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Research Article

IN-SILICO STUDY TO UNDERSTAND THE MOLECULAR MECHANISM OF PURGATIVE ACTION OF IDENTIFIED PHYTOCHEMICAL COMPOUNDS IN *TRIVRT* ROOT (*OPERCULINA TURPETHUM* (L.) SILVA MANSO) THROUGH ADME SCREENING AND MOLECULAR DOCKING

Swethasree K.S[1]*, Deepu Mathew[2], Priyalatha B[3], Vimala K.S[3], Priya S[4]

*[1]PG Scholar, [3]Professor, [4]Professor & HOD, Department of Dravyaguna Vigyana, Amrita School of Ayurveda, Amrita Vishwa Vidyapeetham, Amritapuri, Kollam, Kerala, India.

[2]Professor, Centre for Plant Biotechnology & Molecular Biology, KAU, Thrissur, Kerala, India.

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ABSTRACT

The molecular mechanism behind the pharmacological action of a drug can be analyzed through In-silico studies, which utilize computational techniques to predict interactions, efficacy, and safety profiles. This study investigates the purgative action of *Trivrt* (*Operculina turpethum* (L.) Silva Manso), a perennial twinner in the Convolvulaceae family, through molecular docking techniques and bioinformatics tools. Detailed in Ayurvedic texts, *Trivrt* root is lauded for its efficacy in *Virecana karma*, a therapeutic approach for eliminating vitiated *Pitha dosha*. To identify bioactive phytocompounds from *Trivrt* and their interactions with gastrointestinal motility receptors, comparing these with commercial laxatives through In-silico analysis. Findings indicate that the purgative effects are mediated by compounds such as Operculinosides D, Betulin, and Luteolin, which influence neurohumoral signals and smooth muscle contraction/relaxation via specific receptors. The results underscore the potential of *Trivrt* root's phytocompounds as viable therapeutic agents in gastrointestinal motility, paving the way for future drug discovery endeavors.

INTRODUCTION

Trivrt (*Operculina turpethum* (L.) Silva Manso is a perennial twiner, belonging to the family Convolvulaceae. *Acarya Caraka* explains elaborately about 110 recipes of *Trivrt* in "*Shyamā Trivrt Kalpa Adhyaya"* of *Kalpasthana*. The roots of this plant used in many of the formulations in Ayurveda & according to *Acarya*, *Trivrt* root is the best drug for *Virecana karma* (one among *Panca Shodhana* therapy) and advises the use of *Trivrt* root for *Ritu Shodhana* also.

Virecana karma is a *Shodhana* therapy used for eliminating vitiated *Pitha Dosha*. According to Ayurveda, drugs having *Ushna, Tikshna, Sara, Sukshma, Vikashi, Vyavayi guṇa* are used for *Virecana karma* which is comparable to purgation in modern system of medicines. *Trivrt* (*Operculina tupethum* L. Sila manso) also possess *Tikshna, Ruksha, Laghuguna, Ushna Virya* and is *Pithahara, Recana, Jvarahara, Vatala, Sukha Virecana* and *Kapha Pithahara* in action.

As per Literature review *Trivrt* root possesses anti-inflammatory, laxative, anti-pyretic, anti-cancer, hepato-protective, ulcer protective, anti-bacterial, anti-arthritic. In modern system of medicine, purgation is used to treat various medical conditions related to bowel movements and digestion and is characterized by vigorous evacuation of bowels by a cathartic/ purgative drug. As per modern literature, contraction and relaxation of smooth muscles located at various parts of GIT and the receptors of neurohormonal substances present at different sites, like stomach, small intestine, large intestine are responsible for purgative action. The stimulatory and inhibitory action of smooth muscles by the activation of neurotransmitter signals by the receptors are said to be responsible for the purgative action

Computational techniques, particularly molecular docking, are essential in drug discovery, enabling the design of new drugs and potent phytocompounds by predicting their interactions with target proteins. This study employs molecular docking to identify bioactive phytocompounds from *Trivrt*

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(*Operculina turpethum*) that stimulate gastrointestinal motility, comparing their docking scores with those of commonly prescribed commercial laxatives. By utilizing bioinformatics tools, such as molecular docking, the study screens *Trivrt's* phytocompounds for medicinal properties, facilitating a rapid understanding of their mechanisms of action.

METHODOLOGY

Identification of ligands

A thorough literature survey reveals that *Operculina turpethum* contains a variety of bioactive compounds, many of which have potent pharmacological effects. To further explore the chemical diversity of this plant, metabolic profiling was conducted using LC- MS (Liquid Chromatography-Mass Spectrometry) on samples collected from four locations across the Western Ghats of India: Manoli, Kodkani, Nadpal, and Wayanad. The analysis identified a total of 115 compounds, with 35 compounds identified as ligands that were common across all four locations. These compounds are potentially responsible for the medicinal activities of the plant and could be of interest for further pharmacological investigations

Ligand selection

The 3D structures of the ligands identified from *Operculina turpethum* (L.) Silva Manso were downloaded from the PubChem database and saved in the SDF (Structure-Data File) format. The SDF format is commonly used for storing molecular structures and associated data, making it a suitable format for computational studies, such as molecular docking, structure-activity relationship analysis, or further pharmacological investigations.

