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In silico targeting of osmoporin protein of *Salmonella* to identify anti-Salmonellosis phyto-compounds

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Salmonella

ABSTRACT

Salmonella enterica serotype *typhi* is a gram-negative, rod-shaped bacterium, and has flagella with the human body as its only reservoir. Typhoid fever was found to cause 21.7 million illnesses and 216,000 fatalities worldwide in 2000, and the International Vaccine Institute estimated 11.9 million cases and 129,000 deaths in low- and middle-income countries in 2010. More than 10 million patients were infected with *S. typhi* each year and the mortality rate is associated with more than 0.1 million patients. Moreover, it is also associated with drug resistance globally which makes the disease more dreadful. Other than antibiotics, various flavonoids showed medicinal effects against many diseases including *S. typhi* infection. Flavonoids are a type of plant bioactive metabolite that have potential medicinal efficacy. The goal of this study was to see if certain flavonoids (ellagic acid, eriodictyol, and naringenin) could interact with the outer membrane of osmoporin (PDB ID: 3uu2) receptor in *Salmonella* and helps in inhibiting its growth. To look for probable ligand-receptor binding relationships, we used Pyrxmolecular docking software. The molecular docking results were analyzed using the Biovia discovery studio visualizer. The current study discovered that selected plant-based compounds interacted with an outer membrane of the osmoporin receptor, resulting in minimization of energy in the range of-6.6 to -7.8 Kcal/mol.

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1 Introduction

Salmonella enterica serotype typhi is a gram-negative, rod-shaped bacterium, and has flagella with the human body as its only reservoir (Mahmud et al. 2008). Typhoid fever was found to cause 21.7 million illnesses and 216,000 fatalities worldwide in 2000, and the International Vaccine Institute estimated 11.9 million cases and 129,000 deaths in low and middle-income countries in 2010 (Crump et al. 2004). More than 10 million patients were infected with *S. typhi* each year and the mortality rate is associated with more than 0.1 million patients (Stanaway et al. 2019). Moreover, it is also associated with drug resistance globally which makes the disease more dreadful. Typhoid fever is primarily a travel-related disease in developed countries, affecting travelers such as tourists, temporary workers, military personnel, or travelers visiting friends or relatives (VFR) in endemic areas, with risk varying depending on the geographical region visited, period of travel, integration with local cultures, and traveler concurrent diseases or medications (Masuet-Aumatell and Atouguia 2021). In 2018, an outbreak occurred in Pakistan and the patients became resistant to various generation antibiotics like ampicillin, chloramphenicol, fluoroquinolones, trimethoprim-sulfamethoxazole, and even the third-generation antibiotic cephalosporins (Klemm et al. 2018). Serologically, the bacterium is positive for the lipopolysaccharide antigens O9 and O12, and Vi, a polysaccharide capsular antigen. The effectiveness of the parenteral Vi vaccination in young children has lately been proved, as has the protection of Vi vaccines' unprotected neighbors (Crump et al. 2015; Parry et al. 2002). Vi-rEPA, the first Vi conjugate vaccination to reach clinical trials, has been demonstrated to be immunogenic as well as safe in Vietnamese children from 2 to 5 years of age range, with a 91.5 percent protective efficacy and an estimated 89 percent cumulative efficacy after 3.8 years (Lin et al. 2000). Other than humans, *Salmonella* species like *S. typhimurium*, *S. dublinn*, *S. newport*, *S. hindmarsh*, *S. derby*, *S. agona*, *S. gallinarum*, etc., also cause many diseases in cattle's, sheep, goats, pigs, horses, and poultry (Lewis et al. 2019). Osmoporin (ompC) is a protein found in *Salmonella* and is responsible for the bacteria's survival and pathogenicity, as well as for diffusing hydrophilic chemicals (Valero-Pacheco et al. 2020).

Phytochemicals have recently emerged as one of the most hopeful holistic alternative methods with few adverse effects (Alsheikh et al. 2020; Tiwari et al. 2018). The antibacterial activity of bioactive phytochemicals and essential oils against *Salmonella* spp. was discovered a very long time before (Osaili et al. 2021). The expense of screening compounds with potential bioactivity is high, and it might take a long period. However, computer-aided drug design (CADD) could save time and money by reducing the cost of molecule synthesis, thereby lowering research costs (Abdullahi and Adeniji 2020). One such CADD technology is *in-silico* molecular docking, which can essentially predict binding efficacy as well as structure-based drug design (Abdullahi and Adeniji 2020). Furthermore, molecular docking provides valuable insights into structure-activity relationships, mode of activity, and further analysis of protein-ligand interactions. Such research would lead to the creation of innovative therapeutic compounds against pathogenic infections at a faster rate. Furthermore, the molecule's physicochemical properties would provide crucial information during the early stages of therapeutic development (Abdullahi and Adeniji 2020; Abdullahi et al. 2020). Recently a study conducted by Abishad et al. (2021) concluded that *in-silico* molecular docking studies were helpful in understanding the interaction of phytochemical ligands of thymol, carvacrol, and cinnamaldehyde with pathogen protein motifs and inhibit the growth of *S. typhi* bacteria (Abishad et al. 2021) and *E.coli* (Abishad et al. 2021; Osaili et al. 2021). The present study was conducted to propose the possible molecular interactions of selected phytochemicals with microbial osmoporin receptors to tackle salmonellosis.

