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In-Silico Approach to Assess the Polyphenols from Krishna Tulsi (*Ocimum tenuiflorum* L.) for Keap1/Nrf2 Receptor towards the Treatment of Inflammatory Bowel Disease †

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Abstract: Inflammatory Bowel Disease, is a term used for chronic inflammatory condition that includes two diseases i.e., Ulcerative Colitis and Crohn's Disease, both mostly affect the colon, as well as the mouth, oesophagus, stomach, small intestine, and large intestine, respectively. If untreated, it may cause the gut to become more constricted, rupture, produce holes, fistulas, and—most alarmingly colon cancer. One of the key signalling pathways reported to be important in IBD and Colon Cancer is the Keap1/Nrf2. According to several studies, Keap1/Nrf2 is also implicated in T-cell differentiation and inflammation; it can block IL-17, Th1 and Th17 generation and stop the production of various other pro-inflammatory cytokines. Most fruits and vegetables contain polyphenols, which are recognized by possessing more than one phenolic group. By destroying Keap1, these polyphenols can activate a pathway connected to Nrf2, continuous improvement in polyphenol extraction and purification, as well as research on the molecular mechanism of Keap1/Nrf2 in numerous polyphenol monomers that can control Nrf2 have been found during the past decade. Therefore, a molecular docking research was carried out to assess how Keap1/Nrf2 interacted with the common polyphenols found in Krishna Tulsi (*Ocimum tenuiflorum* L.) such as Syringic acid, Caffeic acid, Ferulic acid, Catechin and Epicatechin. Catechin was found to have least binding energy of -8.2 kcal/mol that indicates the high binding affinity between the chosen receptor and ligand. The contact hydrogen bond includes GLY364; LEU365 and LEU557. To verify these results in IBD, however, more in-vitro, and in-vivo research is necessary.

Keywords: molecular docking; inflammatory bowel disease; polyphenols; Keap1/Nrf2; colon cancer

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1. Introduction

Inflammatory bowel disease (IBD) is a chronic condition that affects the gastrointestinal tract and causes inflammation in the digestive system [1]. The two most common forms of IBD are ulcerative colitis and Crohn's disease, both of which can have a significant impact on an individual's quality of life [2]. Symptoms of IBD may include abdominal pain, diarrhea, rectal bleeding, and weight loss. These symptoms can be debilitating and can lead to serious complications if left untreated [3,4]. The exact cause of IBD is not yet fully understood but it is believed to be a combination of genetic, environmental, and immunological factors that lead to chronic inflammation in the gut [5,6]. Molecular docking is a computational technique used to predict the binding of small molecules to a protein target [7]. It is a powerful tool in drug discovery and can aid in the identification of potential therapeutics for a variety of diseases, including IBD. By simulating the interactions between a small molecule and a protein target, molecular docking can predict the

binding affinity and binding mode of the molecule to the protein. This information can be used to design more effective and selective drugs [8].

Ocimum tenuiflorum, commonly known as holy basil or tulsi, is a medicinal herb that has been used in Ayurvedic medicine for centuries [9]. Studies have shown that *Ocimum tenuiflorum* has anti-inflammatory properties and may have potential therapeutic benefits for IBD [10]. The plant contains several biologically active compounds such as Syringic acid, Caffeic acid, Ferulic acid, Catechin and Epicatechin, which have anti-inflammatory effects [11,12]. Additionally, it has antioxidant, immunomodulatory, and anti-cancer properties, making it a potential candidate for the treatment of IBD [13].

One of the key receptors that *Ocimum tenuiflorum* can target for IBD treatment is the Keap1/Nrf2 receptor. The Keap1/Nrf2 pathway plays a key role in regulating the body's response to oxidative stress and inflammation. *Ocimum sanctum* has been shown to activate the Nrf2 pathway and promote the production of antioxidant and anti-inflammatory molecules [14]. This can help to reduce inflammation in the gut and protect against damage to the intestinal lining. In this, we will discuss the current understanding of IBD and its impact on individuals, introduce the concept of molecular docking, and explore the potential of *Ocimum tenuiflorum* as a treatment for IBD through the application of molecular docking techniques. By understanding the molecular interactions between *Ocimum tenuiflorum* compounds and proteins involved in IBD, including the Keap1 receptor, we can gain insight into the mechanisms of action of the plant and identify new targets for drug development. The use of molecular docking and *Ocimum tenuiflorum* could potentially lead to the development of more effective treatments for IBD that target the underlying causes of the disease.