Ligand filtration

The 3D structures of ligands from *Operculina turpethum* were obtained from the PubChem database in SDF format, which is suitable for computational analyses. These structures can be used in molecular docking studies to simulate interactions with biological targets, aiding in the prediction of binding affinities and activities. Additionally, the SDF files facilitate structure-activity relationship (SAR) analyses, helping to identify chemical features responsible for pharmacological effects. This information is valuable for further pharmacological investigations, including assessments of bioavailability, efficacy, and toxicity profiles. Such comprehensive analyses contribute to the optimization of drug design and development processes.

Protein selection

The peristaltic reflex in the intestine starts with mucosal stimulation that triggers the release of serotonin (5-HT) from enterochromaffin cells. This serotonin activates both intrinsic and extrinsic primary afferent neurons (PAN) in the enteric nervous system (ENS) via the 5-HT4 receptor. The extrinsic PAN send signals to the central nervous system (CNS) through the vagus nerve, which can lead to vomiting when the stimulation is strong. The intrinsic PAN communicate with excitatory and inhibitory interneurons in the ENS to coordinate muscle contractions and relaxation, using neurotransmitters like acetylcholine (Ach) and nitric oxide (NO). Additionally, dopamine (DA) influences smooth muscle contraction by acting on dopamine receptors and muscarinic M3 receptors. The targets selected for the interaction study are:

1. 5-HT4- (5-Hydroxytryptamine receptor 4)
2. 5-HT2A-(5-Hydroxytryptamine receptor 2A)
3. 5-HT2B-(5-Hydroxytryptamine receptor 2B)
4. 5-HT7-(5-Hydroxytryptamine receptor 7)
5. 5-HT1A-(5-Hydroxytryptamine 1A receptor)
6. M1-(Muscarinic acetylcholine receptor M1)
7. M3-(Muscarinic acetylcholine receptor M3)
8. A1-(Adenosine receptor A1)
9. A2-(Adenosine receptor A2)
10. D2-(Dopamine Receptor 2)

Retrieval of required protein files from database

All the structures of purgative targets were retrieved from the Protein Data Bank (PDB) and downloaded in the PDB (Protein Data Bank) format.

Commercially available drugs selected for ADME screening and docking for the comparative study:

- Bisacodyl
- Docusate sodium
- Lactulose
- Methyl cellulose
- Sorbitiol

Molecular docking using CB-Dock

CB-Dock is a web server designed for blind docking, utilizing curvature-based cavity detection (Cur Pocket) and AutoDock Vina to predict protein binding sites and simulate ligand binding poses. It simplifies drug discovery by automatically identifying binding sites and evaluating ligand-protein interactions. Users upload protein files in PDB format and ligand files in SDF format to perform docking and receive the best ligand binding poses. The platform aids researchers in optimizing ligand interactions with target proteins, enhancing drug design efficiency.

Visualization and analysis of docked structure using PyMOL Visualization software

After docking ligand-protein complexes using CB-Dock, the complex with the highest Vina score was selected for detailed analysis. The presence and

number of hydrogen bonds were assessed to evaluate interaction stability, and their distances to interaction residues were measured for spatial and binding effectiveness. The binding site analysis identified key residues contributing to binding affinity and specificity. Visual representations of the docked complex, including hydrogen bonds and interaction residues, were saved as PNG images for documentation.

Results

Filtration of ligand molecule and commercial drugs

Thirty-five phytocompounds and five commercial drugs were evaluated using Swiss ADME software for lipophilicity, solubility, G.I. absorption, Lipinski's rule, and bioavailability. Among the phytocompounds, 28 passed all parameters, with 22 showing high G.I. absorption and solubility ranging from very soluble to poorly soluble. Four commercial drugs met most criteria, with Bisacodyl and Docusate sodium adhering to Lipinski's rule, while Lactulose exhibited high solubility but failed due to two violations.

Docking results of chosen target protein involved in the purgation with phytocompounds in *Trivrt* root

Interaction of phytocompounds with target 5HT4-5-Hydroxytryptamine receptor 4 PDB ID-7XT8

During molecular docking, one Compound (lanosta-5-ene) does not show any interaction with the target. Out of 34 Phytocompounds, Operclinosides D showed lowest or best vina score, -10. The interacting active residue site of Operclinosides D with the target are ASN-237, HIS-62, ASP-322ASP-322, SER-191 with number of hydrogen bonds 8 having lengths 2.1, 2.3, 2.5, 2.6, 2.8Å.

Interaction of phytocompounds with target D2-Dopamine Receptor 2, PDB ID-6VMS

During molecular docking, revealed no interaction for two compounds. Among the 33 interacting compounds, Operculinosides B showed the best binding affinity with a vina score of -10. It interacted with active site residues LEU-152, VAL-276, ARG-150, PHE- 234, LEU-90, and ARG-214 through six hydrogen bonds, with lengths ranging from 2.2 to 2.7 Å.

