

In Silico Investigation of Anti-Anxiety and Anti-Depression Effect of *Salvia Hispanica*

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Abstract

Background: Anxiety and depression are major concerns in this generation that need to be addressed immediately. Chia seeds are an invaluable source of nutrition that has several benefits associated with its intake. This study has been conducted to explore the anti-anxiety and anti-depression effect of *Salvia hispanica*.

Methodology: Computational methods such as molecular docking and molecular dynamics simulations were run using Schrodinger's Glide and Desmond to test the stability of the interactions of the constituents of chia seeds with the protein targets responsible for the mentioned mental disorders.

Results: The studies revealed that the protein-ligand complexes made several stable interactions between them.

Conclusion: In this regard, the work paves the way for more investigations to ascertain the mechanism of interaction between the constituents of chia seeds with the proteins in the human body responsible for anti-depression and anti-anxiety effects.

Keywords: chia, molecular dynamics simulations, anti-depressant, anti-anxiety, therapeutic

Introduction

Over 264 million individuals worldwide currently suffer from Major Depressive Disorder (MDD), which has now become a leading cause of disability. It is linked to almost 200 identified genes and peaks in incidence in the mid-20s. MDD has a wide range of symptoms and possible etiologies, making it difficult to study and treat. The availability of MDD treatments is hampered by societal stigma, inaccurate diagnosis, and income level. MDD and sleep disturbances are related as well. While selective serotonin reuptake inhibitors (SSRIs), a common medical medication, can elevate mood, they can also have serious negative effects. The efficacy of these adverse effects may be limited as a result of decreased patient adherence to prescriptions. When pharmaceutical treatments don't address the right mechanisms underlying a patient's depression, they can have unfavourable side effects and offer little help. In addition, more than one-third of MDD patients may have treatment-resistant depression (TRD), which is unresponsive to both medication and psychological counselling (1). Furthermore, children and teenagers with anxiety suffer from a serious mental illness that disrupts their emotional states. It is a condition of the central nervous system associated with abnormalities in neurotransmitters, with serotonin, dopamine, norepinephrine and gamma-aminobutyric acid (GABA) being important in regulating mood. Serotonin, glutamate, and glycine are inhibitory neurotransmitters, but GABA eases anxiety and stress and aids in neuronal recovery following impulse transmission. Drugs that change neuronal chemistry, such as TCA, MAOIs, and SSRIs, are used in pharmacotherapy for anxiety disorders (2).

Herbal or natural compounds have been extremely popular in study in recent years. Chia, or *Salvia hispanica* L., is an annual herbaceous plant that is majorly planted for its seeds. The seed might be black, grey, black speckled, or white in color. There is very little variance between white and black Chia seeds that most people think they are interchangeable (3). Because of their nutritional qualities and therapeutic benefits, chia seeds have gained popularity as one of the most recognized foods in the world (4–8). Chia, according to Coorey et al.(9), is a great component because it's easy to add to commercial cuisine and has the greatest known level of α -linolenic acid. Numerous research have indicated that chia seeds may be essential for health, antioxidant activity, and antibacterial activity because of their high fatty acid content(4,6,10–12). Moreover, "chian" a word of Spanish origin which means oily, is where the name "chia" originates. Chia is nothing but an oilseed that contains proteins, carbohydrates, dietary fibre, fats, vitamins (A, B, K, E, and D), antioxidants and minerals, according to a variety of publications (8,13,14). As

a dietary supplement, chia seeds offer a plethora of benefits, such as strengthening bones and muscles, supporting the digestive system, encouraging good skin, lowering the risk of diabetes and heart disease, and more (4,5,7,15). The seed is free of mycotoxins and gluten, and it has a high concentration of polyphenolic antioxidants (4). High percentages of lipids (30–33%), carbs (26–41%), proteins (15–25%), dietary fiber (18–30%), vitamins, minerals, and antioxidants (wet basis) in chia seeds. Numerous studies on phytochemicals have been published, emphasizing that polyunsaturated fatty acids (PUFAs:

α -linolenic (ALA, ω -3 fatty acid) and linoleic (LA, ω -6 fatty acid) acids) are the main constituents of chia oil (8). 39% of the dry seed mass of chia seeds is made up of oil, which is made up of up to 68% ω -3 and 19% ω -6 fatty acids (5,13). The fatty acid ratio of ω -3 to ω -6 is 0.3:0.35. Omega-3 fatty acid provides several health benefits for humans, including lowering cholesterol and the amount of three glycerides in the blood, as well as anti-inflammatory, cardioprotective, and hepatoprotective effects. It also has anti-diabetic qualities and guards against cancer, arthritis, and autoimmune diseases. ω -6 has several benefits, including anti-inflammatory, anti-hypertensive, anti-thrombotic, and anticancer effects (4–7,15).

For every 100 grams, chia seeds have 34–40 grams of dietary fibre.