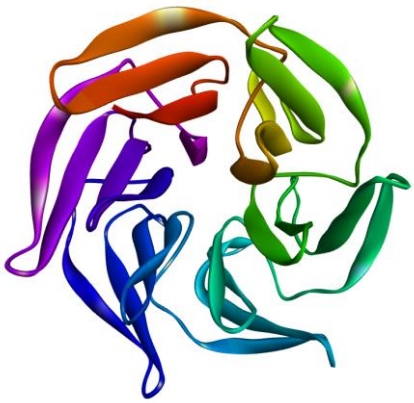


Figure 1. Structure of the kelch domain of Kelch-like ECH Associated Protein-1 (KEAP-1).

2. Materials and Methods

The in silico study was done by the following steps:

1. Protein preparation: The Keap1 receptor was obtained from a protein data bank(4ZY3). The protein was then modeled using the AutoDock Tools and Biovia discovery studio.
2. Selection of ligand—The three-dimensional structures of Syringic acid, Caffeic acid, Ferulic acid, Catechin and Epicatechin were obtained from PubChem database.
3. Active site of target protein—PyMOL software was used to analyze the target binding site of the receptor protein.
4. Molecular docking—The target protein was prepared by AutoDock Tools and Biovia discovery studio. The grid box on the active binding site was generated for docking. The ligands were docked to the Keap1 receptor using the default settings in Autodock Vina. The program was run for a specified number of generations, and the binding affinity and interactions of the compounds to the receptor were analyzed.

5. Data analysis: The results of the docking simulations were analyzed using the Biovia discovery studio, which generates a summary of the best-docked complexes and their binding energies. The binding modes and binding affinities of the *Ocimum tenuiflorum* compounds to the Keap1/Nrf2 receptor were analyzed and compared to identify the most promising compounds for further study.

3. Results

After a successful docking process with the protein, the lowest binding energy was determined for each ligand. The binding site and docked conformations were then visualized for interactions. The inhibition constant (Ki) was calculated using the equation:

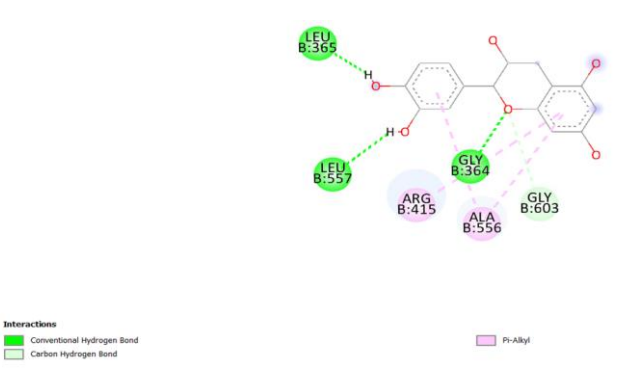
$$Ki = \exp(\Delta G/RT)$$

where ΔG represents the minimum binding energy of the docked conformations, R is the universal gas constant ($R = 1.985 \times 10^{-3}$ kcal mol^{-3} K^{-3}), and T is the temperature (T = 298.15 K) [15].

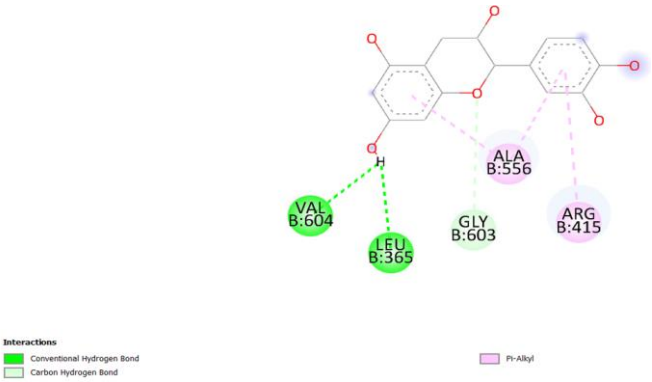
Table 1. The estimated inhibition constants and the minimum binding energies of the ligands docked with Keap1

Ligands	Estimated Free Binding Energy (kcal/mol)	Estimated Inhibition Constant (K_i) (μM)
Catechin	−8.2	0.960
Epicatechin	−7.9	1.595
Caffeic acid	−6.4	20.116
Ferulic acid	−5.9	46.823
Syringic acid	−5.6	77.734