Interaction of phytocompounds with target 5HT1A-Serotonin 1A receptor, PDB ID-7E2X

Docking of 35 phytocompounds, showed no interaction for two compounds. Among the 33 interacting compounds, Betulin exhibited the strongest binding affinity with a vina score of -10.2. It interacted with active site residues LEU-318 and SER-147, forming two hydrogen bonds with lengths of 2.6 Å and 3.1 Å.

Interaction of phytocompounds with target 5HT2A-5-Hydroxytryptamine receptor 2A, PDB ID-P28223.

Molecular docking of 35 phytocompounds revealed no interaction for two compounds. Among the 33 interacting compounds, Lupeol showed the best binding affinity with a vina score of -11.6. It interacted with the active site residue SER-131, forming one hydrogen bond with a length of 2.7 Å.

Interaction of phytocompounds with target, 5HT2B-5-Hydroxytryptamine receptor 2B, PDB ID-P41595.

Docking of 35 phytocompounds, showed no interaction for two compounds. Among the 33 interacting compounds, Luteolin exhibited the strongest binding affinity with a vina score of -9.4. It interacted with active site residues ASP-135, ALA-187, ASN-344, and SER-222, forming four hydrogen bonds with lengths of 2.4 Å, 2.8 Å, 3.1 Å, and 3.2 Å.

Interaction of phytocompounds with target, 5HT7-5-Hydroxytryptamine receptor 7 AF-P34969.

Molecular docking of 35 phytocompounds, revealed no interaction for two compounds. Among the 33 interacting compounds, Luteolin exhibited the highest binding affinity with a vina score of -9.8. It formed interactions with active site residues ASP-162, VAL- 163, THR-167, THR-240, and GLN-235 through five hydrogen bonds, with bond lengths of 2.3 Å, 2.5 Å, 3.0 Å, 3.0 Å, and 3.4 Å.

Interaction of phytocompounds with target, M3-Muscarinic acetylcholine receptor M3(PDB ID-4DAJ)

Docking of 35 phytocompounds, revealed no interaction for two compounds. Among the 33 interacting compounds, Operculinosides D, Turpethic acid B, and Turpethic acid C showed the highest binding affinities, each with a vina score of -10.2. Operculinosides D interacted with residues ASP-517, GLU-256, and LYS-220, forming three hydrogen bonds with lengths of 2.2 Å, 2.3 Å, and 2.8 Å. Turpethic acid B interacted with residues TYR-506, ARG-252, TYR-148, and LYS-254 through four hydrogen bonds, measuring

1.9 Å, 2.5 Å, 2.6 Å, and 2.7 Å. Turpethic acid C formed three hydrogen bonds with residues ARG-171 and LYS-1065, with bond lengths of 2.0Å, 2.5Å, and 2.5Å. These results highlight these compounds as promising candidates for further investigation targeting the M3 receptor.

Interaction of phytocompounds with target, A1-Adenosine receptor A1, AF- P30542

Molecular docking of 35 phytocompounds, revealed no interaction for four compounds. Among the remaining 31 interacting compounds, cycloartenol

exhibited the best binding affinity with a vina score of -10. It interacted with the active site residue ARG-208, forming a single hydrogen bond with a length of 2.6Å.

Interaction of phytocompounds with target, A2- Adenosine receptor A2 AF-X5DNB4

Docking of 35 phytocompounds, showed no interaction for two compounds. Among the 33 interacting compounds, Operculinoside A demonstrated the best binding affinity with a vina score of -8.9. It interacted with the active site residues ASN-42, ARG-102, and ASN-39, forming five hydrogen bonds with bond lengths of 1.7Å, 2.3Å, 2.3Å, 2.4Å, and 2.8Å.

Interaction of phytocompounds with target, M1- Muscarinic acetylcholine receptor M1, AF-P11229

Molecular docking of 35 phytocompounds, revealed no interaction for five compounds. Among the 30 interacting compounds, Operculinoside D exhibited the best binding affinity with a vina score of -9.3. It interacted with the active site residues GLN-177, ASP-393, TYR-85, and GLU-397, forming three hydrogen bonds with lengths of 1.9Å, 2.4Å, 2.5Å, and 2.8Å.

Docking results of commercial drug molecules with target proteins responsible for purgation

Molecular docking analysis of Bisacodyl, compared with five commercial drugs, showed strong binding across various receptor targets. For the 5HT4 receptor, Bisacodyl had a vina score of -8.6, forming four hydrogen bonds with residues ARG-150, LYS-14, SER- 191, and ARG-22. It also demonstrated a vina score of -8.4 with the D2-Dopamine Receptor 2, -7.7 with the 5HT7 receptor, and -7.8 with the 5HT2A receptor, interacting with various residues through hydrogen bonds. Bisacodyl exhibited its strongest binding with the 5HT2B receptor, achieving a vina score of -9, forming seven hydrogen bonds. Additionally, Bisacodyl showed high affinity with the A1-Adenosine receptor, A2- Adenosine receptor, and M1 and M3 Muscarinic receptors, further emphasizing its potent interactions with multiple targets.