2 Material and Method

2.1 Preparation of Receptor structure

Receptor three-dimensional structure with PDB ID: 3UU2 with a resolution of 3.59 Å was downloaded from Research Collaboratory for Structural Bioinformatics, Protein Data Bank (RCSB-PDB), an online database (Figure 1). Heteroatoms and water molecules were excluded from protein structure.

2.2 Preparation of ligands and analysis of ADME properties

Three ligands were selected for molecular docking. 3-D structures of ligands were downloaded from the PubChem database in sdf format and were converted into pdb format by OpenBabel (Figure 1). ADME (Unfavorable absorption, distribution, metabolism, and elimination), an online software tool was used for profiling all the ligands (pH 7) (Jayaram et al. 2012). Physiochemical properties of ligand include Lipinski's rule of five (molar refractivity, molecular weight (<500 Da), H-bond acceptor (<10), H-bond donar (5), LogP (<5), and drug likeliness) were considered (Lipinski 2004) (Figure 1).

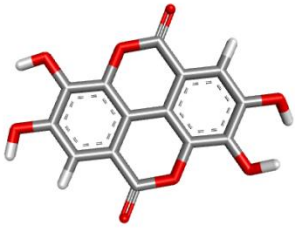
2.3 Molecular docking of ligands

PyRx v0.8 was used for molecular docking studies. Docked structures with high binding affinity were analyzed using PyMOL and Discovery Studio Visualizer.

3 Results and Discussion

In recent years, emergences of drug-resistant microbial strains are considered to be a serious problem and existing challenge for their treatment. Currently, it has been seen that patients are taking self-

A



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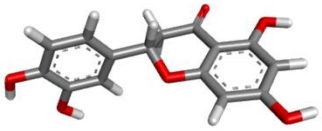

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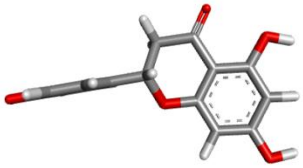

D

S.No.	Ligands	ADME Properties (Lipinski's Rule of Five)		Drug Likeliness
		Properties	Values	
1.	Naringenin	Molecular weight (<500 Da)	272	Yes
		LogP (<5)	2.5	
		H-bond donar (5)	3	
		H-bond acceptor (<10)	5	
		Molar Refractivity	70.19	
2.	Ellagic acid	Molecular weight (<500 Da)	302	Yes
		LogP (<5)	1.2	
		H-bond donar (5)	4	
		H-bond acceptor (<10)	8	
		Molar Refractivity	68.4	
3.	Eriodictyol	Molecular weight (<500 Da)	288	Yes
		LogP (<5)	2.2	
		H-bond donar (5)	4	
		H-bond acceptor (<10)	6	
		Molar Refractivity	71.8	

E

Figure 1 3D view of the protein (A), 3-D structure of Naringenin (B), Ellagic acid (C), Eriodictyol (D), and ADME properties of ligands (E).

medication at a higher dose than normally required which results in the development of drug resistance. Literature suggested that *Salmonella* is developing multi-drug resistance against a diverse range of sophisticated medicine including ciprofloxacin and ampicillin (Ge et al. 2022; Chen et al. 2022). Results of previous investigations have shown that medicinal plant's metabolites and their extract possessed potential toxicity against drug-resistant microbial strains. For instance, Naz et al. (2022) investigated anti-*Salmonella enterica* potential of various plant extracts including *Amaranthus hybridus* leaf >*Aloe barbadensis* leaf >*Adhatoda vasica* leaf, etc (Naz et al. 2022). Similarly, Yang et al. (2022), explored anti *Bacillus subtilis* and anti-*Salmonella typhi* ethyl acetate plant extract with MIC values in the range of 0.78 mg/mL (Yang et al. 2022). Recent data showed that more than 80% of

