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In Silico Molecular Docking Analysis of Phytoconstituents of Lemon as an Antiulcer Agent

Pranjali Srivastava, Bhavesh Kapadi, Jay Khatri, Sudip Mahto, Kinjal Patel*
Department of Pharmacognosy, K. B. Raval College of Pharmacy, Shertha, Gandhinagar,Gujarat, India
***Corresponding author**
Ms. Kinjal Patel

ABSTRACT
In silico analysis is a computational method that allows the study of molecules and their interactions with biological systems. This approach has been widely used in drug discovery and development. In this essay, we will discuss the in-silico analysis of phytoconstituents of lemon to ascertain their antiulcer properties. Ulcers are a common gastrointestinal disorder that affects millions of people worldwide. The conventional treatment of ulcers involves the use of acid suppressants, proton pump inhibitors, and antibiotics. However, the long-term use of these drugs can lead to adverse side effects. Therefore, the search for alternative treatments for ulcers has gained significant attention in recent years. This study utilizes computational tools to predict the molecular structures and properties of the phytoconstituents, including their binding affinities and interactions with relevant biomolecules. The phytochemical analysis revealed that these ingredients contain numerous compounds such as terpenoids, flavonoids, and phenolic acids, which are known for their antiulcer or anti-inflammatory properties. That highlights the potential of these ingredients for developing new drugs and functional foods, based on their interactions with specific biomolecules. The findings of this in-silico analysis can provide a starting point for future experimental studies to validate the therapeutic properties of these phytoconstituents and their potential applications in the healthcare industry.
KEYWORDS: *Lemon, Ulcer, Docking, In silico analysis, alpha-thujene, ascorbic acid, Limonene, Citral*

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INTRODUCTION

Gastric ulcer is a serious illness of the digestive system and affects 5–10% of the adult population. It has become a global problem due to its higher morbidity and mortality, as well as their effects on health, society, and the economy. [1-3] Lemon (Citrus limon) is a citrus fruit that is commonly used as a food flavor. It is known for its numerous health benefits, including its ability to neutralize acid and reduce inflammation. Several phytoconstituents have been identified in lemon, including limonene, citral, ascorbic acid, alpha-thujene, etc. These compounds have been reported to possess anti-inflammatory, antioxidant, and antiulcer properties. [4-6] Lemons are known for their high vitamin C content and acidic nature. However, recent research also suggests that lemons may possess antiulcer properties. Flavonoids are naturally occurring compounds that are found in various fruits and vegetables, including lemons. These compounds possess antioxidant properties and are known to have anti-inflammatory effects. Limonoids, on the other hand, are a class of terpenoid compounds found in citrus fruits and other plants. These compounds possess potent anti-inflammatory and anticancer properties. Studies have shown that lemons and lemon juice may help to alleviate symptoms of peptic ulcer disease (PUD) by reducing acid production, protecting the stomach lining, and inhibiting the growth of Helicobacter pylori, a bacterium that is known to cause PUD. Review of several studies found that consuming flavonoid-rich foods, such as lemons, may help to prevent the development of PUD. While more research is needed in humans, these findings suggest that incorporating lemons into your diet may be a helpful antiulcer agent. However, it is important to note that consuming too much lemon juice may aggravate symptoms in those with acid reflux or GERD, so moderation is key. [5,7]

Thus the present study aims to recognize the mechanism of action of a few antiulcer Phytoconstituents obtained from *Citrus lemon* with the computational approach of phytochemical search, molecular docking simulation to predict the pocket region of the protein. [8]

MATERIAL AND METHODS

Software and Tools

Protein Data Bank (PDB), PubChem, Zinc 15, ChemDraw Ultra 12.0, Auto Dock 1.1.2, Discovery Studio Visualizer, PyRx. [9]

Docking studies

Ligand preparation: The 2D and 3D structures of the Phytoconstituents were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in Sdf format. The open babel in PyRx software was used to convert a sdf file into a pdb file. [10]

Protein preparation: The protein was retrieved from PDB [Protein data base] (https://www.rcsb.org/).in PDB format. Before docking, the protein was prepared by removing all the heterogeneous atoms, adding polar hydrogen atoms and by removing water molecules. Receptor files in the PDB file format are converted to PDBQT file format using the PyRx or Open Babel GUI program. [11,12]