Table 1: High vina scores obtained by the interaction of phytocompounds of *Operculina turpethum* (L.) Silva Manso with target proteins of purgation.

S.No.	Target	Compound Name	Vina score
1.	5HT4- 5-Hydroxytryptamine receptor 4 PDB ID-7XT8	Operculinosides D PubChem CID- 54671548	-10
2.	D2 Dopamine Receptor 2 PDB ID-6VMS	Operculinosides B PubChem CID- 54671548	-10
3.	M3 Muscarinic acetylcholine receptor M3 PDB ID- 4DAJ	Betulin PubChem CID-72326	-10.2
4.	5HT7- 5-Hydroxytryptamine receptor 7 AF-P34969	Luteolin PubChem CID- 5280445	-9.8
5.	5HT1A Serotonin 1A receptor PDB ID-7E2X	Betulin PubChem CID-72326	-10.2
6.	5HT2A 5-Hydroxytryptamine receptor 2A PDB ID-P28223	Lupeol PubChem CID-259846	-11.6
7.	5HT2B 5-Hydroxytryptamine receptor 2B PDB ID-P41595	Luteolin PubChem CID-5280445	-9.4
8.	A1 Adenosine receptor A1 AF-P30542	Cycloartenol PubChem CID- 92110	-10
9.	A2 Adenosine receptor A2	Operculinosides A	-8.9

	AF-X5DNB4	PubChem CID- IMPHY001133	
10.	M1 Muscarinic acetylcholine receptor M1 AF-P11229	 Operculinosides D PubChem CID- 54671548	-9.3

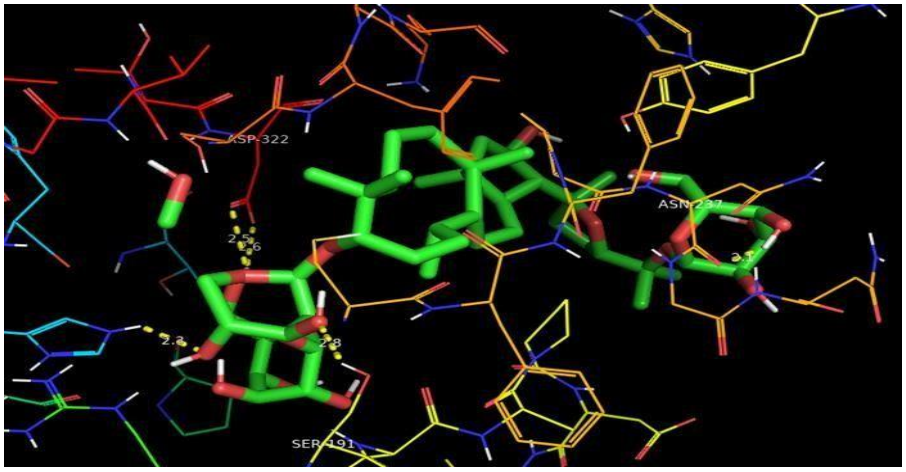

Figure 1: Docking image of phytocompound Operculinosides D with the target protein 5HT4,5-Hydroxytryptamine receptor 4

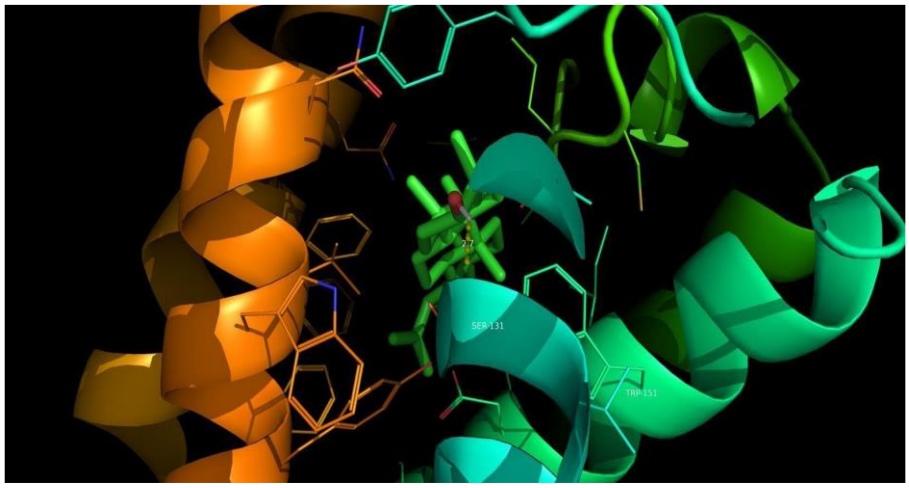

Figure 2: Docking image of phytocompound Lupeol with the target protein 5HT2A, 5-Hydroxytryptamine receptor 2A