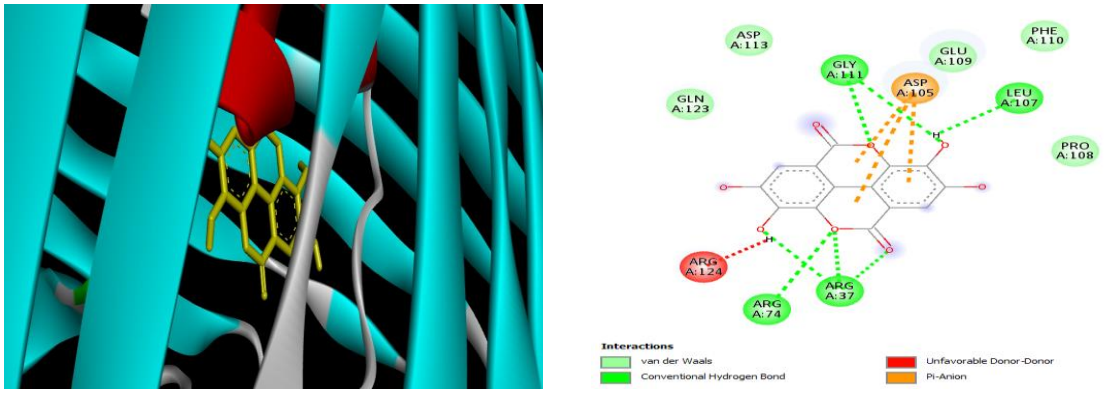

Figure 2 Binding of osmoporin and Ellagic acid. Best binding mode in the pocket of osmoporin and interacting amino acid of the target with a ligand.

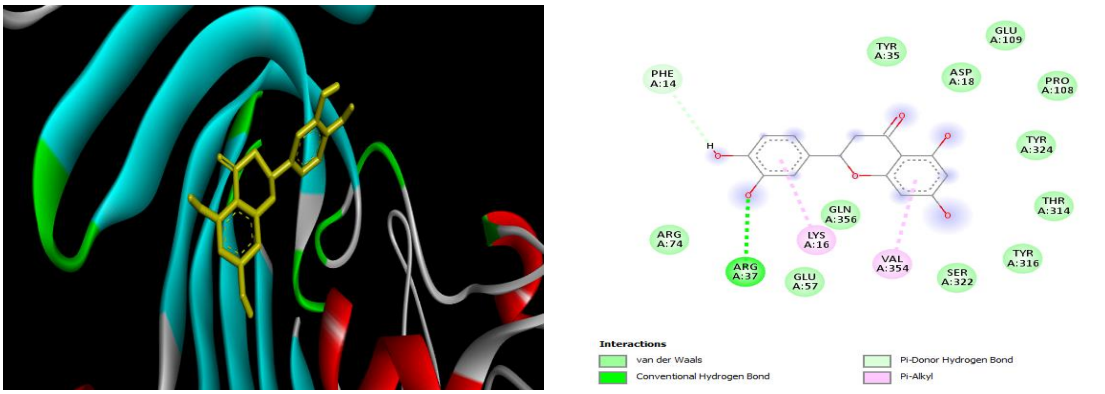

Figure 3 Interactions of osmoporin and Eriodictyol. Best binding mode in the pocket of osmoporin and interacting amino acid of a target with ligand

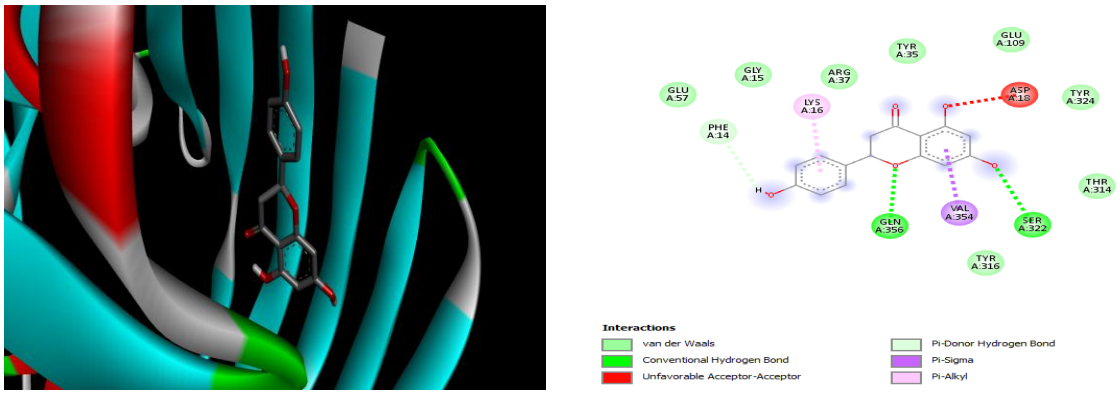

Figure 4 Binding of osmoporin and Naringenin. Best binding mode in the pocket of osmoporin and interacting amino acid of a target with ligand

