In Silico Approach to Assessing the Polyphenols from Krishna Tulsi (Ocimum tenuiflorum L.) as a Keap1/Nrf2 Receptor for the Treatment of Inflammatory Bowel Disease †

Satish Kumar and Biswatrish Sarkar *

Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi 835215, India; 8709489546satish@gmail.com
* Correspondence: biswatrishtarkar@bitmesra.ac.in

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, they 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 pathway. According to several studies, Keap1/Nrf2 is also implicated in T-cell differentiation and inflammation; it can block generation of IL-17, Th1 and Th17, and stop the production of various other pro-inflammatory cytokines. Most fruits and vegetables contain polyphenols, which are recognized by their possession of more than one phenolic group. By destroying Keap1, these polyphenols can activate a pathway connected to Nrf2. We have seen continuous improvements in polyphenol extraction and purification, and in research on the molecular mechanism of Keap1/Nrf2 in numerous polyphenol monomers that can control Nrf2, over the past decade. Therefore, 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 the lowest binding energy (−8.2 kcal/mol), which indicates the high binding affinity between the chosen receptor and ligand. 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

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, diarrhoea, 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 shown in Figure 1. 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 study, 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 the 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).](image)

**2. Materials and Methods**

The in silico study was carried out in the following steps:

1. **Protein preparation**: the Keap1 receptor was obtained from a protein data bank (4ZY3). The protein was then modeled using AutoDock Tools and Biovia discovery studio.
2. **Selection of ligand**: 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 using 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 with 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 ($K_i$) shown in Table 1 was calculated using the following equation:

$$K_i = \exp(\Delta G/RT)$$  \hspace{1cm} (1)

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

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Estimated Free Binding Energy (kcal/mol)</th>
<th>Estimated Inhibition Constant ($K_i$, µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>$-8.2$</td>
<td>$0.960$</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>$-7.9$</td>
<td>$1.595$</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>$-6.4$</td>
<td>$20.116$</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>$-5.9$</td>
<td>$46.823$</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>$-5.6$</td>
<td>$77.734$</td>
</tr>
</tbody>
</table>

The docking results shown in Figure 2 were studied through Biovia discovery studio software to give a two-dimensional and three-dimensional view shown in Figure 3 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 the Vina result, based on which the estimated inhibition constant ($K_i$) (µM) was calculated using the above equation, i.e., $K_i = \exp(\Delta G/RT)$. 

![Figure 2. Cont.](image-url)
Figure 2. 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. Cont.
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 be associated with 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.

A molecular docking study of the polyphenolic compounds of *Ocimum tenuiflorum* with Keap1 was successfully conducted. The minimum binding energy was found to be 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 lowest among all the other ligands, suggesting that this ligand may be a potent inhibitor of Keap1/Nrf2, even at its lowest concentrations. Therefore, catechin may be an effective choice of compound for the management of IBD. However, further in vitro and in vivo studies are required to confirm its activity.

**Author Contributions:** Conceptualization, B.S. and S.K.; methodology, S.K.; software, S.K.; validation, B.S. and S.K.; formal analysis, B.S.; investigation, S.K.; resources, B.S.; data curation, S.K.; writing—original draft preparation, S.K.; writing—review and editing, B.S.; visualization of the interactions, B.S. and S.K.; supervision, B.S.; project administration, B.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Data Availability Statement:** Data will be made available upon reasonable request.

**Acknowledgments:** All the authors are thankful to Birla Institute of Technology, Mesra, India for providing all the necessary equipment and constant support throughout the study.

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

**References**

1. Tsopmejio, I.S.N.; Ding, M.; Wei, J.; Zhao, C.; Jiang, Y.; Li, Y.; Song, H. *Auricularia polytricha* and *Flammulina velutipes* ameliorate inflammation and modulate the gut microbiota via regulation of NF-kB and Keap1/Nrf2 signaling pathways on DSS-induced inflammatory bowel disease. *Food Biosci.* 2022, 47, 101426. [CrossRef]


**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.