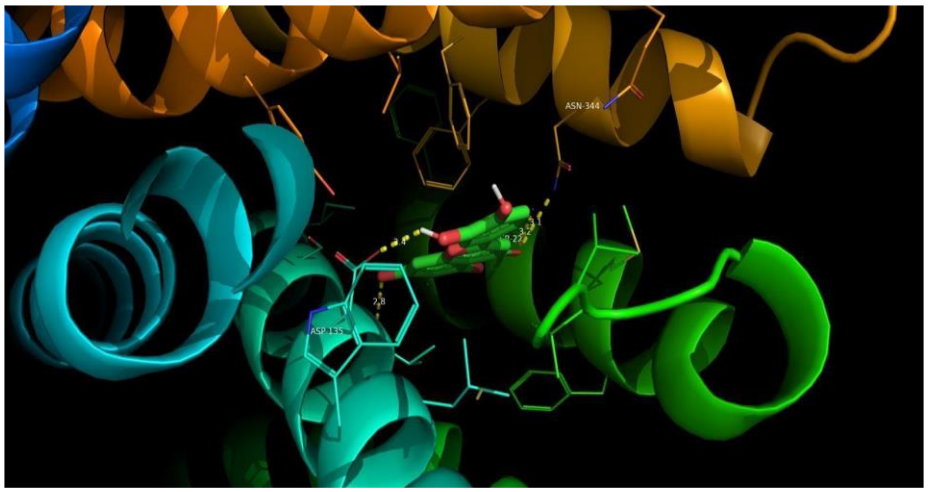

Figure 3: Docking image of phytocompound Luteolin with the target protein 5HT2B, 5-Hydroxytryptamine receptor 2B.

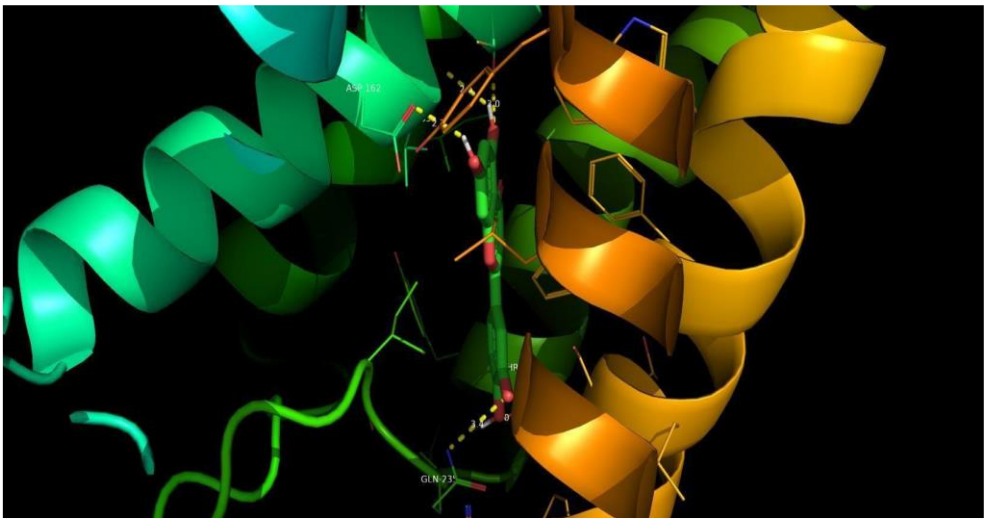

Figure 4: Docking image of phytocompound Luteolin with the target protein 5HT7- 5-Hydroxytryptamine receptor 7

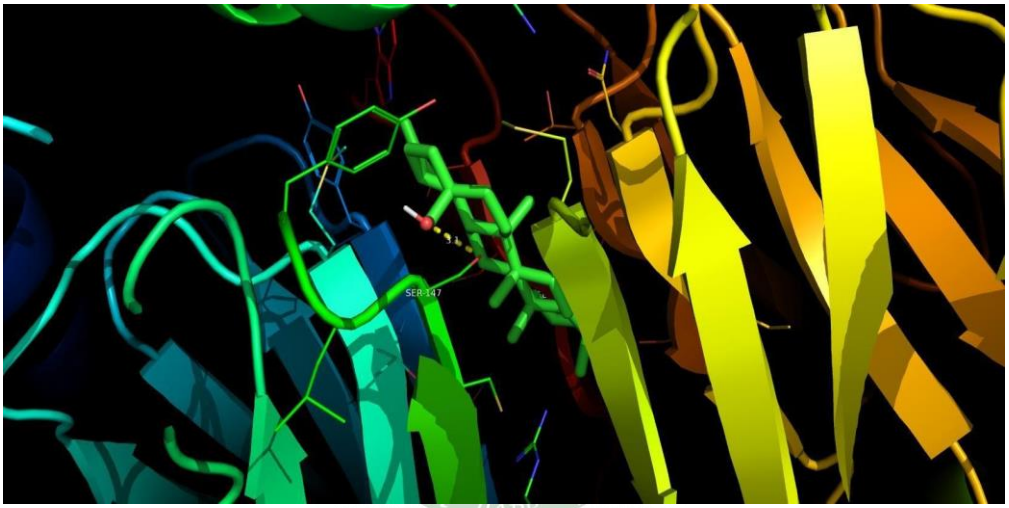

Figure 5: Docking image of phytocompound Betulin with the target protein HT1A, 5-Hydroxytryptamine 1A r eceptor