The study done by Reyes-Caudillo et al. (11) states that consuming a lot of fibre reduces the incidence of coronary heart disease, type 2 diabetes, and numerous cancers. It also reduces appetite after meals when a high-fibre diet is consumed. Chia seeds also contain vitamins (A, B, D, E and K, notably vitamins B1, B2, and niacin) and minerals such as potassium, calcium, phosphorus, magnesium, and so on. For example, there is more calcium in it than in rice, barley, corn, and oats.

Chia seeds also contain higher amounts of various nutrients than other cereals, including magnesium, potassium, and phosphorus (4,16).

Applying an analysis of phenolic content, dried chia seeds were examined to have an 8.8% of phenolic component concentration. In addition, elevated concentrations of gallic, cinnamic, myricetin, kaemferol, querencetin, rosmarinic acid, caffeic acid, and chlorogenic acid have been recorded. Additionally, trace levels of isoflavones such as genistein, glycitein, and daidzein are present. According to Rahman et al. (17), the main ingredients in chia seeds include daidzein and rosmarinic acid, along with other substances like quercetin, myricetin, and caffeic acid. Additionally, vitamins A, B1, B2, and B3 were detected in chia seeds in the same study. Research has demonstrated the anti-carcinogenic, anti-hypertensive, and neuron protecting properties of the flavonoids quercetin, chlorogenic acid, and caffeic acid thus making them a good subject of this research (5). It was noted that chia seeds are free of gluten and potentially harmful mycotoxins (4,6). Moreover, chia seeds have been studied to be a great example of a diet high in antioxidants (18).

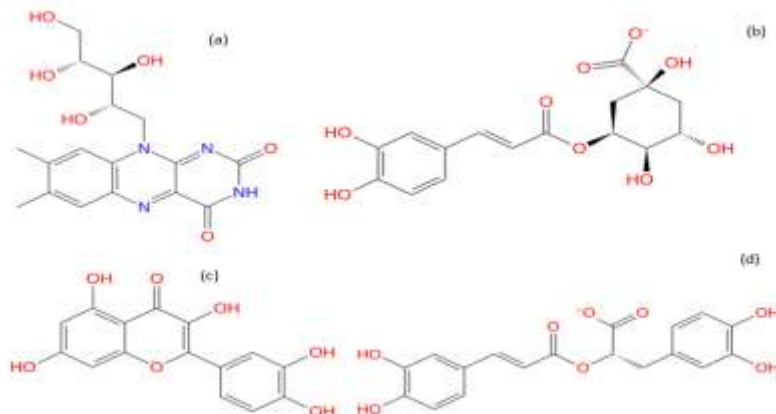
Salba-chia has been found to have significant effects on body weight, visceral obesity, and obesity-related risk factors in overweight and obese adults with type 2 diabetes. It has the ability to convert glucose into a slow-release carbohydrate and affect satiety more than flax due to its higher fiber viscosity. Chia seed oil has been found to increase plasma ALA levels, but no discernable benefits were observed for athletes. Chia supplementation did not cause any gastrointestinal, hepatic, or renal disorders, and it decreased blood pressure in hypertensive individuals. Milled and whole chia seeds were found to be effective in changing disease risk elements in overweight and postmenopausal women, but had no consequence on inflammation or disease risk factors. A dietary arrangement with nopal, soy protein, chia seed, and oat showed no changes in body weight, BMI, or WC post-dietary treatment (19).

The potential impact on human health stems from the structure and practical properties of the protein and peptides found in chia seeds (*S. hispanica* L.). The information was used to investigate the functional potential of the peptides. Twenty of the total proteins are found in chia seeds. Plant basic metabolism consistently includes these (20). An analysis of amino acid sequence revealed that peptides of functional capacity possess inhibitors of dipeptidyl peptidase-IV, inhibitors of angiotensin-converting enzyme, and antioxidant properties. Due to their antioxidant properties, antihypertensive, antihyperglycemic, and anticholesterolemic effects, chia seeds are useful to humans. Peptide and chia protein architectures may be connected to these relationships (15). These constituents possess the capacity to mitigate the likelihood of chronic ailments, such as disorders associated with the gastrointestinal tract, cardiovascular illness, and diverse forms of cancer. Cardiovascular disorders are prevented by fiber, polyphenols, omega-3 fatty acids, vitamins, protein, phytosterols, and minerals that regulate bad cholesterol, hypertension, and platelet aggregation. Chia seed extracts reduce blood sugar and improve GI tract beta-cell function, which reduces type 2 diabetes. Additionally, because chia seeds have an extraordinary fibre content that contributes to stool volume, they can help prevent constipation. However, the phenolic content and antioxidants in these seeds promote

oxidation and help reduce the risk of certain malignancies (21).