The docking results were studied through Biovia discovery studio software to give a two-dimensional and three-dimensional view of the interactions between the ligands and the residues of the binding site of the protein molecule. The estimated free binding energy(kcal/mol) was derived from vina result and based on that estimated inhibition constant(Ki) (μM) was calculated using the above equation i.e., Ki = exp(ΔG/RT)



(**a**)



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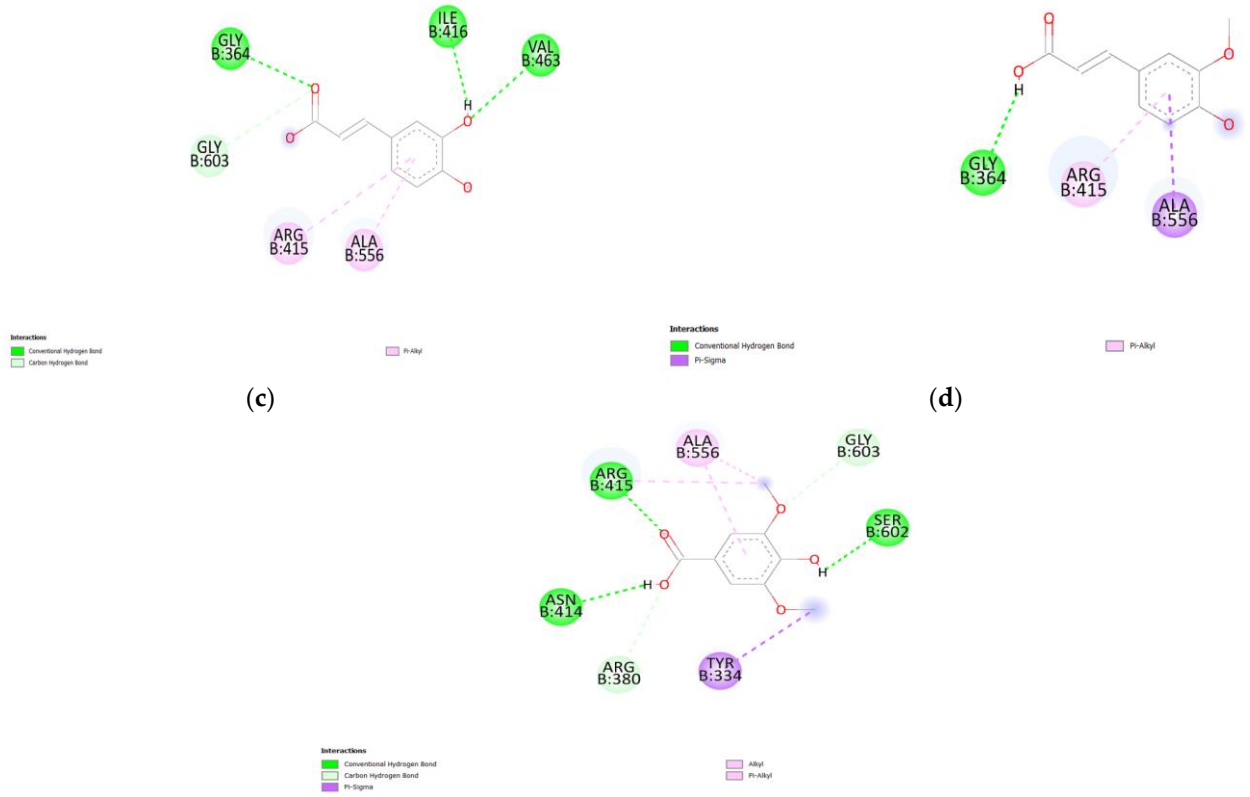

Figure 2. The two-dimensional representation of the docked ligand's and the target's binding site. (**a**) Catechin; (**b**) Epicatechin; (**c**) Caffeic acid; (**d**) Ferulic acid; (**e**) Syringic acid.