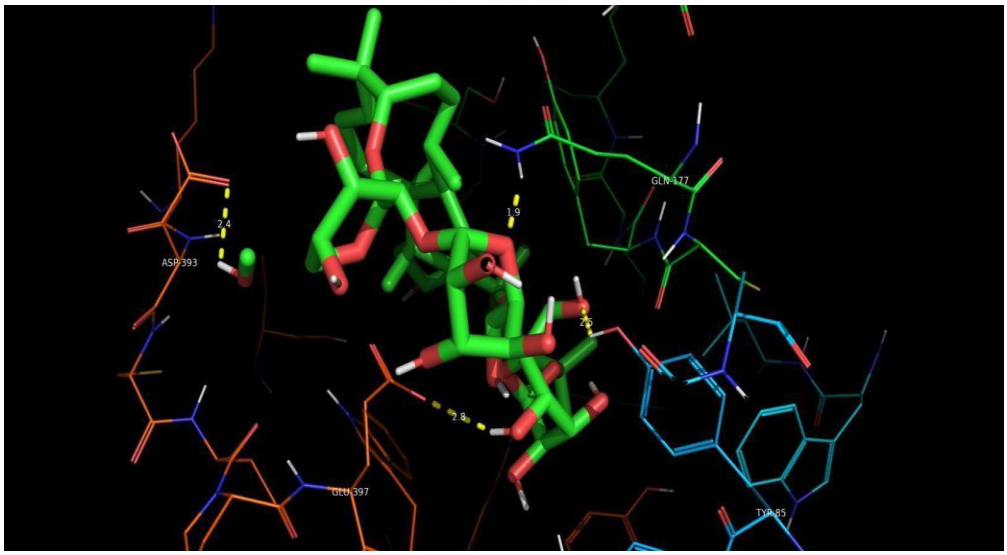

Figure 6: Docking image of phytocompound Operculinosides D with the target protein M1-Muscarinic acetylcholine receptor M1

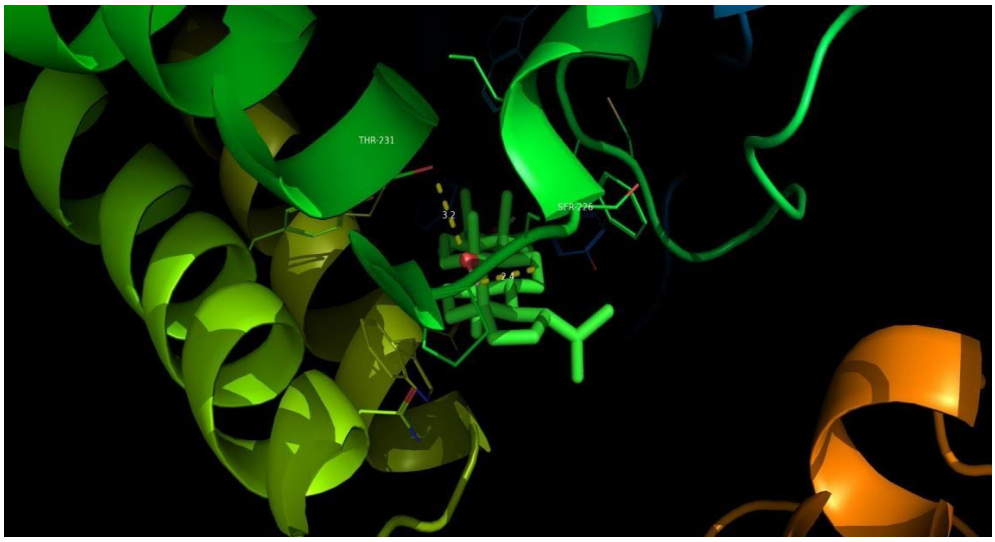

Figure 7: Docking image of phytocompound Betulin with the target protein M3- Muscarinic acetylcholine receptor M3

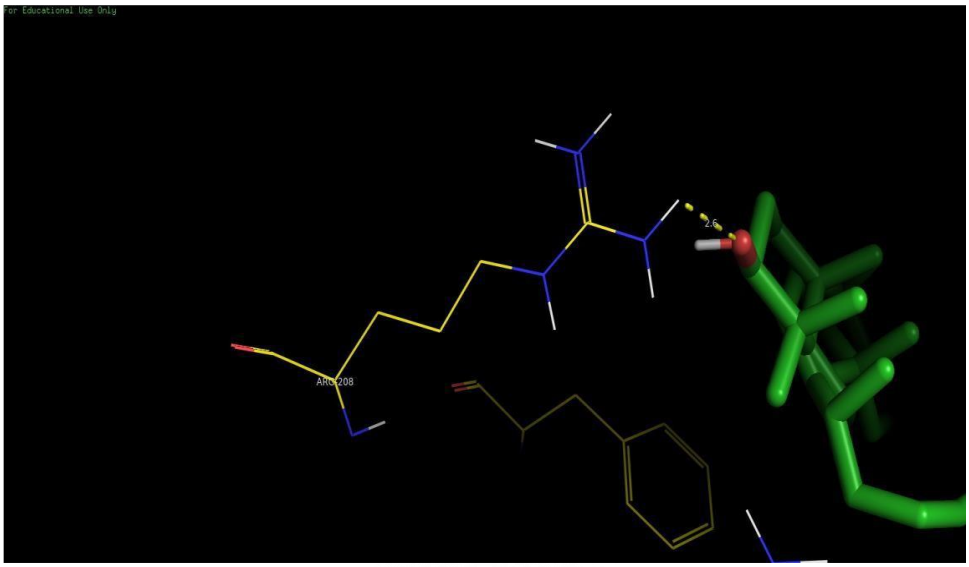

Figure 8: Docking image of phytocompound cycloartenol with the target protein A1 Adenosine receptor A1