Docking studies using Auto dock vina

Using Auto Dock Vina 1.1.2, low-energy compounds in the pdbqt format were simulated molecular docking with target proteins. With a box spacing of 1 Å, the docking process is conducted in receptors. This aims to keep the receptors stiff and ligand flexible because the molecules that interact remain flexible. A binding affinity score (kcal/mol) is generated from the interaction energy between the ligand and receptor for each binding site. [13,14]

Protein–ligand interactions:

The Discovery studio visualizer program was employed to explain interactions between target receptors and ligands. 2D interactions were used to visualize docking result files. Receptor ligand interactions are able to assess intermolecular interactions, such as hydrogen and hydrophobic bonding types that attach to the receptor binding pocket's active side. [15]

RESULTS AND DISCUSSION

Ligand preparation:

PubChem software was used to study the physicochemical properties of selected active compounds. Details of all phytoconstituent are shown in Table 1

Table 1: Physicochemical properties of ligands

Sr. No	Molecule Name	Pub-chem ID	Molecular formula	Molecular weight ($gmol^{-1}$)	Hydrogen bond donors	Hydrogen bond acceptor	Rotatable bond count	X logp
1.	Alpha-thujene	17868	$C_{10}H_{16}$	136.23	0	0	1	2.8
2.	Beta-bergamotene	6427473	$C_{15}H_{24}$	204.35	0	0	3	5.1
3.	Citral	638011	$C_{10}H_{16}O$	152.23	0	1	4	3
4.	Limonene	22311	$C_{10}H_{16}$	136.23	0	0	1	3.4
5.	Ascorbic acid	54670067	$C_6H_8O_6$	176.12	4	6	2	-1.6

Docking studies using Auto-dock vina

Docking of phytoconstituent into PDB structure of D-alanine-D-alanine ligase receptor (PDB ID: 2PVP)

Data on the output of ligand and receptor interactions of Alpha thujene, Beta-bergamotene, Citral, Limonene and Ascorbic acid ligands against the D-alanine-D-alanine ligase receptors had similarities in amino acids VAL 213, PHE 290, PHE 209, LEU 145, ILE 210, ILE 169. The docking molecular evaluation of the binding activity score can be concluded that Bea-bergamotene has a value of -5.5 kcal/mol better compare to other ligands. This can explain that Beta-bergamotene has activity as an anti-ulcer compound through the inhibitory mechanism of D-alanine-D-alanine ligase receptor. Phytoconstituents bound to the target receptor protein and rated based on their docking results. For a detailed review, refer to (Table 2). All compounds were chosen based on ligand protein binding interactions. (Figure 1)

Docking of phytoconstituent into PDB structure of H2 receptor (PDB ID: 7UL3)

Data on the output of ligand and receptor interactions of Alpha thujene, Beta-bergamotene, Citral, Limonene and Ascorbic acid ligands against the H2 receptors had similarities in amino acids TYR 250, TRP 247, CYS 102. Evaluation of docking scores for binding activity interactions between Alpha-thujene ligands and H2 receptors have a strong binding affinity with a score of -6 kcal/mol, stronger than other ligands. This data can reinforce the reason that Alpha-thujene compounds have anti-ulcer activity through the mechanism of H2 receptor inhibition. Phytoconstituents bound to the target receptor protein and rated based on their docking results. For a detailed review, refer to (Table 2). All compounds were chosen

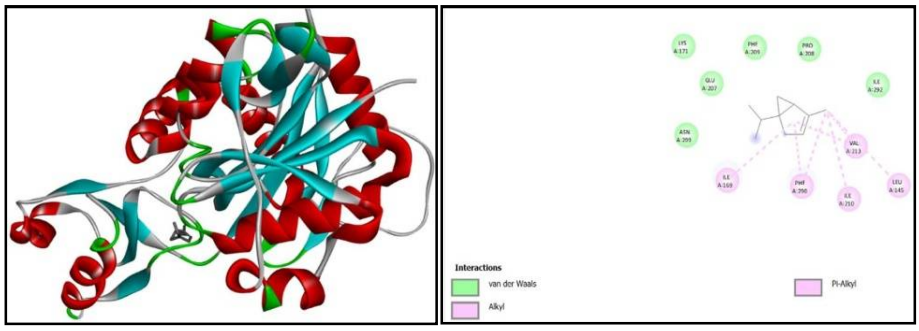
based on ligand protein binding interactions. (Figure 2)

Docking of phytoconstituent into PDB structure of CeU receptor (PDB ID: 4INP)