drugs used globally either have plant-based chemical structures or derivatives (Mahmud et al. 2008). In comparison to previous laborious drug discovery methods, computational-based drug discovery approaches are gaining importance. In this method, best-suited interactions between drugs and receptors are calculated based on their binding efficacy. Minimum binding energies between drugs and receptors are considered to be best for drug discovery (Tuli et al. 2021; Tuli et al. 2022). As per the literature, minimum energies correspond to the maximum stability of the complex. Therefore, computational studies are considered to be important to screen out active drug moieties from various drug libraries. Results of the present study described that ellagic acid, eriodictyol, and naringenin interact with the osmoporin receptor (PDB ID: 3uu2) protein of *Salmonella* in the range of -6.6 to -7.8 Kcal/mol (Figure 2, 3 & 4). The selected ligands interacted with various amino acids of osmoporin receptor protein including GLY

Table 1 Binding affinity and RMSD value of Ellagic acid

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
ellagic acid	-7.8	0	0
ellagic acid	-7.8	6.183	0.041
ellagic acid	-7.6	12.08	10.001
ellagic acid	-7.5	11.397	9.98
ellagic acid	-7.4	12.16	10.316
ellagic acid	-7.4	16.143	14.334
ellagic acid	-7.4	4.225	2.183
ellagic acid	-7.3	16.176	14.515
ellagic acid	-7.3	4.601	2.812

Table 2 Binding affinity and RMSD value of Eriodictyol

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
eriodictyol	-7.2	0	0
eriodictyol	-7.1	13.608	10.507
eriodictyol	-7.1	6.662	4.21
eriodictyol	-7	18.534	14.143
eriodictyol	-6.9	11.359	8.849
eriodictyol	-6.9	11.008	9.141
eriodictyol	-6.9	12.993	11.341
eriodictyol	-6.9	11.758	9.48
eriodictyol	-6.8	18.456	14.358

111, ASP 105, LEU 107, ARG 124, GLN 356, LYS 16, VAL 354, ASP 18, and SER 122. Further, the interactions with amino acid residue and distance between the amino acid and the ligand pole (Å) of selected ligands i.e., ellagic acid, eriodictyol, and naringenin are provided in Tables 1, 2 & 3 against docked osmoporin receptor protein. The docked pose of ligand and osmoporin receptor protein receptor has been shown in Figure 2, 3 & 4.

Table 3 Binding affinity and RMSD value of Naringenin

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
naringenin	-6.8	0	0
naringenin	-6.8	15.549	11.673
naringenin	-6.7	17.727	14.426
naringenin	-6.7	14.037	12.004
naringenin	-6.7	6.422	4.001
naringenin	-6.7	18.755	14.659
naringenin	-6.6	18.697	14.946
naringenin	-6.6	13.4	11.023
naringenin	-6.6	29.285	27.715

Previous studies have suggested the promising role of phytochemicals to modulate cellular signaling to combat dreadful diseases. It has been observed that several bioactive phyto metabolites that are present in our diet possess a diverse range of therapeutic properties (Gupta et al. 2022; Aggarwal et al. 2022). Researchers are in progress to suggest diet associated modifications to fight infectious diseases. In addition, computational drug designing methods can play an important role to screen out the biological efficacy of plant metabolites with recognized cellular targets. As the spanning of dreadful diseases is increasing day by day, and discovery of novel therapeutic agents are not increasing at the same pace. Therefore, the utilities of computational drug discovery methods may not only speed up the conventional approaches but also enhance the success rate of novel therapeutic findings (Tuli et al. 2022). In the present study, we investigated the binding interactions of plant-based therapeutics with the osmoporin receptor of *Salmonella*. The results of our findings are in good agreement with previous reports. For instance, Abishad et al. (2021) investigated binding free energy for carvacrol, cinnamaldehyde, and thymol in the range of − 5.65 to − 4.97 kcal/mol against osmoporin receptor protein (Abishad et al. 2021). In another study, researchers explored the utility of 20 phytochemicals to interact with pathogenic microbial receptor (Mur B) protein to inhibit the growth of multi-drug resistance *Vibrio cholera* (Ragunathan et al. 2018).

Conclusion

Salmonellosis is considered to be an emerging microbial infection towards human and animal health. The results of the current study explored molecular docking interactions between ligands (ellagic acid, eriodictyol, and naringenin) and the osmoporin receptor protein of *Salmonella*. In the present investigation, molecular interactions between pathogenic microbial receptors and selected phytochemicals were found in the range of -6.6 to -7.8 Kcal/mol. Therefore, plant-based metabolites can be investigated in near future to design novel anti salmonellosis agents.

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