According to earlier research, adding chia to beef patties improved the antioxidant bioavailability and the omega-6/omega-3 ratio (22). These advantages, together with chia's high tryptophan content, suggest that adding chia to one's diet may be effective in lowering symptoms of depression and insomnia by enhancing serotonin production and brain function (1). Another study done by Tai (1) on the effect of chia consumption on sleep quality, it was not shown to have a direct implication on enhanced mood or sleep quality, but the control group experienced higher reductions in depressed symptoms and improvements in sleep quality than the chia consumption group. This suggests that maintaining adequate hydration may be more important for enhancing mood and sleep quality than chia eating.

Figure 1 Compounds (a) Vit B2 (b) Chlorogenic acid (c) quercetin (d) Rosmarinic acid present in chia seeds



S. hispanica seed therapy may be beneficial for individuals suffering from non-alcoholic fatty liver disease (NAFLD). This is explained by the dietary fiber, flavonoids, and omega-3 EFAs found in chia seeds. In patients with nonalcoholic fatty liver disease (NAFLD), incorporating chia seeds into the diet may help reduce intrahepatic fat. A study by Medina-Urrutia et al. found that supplementing the diet with 25 g/d of pulverized chia seeds for eight weeks decreased the waist circumference and body weight (median -4%) in 25 individuals with NAFLD and insulin resistance (23).

The outcomes demonstrated that while depression-like behaviors were lessened with both pretreatment and treatment, chia supplementation was ineffective against anxiety related to Alzheimer's disease. The chia therapy had no effect on memory or learning. Instead, when compared to their non-treated disease-induced peers, the cognitive function of the chia-pretreated mice was noticeably lower. In contrast to the controls, all disease-induced animals had hippocampal concentrations of amyloid- β 42, amyloid precursor protein, and total tau protein that were also elevated (even with chia supplementation). These results suggest that taking chia supplements while Alzheimer's develops could make the condition worse. Even though the results shown here are from an experimental and preclinical study, we advise using chia with caution and caution, especially in individuals with Alzheimer's disease who are in the early stages of the disease, until further research in various experimental settings is carried out (24). The search for bioactive peptides that can strengthen the immune system or lower the risk of chronic diseases has gained momentum in recent years. Peptidomics and bioinformatics developments in food science have made it possible to conduct integrated research for the quick development of bioactive peptides derived from food (25). Aguilar-Toala et al. offered a pipeline based on in silico analysis. They screened for probable bioactivities and connected them to pharmacological (drug) targets using chia seed peptides which were discovered by peptidomics and bioinformatics. Descriptors are predicted physicochemically and structurally, and then their intermolecular interactions are virtually screened via ensemble docking with likely molecular targets and molecular dynamics simulations. By using this method, they were able to find ten multifunctional chia seed peptides that had stable intermolecular contacts during the development of a ligand receptor complex. It also predicts sequences present in the peptide fractions that may offer multifunctional bioactivities towards human protein receptors implicated in chronic diseases like inflammation and hypertension. These findings support the good in vitro biological activity which had been previously reported for chia seed peptides (26).

Methodology

Protein preparation

PDB IDs - 4COF, IS3E, 8AXD, 5I71, 2Z5Y (2.17 Å) were used for the protein structure of GABA, 5HT3A and HMO-A, MAO-A, MAO-B and the ts3 human serotonin transporter and were accessed from RCSB PDB. The "Protein preparation Wizard" tool was utilized to prepare the proteins. There are three processes involved in this process: import and processing, review and modification followed by protein refining. The protein was first pre-processed by adding hydrogen atoms, removing water molecules from the hetero group that were greater than 5 Å, and adding side chains and loops that were absent. The pre-processed protein was then further improved utilizing the OPLS3e force field for energy minimization and PROPKA for hydrogen bond assignment at neutral pH. The module SiteMap of Schrödinger was used to predict the binding pockets of the test ligands in the protein structures (27).

Docking studies

Using the "LigPrep" tool, the ligands shown in figure 1 were ready for docking analysis. This involved converting them into three-dimensional structures and producing potential tautomers and conformers. Using Epik, ligand preparation was carried out at neutral pH 7 ± 2 , and the OPLS3e (Optimized potentials for liquid simulations_3e) force field was used to minimize the ligands' energy. The Glide software of Schrödinger Maestro Version 12.5.139 was used to carry out docking studies (28).

Taking into account the protein-bound inhibitor, the receptor grid construction tool of the GLIDE module was used to create the grid in the protein. Docking and scoring come after GLIDE's quick, precise, and methodical search for the conformational, positional, and orientational space. Using the 'Ligand docking' tool in the GLIDE module and the XP (Extra precision) method of docking, also referred to as stiff docking, the produced ligands were then docked to the active site of the protein. For this investigation, only hydrogen bonds (H-bonds) that form between acceptor and donor atoms at a distance of less than 3.3 Å were taken into account. The more complex docking method that makes use of the improved scoring function is computationally known as XP docking. Furthermore, the likelihood of receiving false-positive results is lower than with high-throughput virtual screening and standard precision docking.