Data on the output of ligand and receptor interactions of Alpha thujene, Beta-bergamotene, Citral, Limonene and Ascorbic acid ligands against the CeU receptor target had many similarities in the interactions of amino acid PHE 123, ILE 284, LEU 208. While, other ligands contain few amino acid's interaction with the receptors. The docking molecular evaluation of the binding activity score can be concluded that Beta-bergamotene has a value of -6.6 kcal/mol better compare to other ligands. This can explain that Beta-bergamotene has activity as an anti-ulcer compound through the inhibitory mechanism of CeU receptor. Phytoconstituents bound to the target receptor protein and rated based on their docking results. For a detailed review, refer to (Table 2). All compounds were chosen based on ligand protein binding interactions. (Figure 3)

Table 2: Binding energy of ligand with receptors

Sr. No.	Ligands	Receptor	BindingAffinity	RMSD
1	Alpha-thujene	D-alanine-D-alanine Ligase (2PVP)	-5.2	0
		H2 (7UL3)	-6	0
		CeU(4INP)	-5.5	0
2	Beta-bergamotene	D-alanine-D-alanine Ligase (2PVP)	-5.5	0
		H2 (7UL3)	-5.5	0
		CeU(4INP)	-6.6	0
3	Citral	D-alanine-D-alanine Ligase (2PVP)	-5	0
		H2 (7UL3)	-5.4	0
		CeU(4INP)	-5.5	0
4	Ascorbic acid	D-alanine-D-alanine Ligase (2PVP)	-5.2	0
		H2 (7UL3)	-5.2	0
		CeU(4INP)	-5.6	0
5	Limonene	D-alanine-D-alanine Ligase (2PVP)	-5.1	0
		H2 (7UL3)	-5.8	0
		CeU(4INP)	-5.8	0



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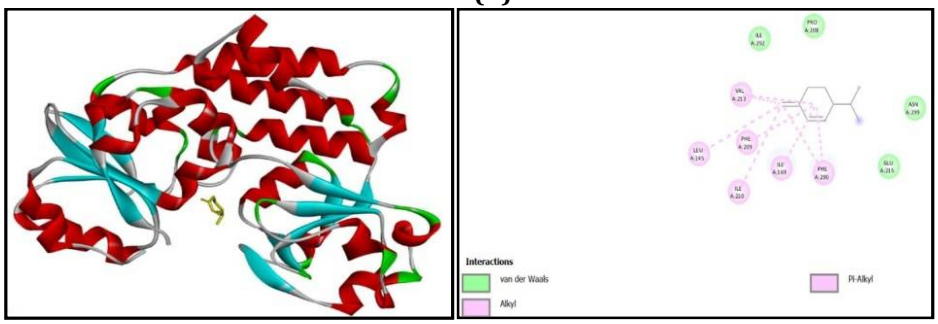

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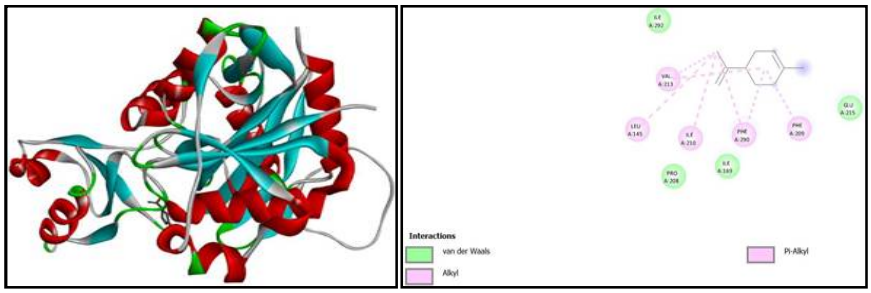

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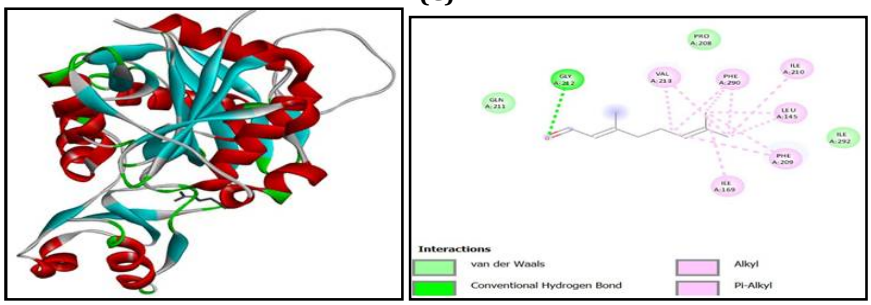