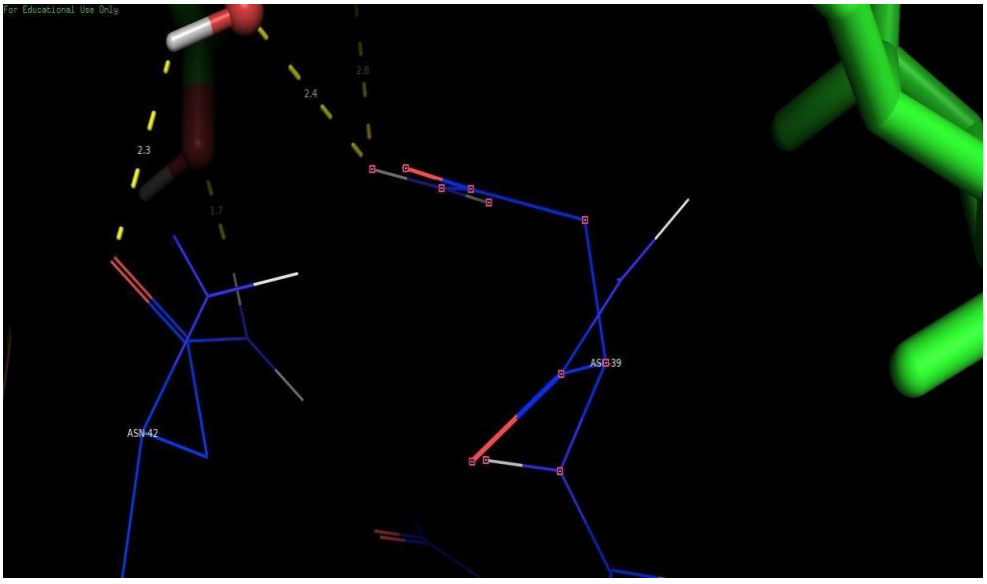

Figure 9: Docking image of phyocompound Operculinosides A with the target protein A2- Adenosine receptor A2

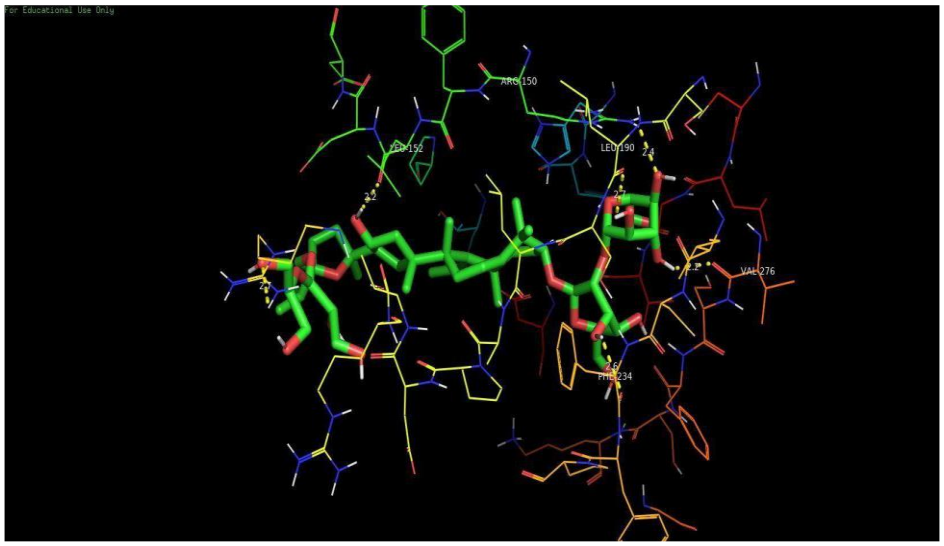

Figure 10: Docking image of phytocompound Operculinosides B with the target protein D2 Dopamine Receptor 2