Molecular Dynamics (MD) simulations

Desmond (29) of Schrödinger suite 12.5 was used for the MD simulation, which was run using the following procedure:

Using molecular dynamics (MD) simulations, the thermodynamic behavior and stability of the protein ligand complexes were examined. The Desmond-v7.2 program (29), which is integrated with the Maestro suite (Schrödinger, Inc. LLC, New York, NY, USA), was used to conduct these simulations. The complexes were filled with specific water molecules using a simple point charge (SPC) water model, and they were arranged in an orthorhombic box with periodic boundary conditions of 10 Å. The OPLS3 force field was used in the MD computations (30). The system was solvated in a SPC water model and 0.15 M NaCl to simulate a physiological ionic concentration in order to replicate physiological circumstances. The NPT ensemble was used to execute the simulations, with a constant pressure of 1.01325 bar and temperature of 300 K. The simulation's duration was set at 100 ns, and data were collected while the energy (kcal/mol) measured at 1.2 ps intervals. A cut-off radius of 9.0 Å was used to determine short-range interactions. The Nose-Hoover thermostat (31) and the Martyna-Tobias-Klein (32) techniques were used to control the temperature and pressure, respectively. For both bonded and non-bonded interactions within the cut-off range, the equations of motion were integrated using the RESPA integrator at a time step of 2.0 fs (33). Prior to initiating the simulations, the system underwent minimization and equilibration procedures following Desmond's default methods. The simulated interaction diagram protocol from the Desmond package was used to analyze the trajectory.

Results and discussion

Docking results of the polyphenols present in chia seeds with the targets responsible for anxiety and depression were studied. The most stable docking pose with the best score and favorable interactions was selected for further MD analysis. The protein structure of all the selected protein complexes was found to be stable as per the RMSD and RMSF studies. Characterizing alterations in the locations of the ligand atoms which can be done with the use of the Ligand Root Mean Square Fluctuation (L-RMSF) was also calculated. The ligand torsions plot which summarizes

the conformational evolution of every rotatable bond (RB) in the ligand throughout the simulation trajectory (0.00 through 100.00 nsec) was also examined from the structural stability of each ligand in the protein. In this paper we shall mainly focus on the protein-ligand interactions found in each complex and will be discussed further.

GABA complex

Figure 2. Interactions of Vit B2 with the GABA complex (PDB ID: 4COF)

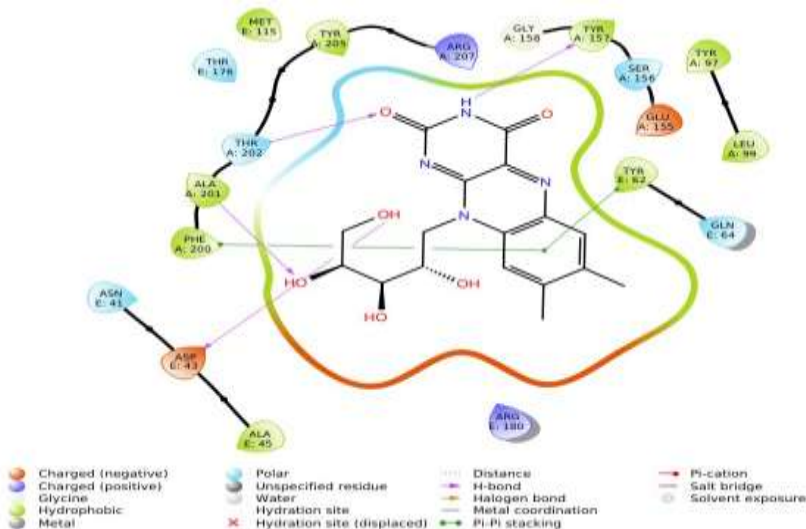
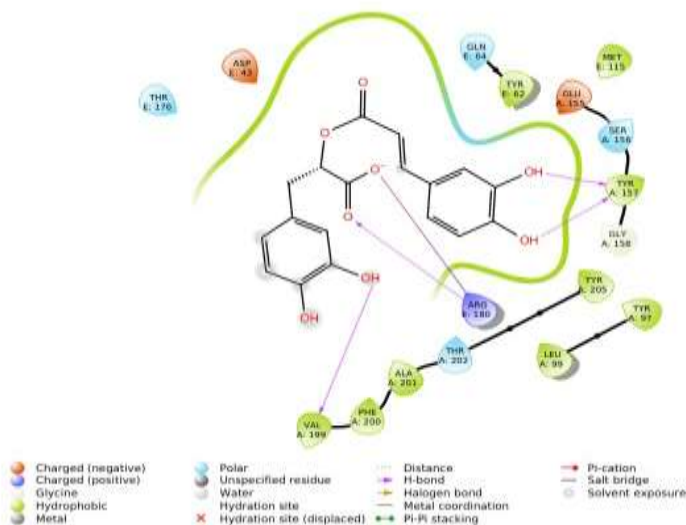


Figure 2 shows the docking pose of Vit B2 with the GABA complex. Docking results showed H-bond interactions with Tyr157, Thr202 and Asp43; pi-pi interactions with Phe200, Tyr62 meanwhile the MD results showed major interactions with Thr202, Asp162, Asn80.