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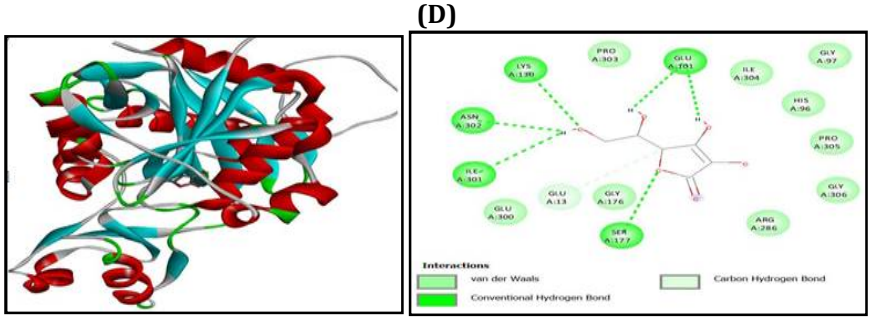

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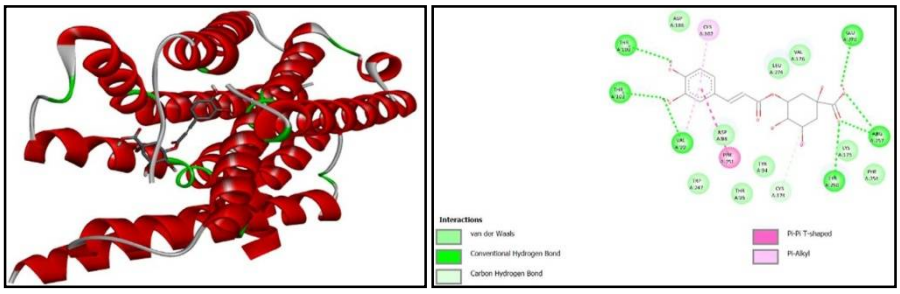
Figure 1: Interaction of ligands with D-alanine-D-alanine Ligase (2PVP) receptor (A) Alpha thujene (B) Beta-bergamotene (C) Limonene (D) Citral (E) ascorbic acid



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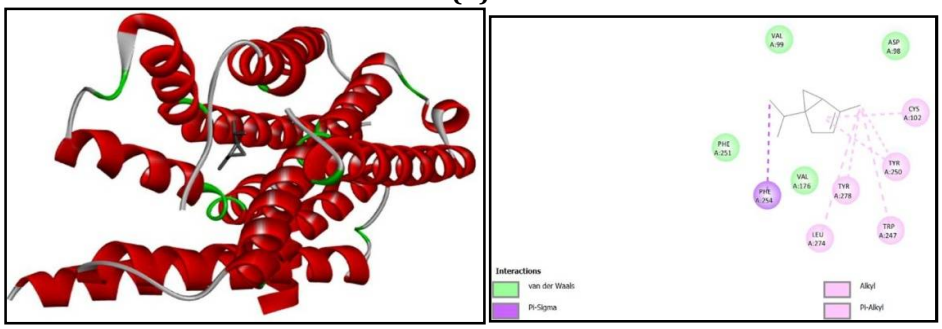

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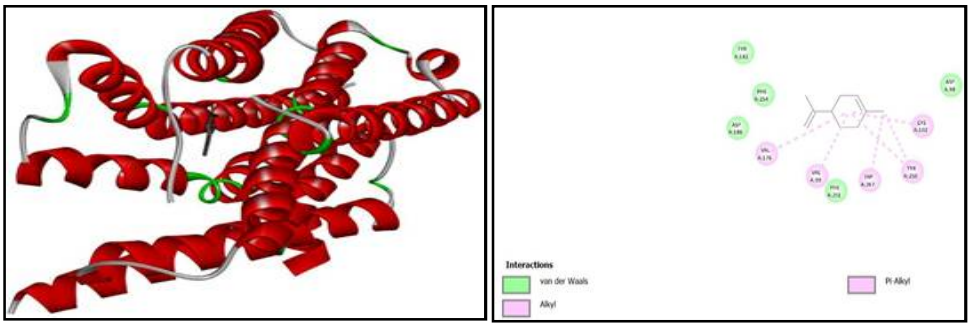