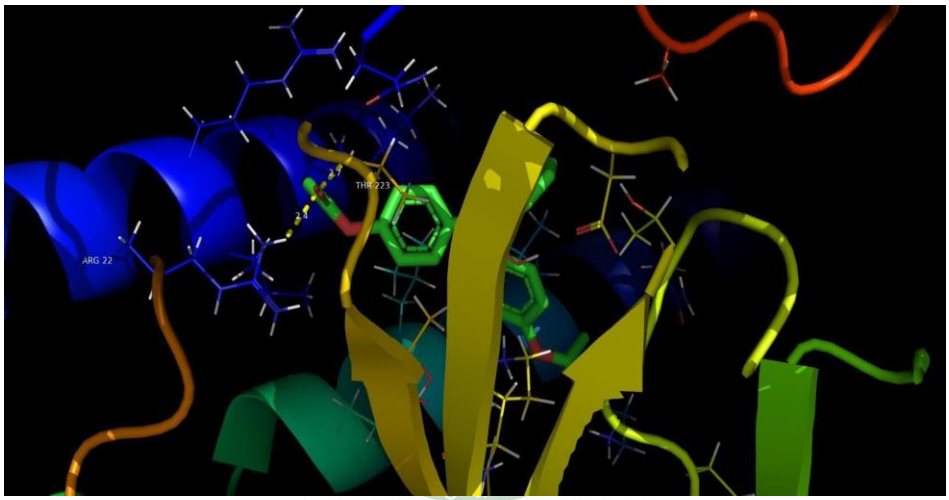

Figure 10: Docking image of phytocompound Bisacodyl with the target protein, 5HT4-5-Hydroxytryptamine receptor 4

DISCUSSION

In this study, *In-silico* docking analysis was employed to identify potent phytocompounds from the root of *Trivrt* (*Operculina turpethum*) and elucidate their mechanisms in promoting purgation. Ten protein targets involved in the purgative pathway were examined, with a focus on the 5-HT_4 receptor, a serotonin subtype crucial for gastrointestinal motility. Activation of 5-HT_4 receptors stimulates peristalsis and fluid secretion, facilitating bowel movements. The phytocompound Operculinosides D exhibited a strong interaction with the 5-HT_4 receptor, achieving a vina score of -10 and forming seven hydrogen bonds with the residue ASN-237. This interaction was superior to that of commercial drugs like Docusate sodium, Lactulose, Sorbitol, and Methyl cellulose, which showed lower vina scores and fewer interactions with ASN-237. These findings suggest that Operculinosides D may be a more effective stimulator of purgation by enhancing motility.

Additionally, the study explored interactions with other receptors involved in smooth muscle relaxation and contraction during purgation. Lupeol demonstrated a strong binding affinity for the 5-HT_2A receptor, while Luteolin interacted effectively with the 5-HT_2B receptor. Both compounds showed better docking scores compared to commercial drugs, indicating their potential as agents for smooth muscle relaxation. For receptors associated with muscle contraction, Betulin exhibited a strong interaction with the 5-HT_1A receptor, and Luteolin with the 5-HT_7 receptor, outperforming commercial drugs in binding affinity. Furthermore, Operculinosides D and Betulin showed promising interactions with M1 and M3 muscarinic receptors, which are involved in enhancing motility and stimulating gastric secretions. Overall, the comparative docking analysis highlighted the superior performance of *Trivrt* root phytocompounds over commercial laxatives in binding affinity, suggesting their potential as more effective agents for purgation.

The comparative docking analysis highlights the

superior performance of *Trivrt* (*Operculina turpethum*) root phytocompounds over commercial laxatives in binding affinity. For the 5HT4 receptor, Trivrt's Operculinosides D achieved a vina score of -10, surpassing Bisacodyl's -8.4. Similarly, Lupeol and Luteolin showed better scores (-11.6 and -9.8) against 5-HT2B and 5-HT2A than Bisacodyl (-7.8 and -9). Betulin and Luteolin also outperformed Bisacodyl in 5-HT1A and 5-HT7 docking (-10.2 and -9.8 vs. -8.3 and -7.7). M1 and M3 receptors, Operculinosides D and Betulin (-9.3 and -10.2) demonstrated better binding than Bisacodyl (-9 and -8.3). These findings suggest *Trivrt's* phytocompounds are more effective and could be preferred for purgation.

CONCLUSION

The dissertation titled "*In-silico* study to understand the molecular mechanism of purgative action of identified phytochemical compounds in Trivrt root (*Operculina turpethum* (L.) Silva Manso) through ADME screening and molecular docking" concluded that the purgative mechanism of *Trivrt* root is a synergistic effect of its phytocompounds. Operculinosides D activates neurohumoral signals through 5-HT_4 receptors, enhancing peristaltic reflex via acetylcholine, CGRP, NANC, and NO release. Betulin and Luteolin facilitate smooth muscle contraction by targeting 5-HT_1A and 5-HT_7 receptors, while Lupeol and Luteolin promote relaxation by stimulating 5-HT_2A and 5- HT_2B receptors. Operculinosides D and Betulin also enhance motility, gastric secretions, and neural activity via M1 and M3 receptors. ADME screening revealed that seven phytocompounds (Operculinosides A-D and Turpethic acids A-C) from *Trivrt* root and one commercial drug (lactulose) did not, deeming it unsuitable for standalone drug development. This study highlights the potential of *Trivrt* root phytocompounds as effective agents in purgative mechanism.

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***Address for correspondence**
Dr. Swethasree K. S
PG Scholar,
Department of Dravyaguna Vigyana,
Amrita School of Ayurveda, Amrita Vishwa Vidyapeetham, Amritapuri, India.
Email: swethasreerag@gmail.com