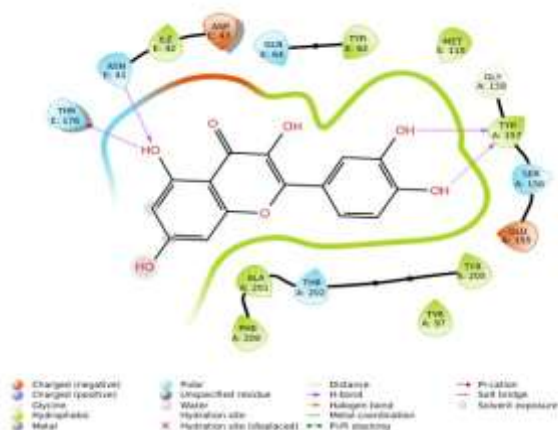
Figure 3. Interactions of rosmarinic acid with GABA complex (PDB ID: 4COF)



The docking pose of rosmarinic acid with GABA receptor as seen in figure 3 showed interactions with Val199, Arg180 and Tyr157. Out of these Arg180 and Val199 were present in MD but as weak interactions; instead strong H-bonds were observed with Arg312 and Asn80.

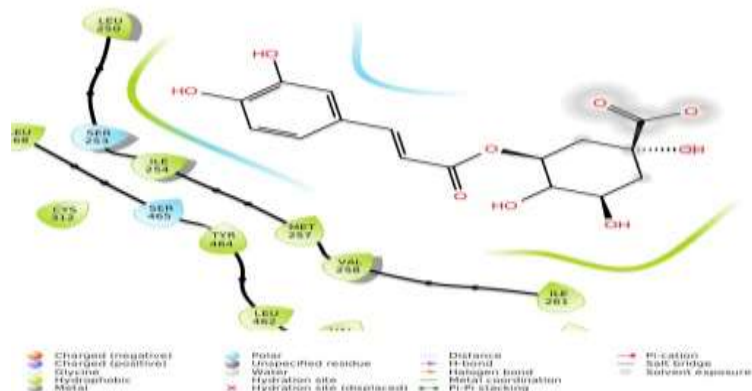
HT complex

Figure 4. Interactions of quercetin with serotonin 5HT-3A receptor (PDB ID – 8AXD)



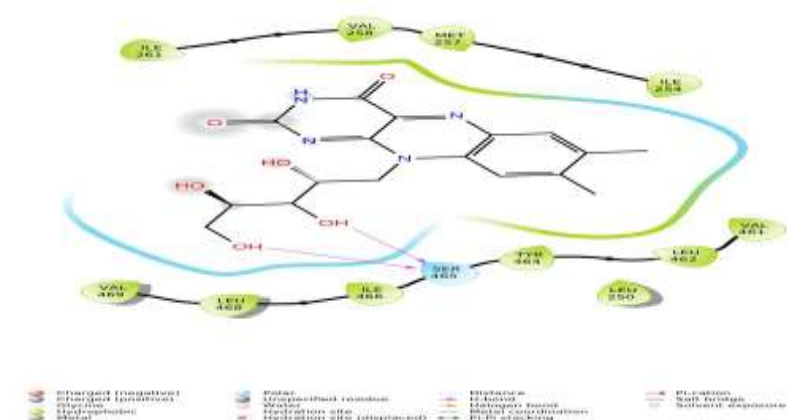
Docking of HT receptor along with quercetin showed H-bond interactions with Tyr157, Asn41 and Thr176 as seen in figure 4.

Figure 5. Interaction of chlorogenic acid with serotonin 5HT-3A receptor (PDB ID – 8AXD)



Water mediated interactions were found with Ser253, Ser465 and Leu458 as observed in the protein-ligand complex of figure 5.

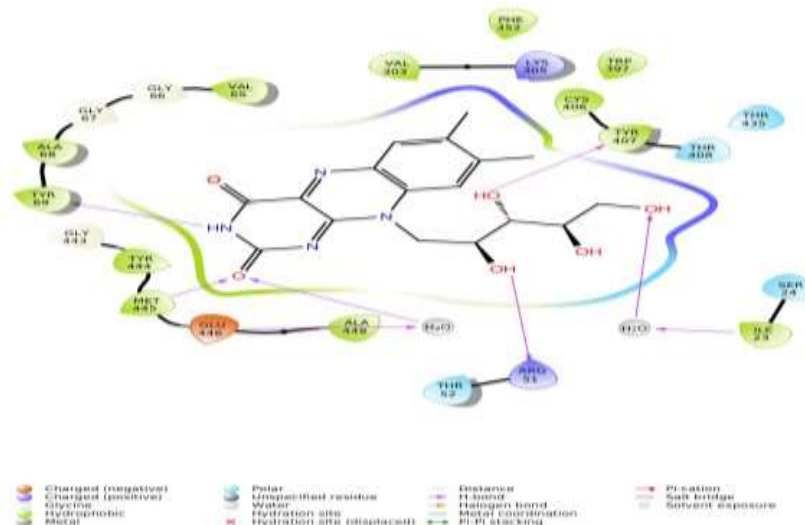
Figure 6. Interactions of Vit B2 with serotonin 5HT-3A receptor (PDB ID – 8AXD)



Docking pose seen in figure 6 indicates an interaction with Ser465 but is found to be very weak in MD. A strong hydrophobic interaction with Trp472 is observed.