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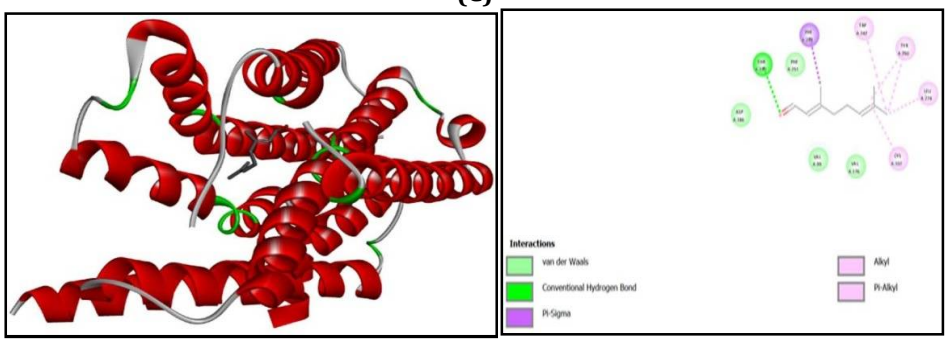

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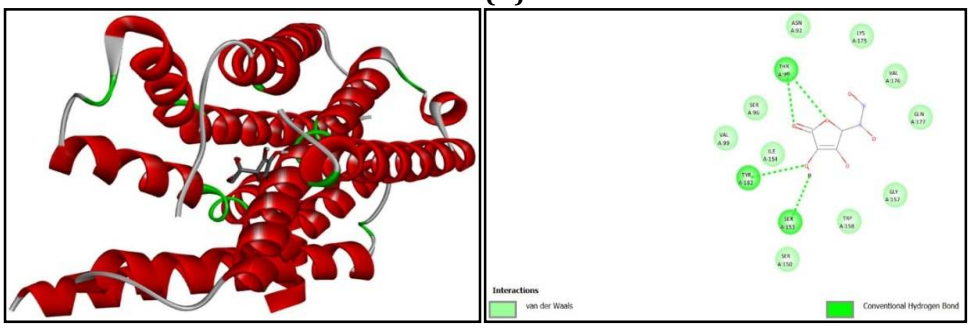

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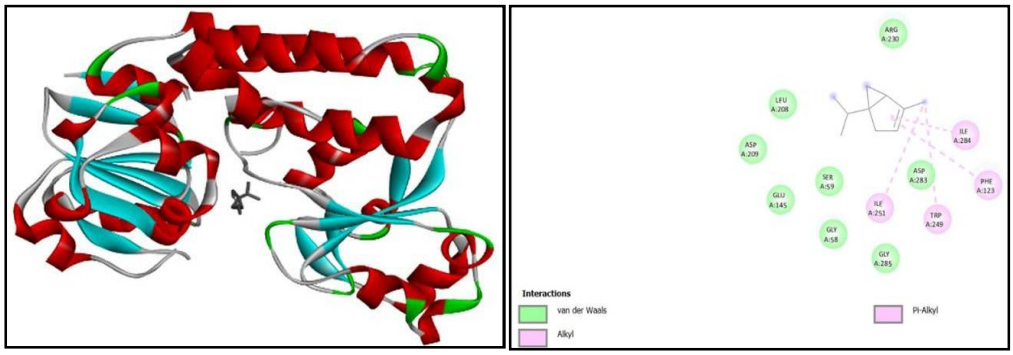
Figure 2: Interaction of ligands with H2 receptor (7UL3). A) Alpha thujene (B) Beta-bergamotene (C) Limonene (D) Citral (E) ascorbic acid



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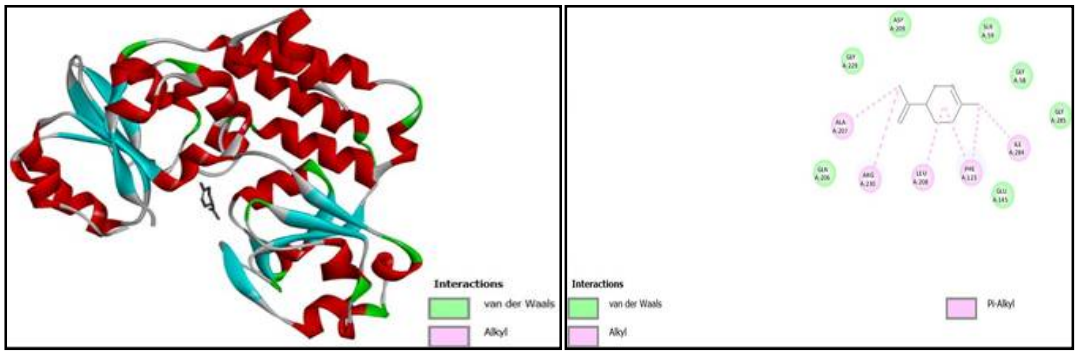