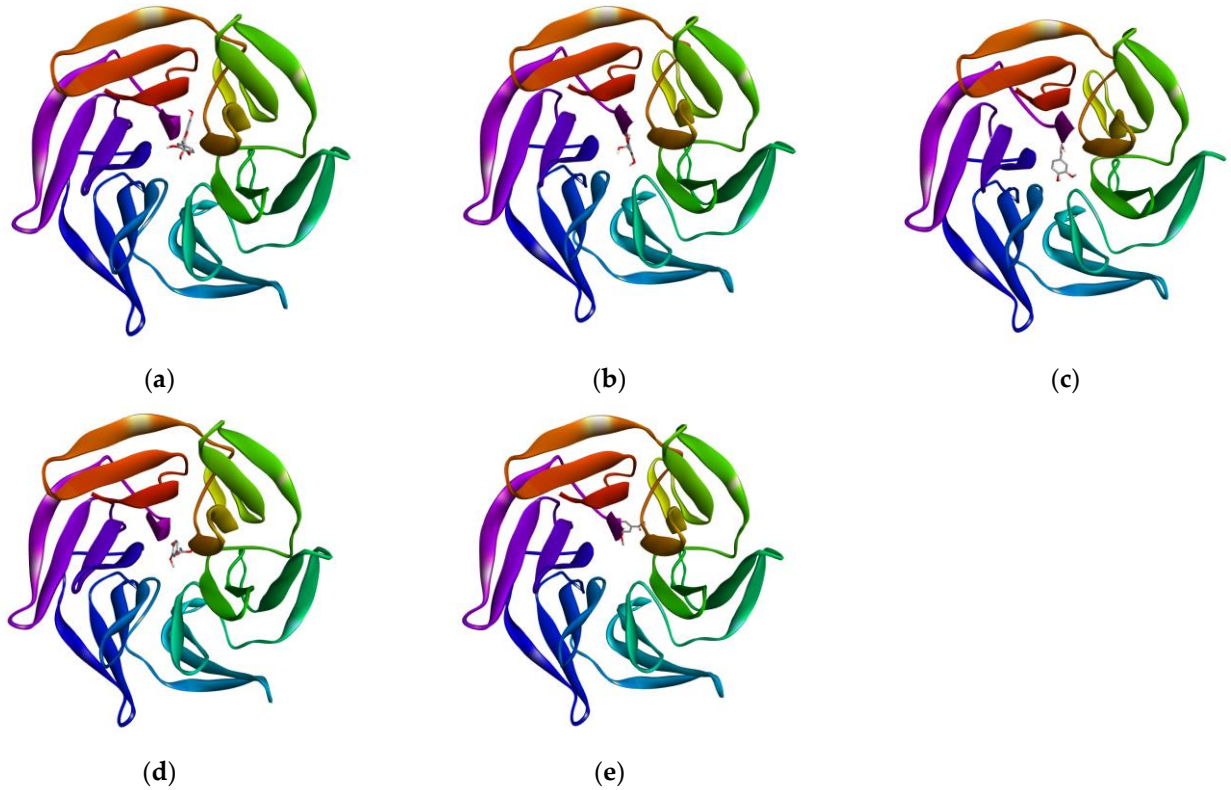

Figure 3. The three-dimensional representation of binding site of selected polyphenolic compounds of *Ocimum tenuiflorum* with Keap1. (**a**) Catechin; (**b**) Epicatechin; (**c**) Caffeic acid; (**d**) Ferulic acid; (**e**) Syringic acid.

4. Discussion

The Nrf2-Keap1 connection is thought to be disrupted by many inhibitors that directly bind to the Kelch domain of Keap1, which in turn promotes Nrf2 nuclear translocation [16]. We thus anticipated that the Nrf2 activation caused by our screened compounds may possibly be connected to direct binding to the Keap1 Kelch domain. Keap1 is thought to limit the degradation of Nrf2. Our findings shown that Keap1 and Nrf2 may be effectively separated by binding our ligands with the Keap1 protein.

The molecular docking study of the polyphenolic compounds of *Ocimum tenuiflorum* with the Keap1 was successfully conducted. The minimum binding energy was found between −8.2 and −5.6 kcal/mol, showing a good binding affinity between the ligands and the protein. The binding energy of catechin was found to be −8.2 kcal/mol, which was the least among all the other ligands, suggesting that this ligand can be a potent inhibitor of Keap1/Nrf2 even at the lowest concentrations. Therefore, catechin can be the choice of compound in the management of IBD. However, further In-vitro and In-vivo studies are required to confirm the activity.

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Conflicts of Interest: The authors declare no conflict of interest.

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