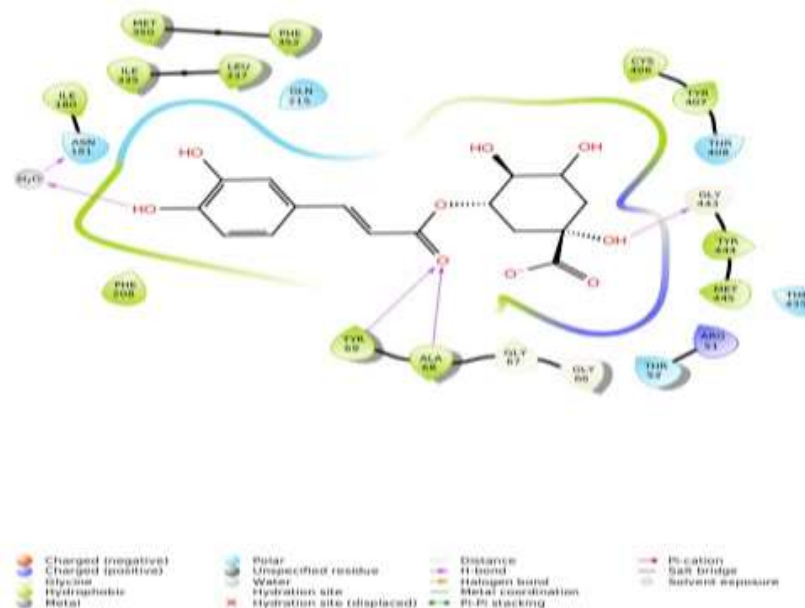
HMO A complex

Figure 7. Interactions of Vit B2 with HMO-A (PDB ID - 2Z5Y)



H-bonds with Tyr407, Arg51, Met445 and Tyr69 were found in the docking pose shown in figure 7. All of them were present in the MD run except for Arg51 which was present as a weak water mediated H-bond and tyr407 was found as a hydrophobic interaction. Water mediated interactions can also be seen with Ile23 and Glu446 and both of them were present in MD.

Figure 8. Interactions of Chlorogenic acid with HMO-A (PDB ID - 2Z5Y)



In the docking pose (figure 8), water mediated interaction can be seen with Asn181 but found to be quite negligible in MD. H-bonds were seen with Ala68 and Tyr69 but negligible during the simulation; Gly443 is seen in the docking pose but not present in MD.

Figure 9. Interactions of rosmarinic acid with HMO-A (PDB ID - 2Z5Y)

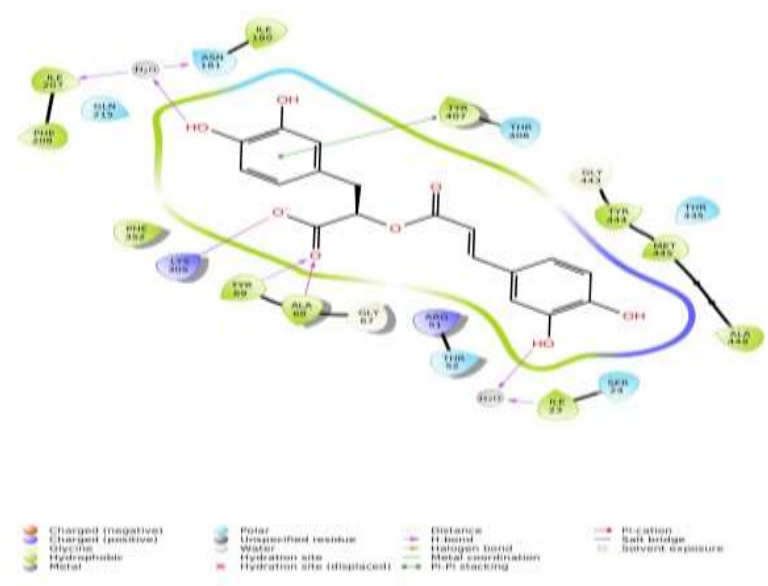
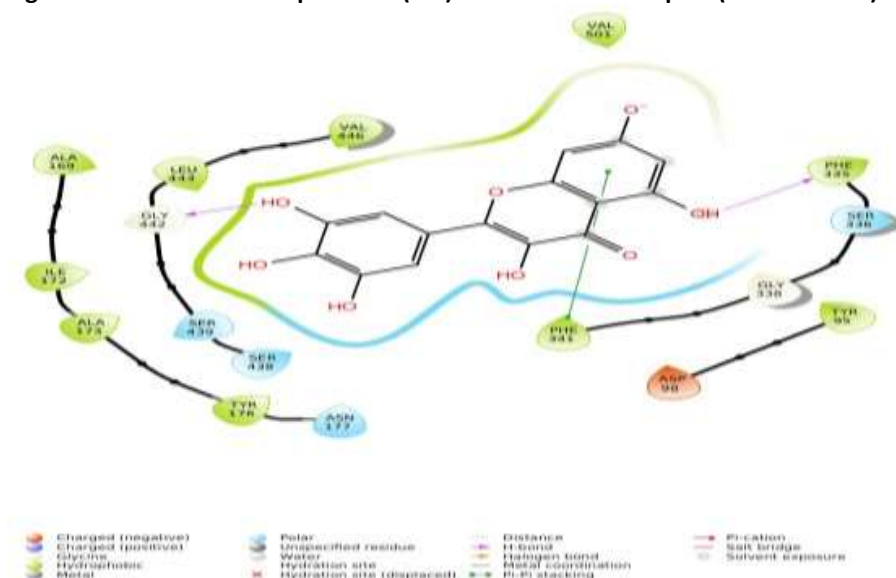