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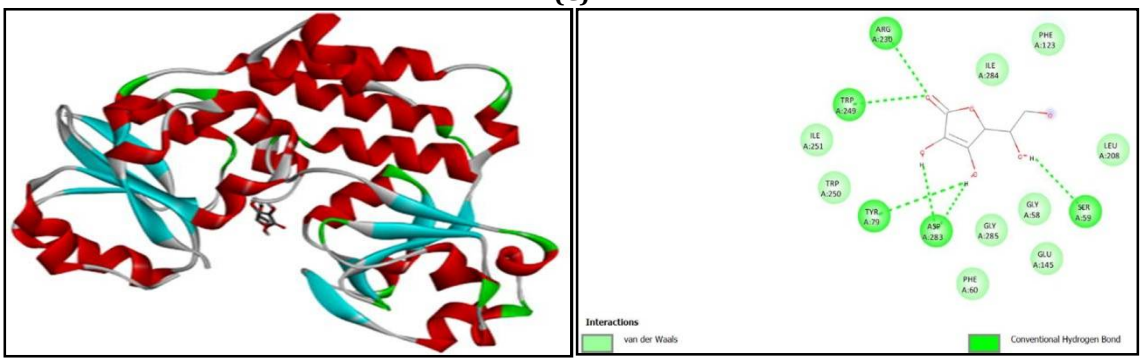

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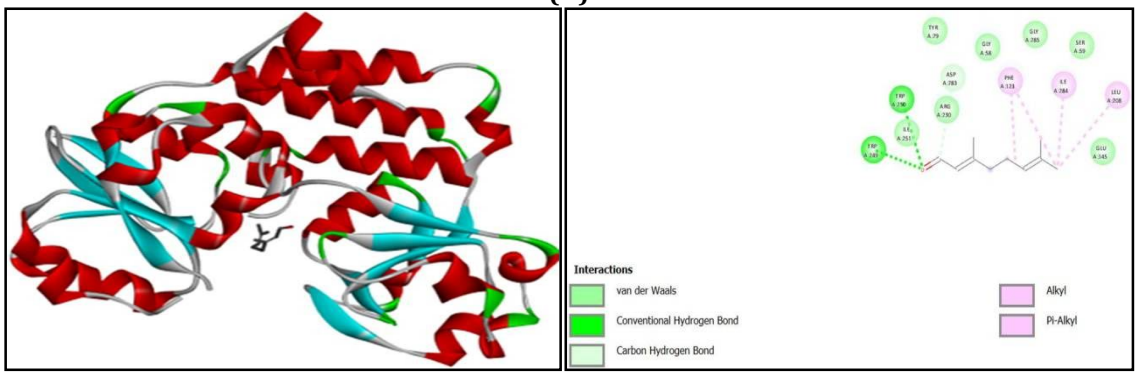

(E)

Figure 3: Interaction of ligands with CeU(4INP) receptor. A) Alpha thujene (B) Beta-bergamotene (C) Limonene (D) Citral (E) ascorbic acid

CONCLUSION

The docking study of lemon as an antiulcer agent has provided valuable insights into the potential mechanisms of action of lemon compounds in inhibiting gastric ulceration. The results of this study suggest that the active compounds in lemon like Isopugeol, alpha- bergamontene, alpha-piene, alpha-terpiene, alpha-thujene, beta-bisolobene, beta- bergamontene, beta-phellandrene, citral, limonene, labinene, pectin, vitamin C (ascorbic acid),may act by binding to key enzymes and receptors. D-alanine-D-

alanine ligase (2PVP), H2 (7UL3) and CeU (4INP) receptor involved in promoting ulceration, thereby preventing their activity can reduce the risk of ulcer formation. We found 5 molecules having highest binding affinity (ΔG) i.e., largest negative value in kcal/mol in efficacious interaction with D-alanine-D-alanine ligase (2PVP), H2 (7UL3) and CeU (4INP) receptor. Among them, the strongest binding affinity is shown by alpha-thujene with H2 receptor (7UL3) and Bea-bergamotene with D-alanine-D-alanine Ligase (2PVP) and CeU receptor (4INP). The use of in-silico methods provides a promising approach to screen potential antiulcer agents from natural sources, which can be further explored through experimental and clinical studies. Overall, the results of this study suggest that lemon can be a useful dietary supplementin the management of gastric ulcers.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest regarding the publication of this research paper.

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