cases but the exact mechanism was still unclear.

Dietary modifications participate in well-being of an individual.

Since synthetic medicines are posing a larger threat in our society for long term illnesses, it will always be wise to choose plant products instead of synthetic ones.

Combination therapies of synthetic and herbal compounds will open a new dimension in research in pharmaceutical sciences and will promise medications with lesser side effects.

## CONCLUSION

The present study predicts that out of the all classes of flavonoids, food items containing Flavan-3-ol family have the potential to improve brain health by reducing amyloid beta aggregates.

Also Flavan-3-ol family candidates exhibit better binding energies than marketed drugs for AD. Our ADME studies indicates that most of the Flavan-3-ol members can cross Blood Brain Barrier but due to high molecular weight Theaflavin-3'-gallate cannot cross BBB.

This can be improvised by using advanced drug delivery methods.

Further preclinical and clinical trials need to be carried out to support the utilization of Theaflavin-3'-gallate for Alzheimer's disease.

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