Figure 9 shows the interactions between rosmarinic acid and HMO-A. H bonds with Ala68, Tyr69, Lys305 are present in both docking and MD, water-mediated interactions with Asn181 is strong, Ile207 is present only as a normal H-bond; Ile23 was found as a weak interaction; one pi-pi interaction with Tyr407 was found in both docking and MD.

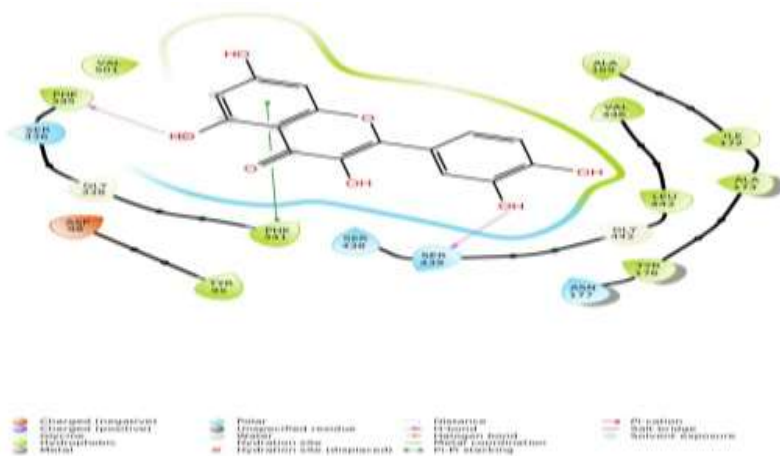
SERT complex

Figure 10. Interactions of quercetin (ion) with the SERT complex (PDB ID - 5I71)



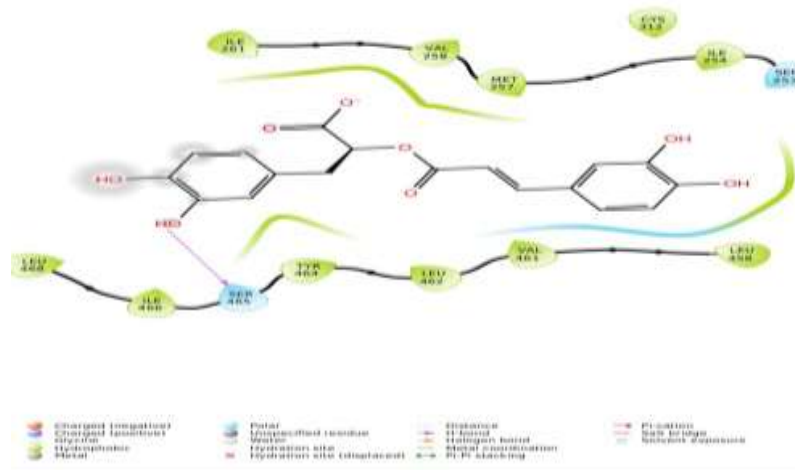
Docking of quercetin with the SERT complex showed interactions with Gly442 (as seen in figure 10) which was not present in MD while other interactions of Phe335 and a pi-pi interaction of Phe341 was present.

Figure 11. Interactions of quercetin with the SERT complex (PDB ID - 5I71)



H-bond interactions with Ser439 and Phe335 and pi-pi interactions with Phe341 can be seen in the docking analysis and MD simulation as seen in figure 11.

Figure 12. Interactions of rosmarinic acid with the SERT complex (PDB ID - 5I71)



One H bond interaction was observed with Ser465 as can be seen in figure 12.

Figure 13. Interactions of Vit B2 with the SERT complex (PDB ID - 5I71)

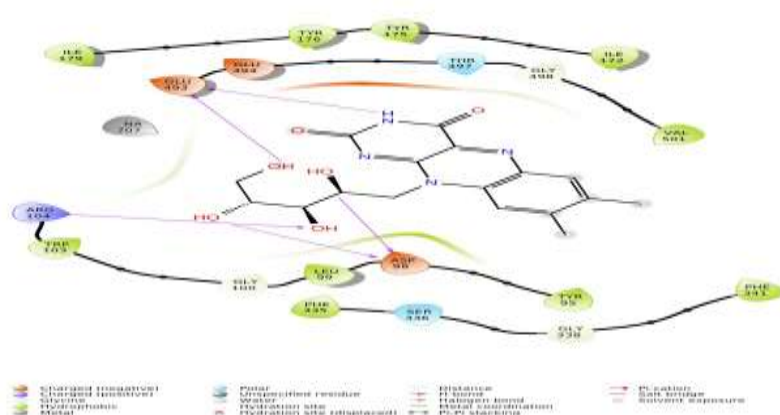
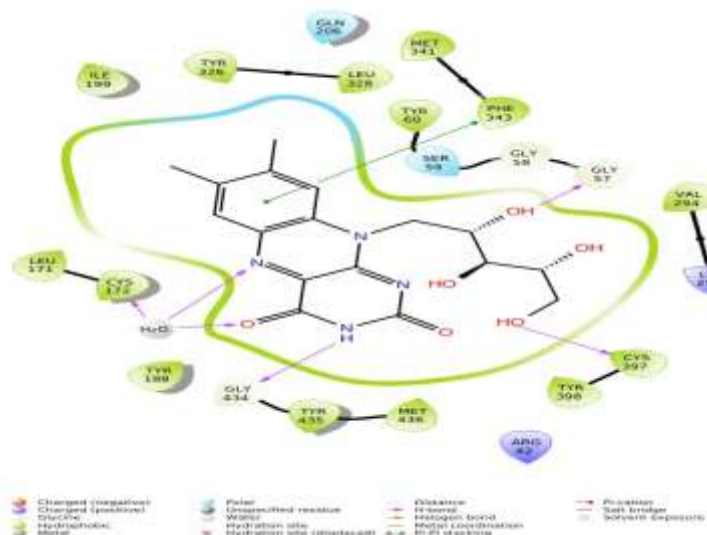


Figure 13 shows H-bond interactions with Asp98, Arg104 and Glu493 were present in docking and MD simulations.

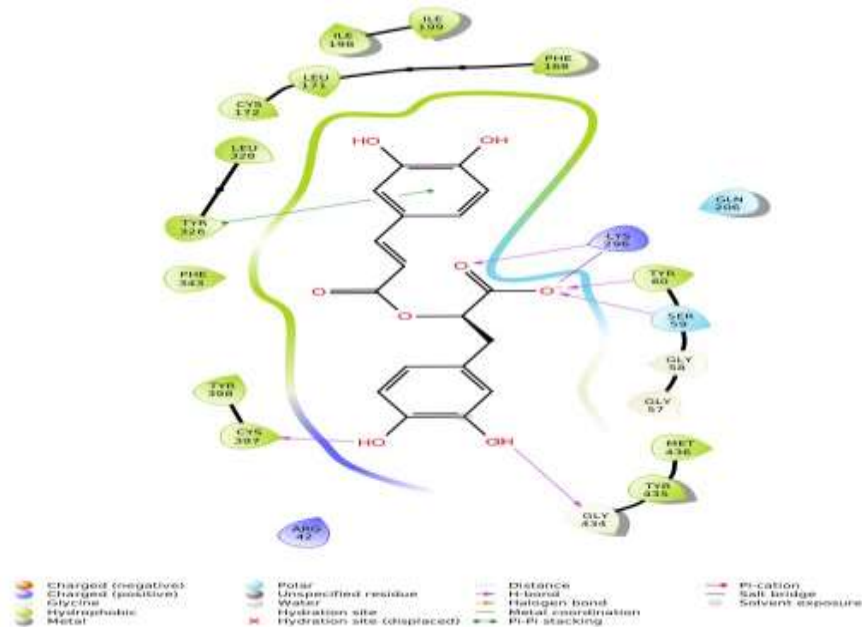
HMO B complex

Figure 14. Interactions of Vit B2 with MAOB (PDB ID - 1S3E)



A weak water bridge was observed as seen in figure 14 with Cys397 while the interaction with Cys172 was not found in MD. A negligible interaction was observed with Gly57, a strong H-bond with Gly434 and one strong pi-pi interaction with Phe343.

Figure 15. Interactions of rosmarinic acid with MAOB (PDB ID - 1S3E)



Water mediated interaction with Gly434, H-bond interaction with Cys397, Tyr60, Ser59, Lys296 were present meanwhile the pi-pi interaction with Tyr326 turned out to be weak as shown in figure 15.

Figure 16. Interactions of chlorogenic acid with MAOB (PDB ID - 1S3E)

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