JOURNAL MEDICAL INFORMATICS TECHNOLOGY
medinftech.org ISSN 2988-7003 (online)

Computational Pharmacology Approach to Identify Antidiarrheal Candidates from *Eleusine indica* L.: Molecular Docking Simulation and Drug-likeness Prediction Targeting 5ZHP Receptor

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ARTICLE HISTORY
Received: 09 May 26
Final Revision: 06 June 26
Accepted: 14 June 26
Online Publication: 30 June 26

KEYWORDS
Antidiarrheal, Druglikeness, *Eleusine indica*, Molecular Docking, Muscarinic M3 Receptor

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DOI
10.37034/medinftech.v4i2.142

A B S T R A C T

Diarrhea remains a significant global health concern with high morbidity and mortality rates, particularly in developing countries. The use of synthetic antidiarrheal drugs is associated with various adverse effects, necessitating the exploration of safer therapeutic alternatives derived from natural sources. This study aimed to evaluate the potential of secondary metabolite compounds from *Eleusine indica* as antidiarrheal candidates through an in silico approach, employing drug-likeness analysis and molecular docking simulation against the muscarinic acetylcholine M3 receptor (PDB ID: 5ZHP). Drug-likeness analysis was performed using the SwissADME web tool based on Lipinski's Rule of Five. Molecular docking simulation was conducted using Molegro Virtual Docker, with the root mean square deviation (RMSD) value employed as a validation parameter. The results revealed that the majority of the compounds satisfied Lipinski's criteria, indicating their potential as oral drug candidates. Docking method validation yielded an RMSD value of 0.777437 Å, confirming the validity and reliability of the docking procedure. Docking results suggested that compound M15 (andrographolide) and compound M13 [4-(1-hydroxyethyl)-2,6-bis(3-methylbut-2-en-1-yl)phenol] exhibited the lowest Rerank Scores of −116.686 and −114.300, respectively, which were notably lower than that of the positive control loperamide (−41.9287), suggesting potentially stronger binding affinity and more favorable interaction with the target receptor. However, these findings are predictive in nature and require further validation through experimental biological studies and molecular dynamics simulations to confirm actual binding stability and pharmacological effectiveness.

1. Introduction

Diarrhea remains a major global public health problem, causing high morbidity and mortality, particularly in developing countries. According to the World Health Organization (WHO), it accounts for approximately 1.7 billion cases annually and nearly 525,000 deaths among children under five, making it the second leading infectious cause of child mortality [1], [2]. Clinically, diarrhea is defined as the passage of three or more loose stools per day resulting from impaired intestinal fluid balance [3]. In low- and middle-income countries, recurrent diarrhea contributes not only to dehydration and electrolyte imbalance but also to long-term consequences such as malnutrition, stunting, and cognitive impairment [4]. Its multifactorial etiology,

encompassing both infectious and non-infectious factors, complicates treatment strategies and increases the socioeconomic burden, particularly in regions with limited access to clean water and adequate healthcare services [5], [6].

Diarrhea may be caused by bacterial, viral, parasitic, and protozoal infections transmitted via the fecal–oral route, as well as other contributing factors such as medication use, food allergies, digestive and absorptive disorders, nutritional deficiencies, and psychological stress. The primary pathophysiological mechanisms underlying diarrhea include increased intraluminal osmotic pressure, which impairs water and electrolyte reabsorption, and enhanced fluid and electrolyte secretion, both of which may ultimately lead to dehydration and nutritional disturbances [7].

Muscarinic acetylcholine receptors (M-AChRs), particularly the M3 subtype, play a pivotal role in the pathophysiology of diarrhea-predominant irritable bowel syndrome (D-IBS). Elevated acetylcholine levels within the nervous system may affect smooth muscle, cardiac muscle, and endocrine and exocrine functions, thereby triggering diarrheal episodes. Consequently, muscarinic acetylcholine receptor subtype M3 (M3-AchRs) have been targeted therapeutically using anticholinergic agents, including loperamide, loratadine, baclofen, amitriptyline, oxybutynin, and chlorphenamine, to reduce gastrointestinal motility and secretion. Nevertheless, prolonged use of these agents is associated with adverse effects such as headache, memory impairment, cognitive decline, behavioral changes, anxiety, and insomnia [8].

Medicinal plants exhibit pharmacological activities largely attributable to their secondary metabolites. These compounds hold considerable potential as antidiarrheal and antibacterial agents against diarrhea-causing microorganisms, with such activities generally attributed to alkaloids, flavonoids, tannins, steroids, terpenoids, essential oils, and saponins [9]. One herbal preparation reported to exhibit antidiarrheal activity through inhibition of the M3-AChRs receptor is papaya leaf infusion (*Carica papaya* L.). This activity is attributed to the presence of quercetin-3-rutinoside, a bioactive compound that contributes to antidiarrheal effects [8].

Eleusine indica L. (commonly known as goosegrass) is a weed belonging to the family Graminae that grows abundantly in agricultural fields and gardens. This plant has long been utilized in traditional medicine and is known to contain diverse bioactive compounds, including tannins, flavonoids, triterpenoids, alkaloids, steroids, quinones, and phenols. Among the compounds successfully isolated are β-sitosterol glucoside, schaftoside, vitexin, and isovitexin. Numerous studies have demonstrated its biological activities, including antipyretic, antimicrobial, and antiviral effects [10]. An in vivo study demonstrated that administration of 70% ethanol extract of *Eleusine indica* at a dose of 40 mg/kg body weight exhibited antidiarrheal activity in mice, as evidenced by diarrhea frequency, fecal weight, diarrhea duration, and fecal consistency that were comparable to those observed in the positive control group receiving 2 mg of loperamide [10].

In silico approaches, particularly molecular docking, enable efficient and cost-effective prediction of interactions between bioactive compounds and target proteins. These computational methods facilitate the screening of potential drug candidates prior to laboratory experimentation, thereby accelerating the drug discovery and development process [11]. The three-dimensional crystal structure of the M3-AChR (PDB ID: 5ZHP) provides a suitable and well-characterized model for such investigations. Furthermore, the molecular basis underlying the potential antidiarrheal activity of *E. indica* remains poorly understood. Therefore, the present study aims to perform molecular docking of the major phytochemical compounds identified in the ethanol extract of *E. indica* against the 5ZHP receptor, with the objective of identifying potential antidiarrheal candidates exhibiting favorable binding profiles.

2. Research Method

2.1. Materials

This study was computational in nature and utilized protein structural data obtained from the Protein Data Bank (PDB) repository. The crystallographic structure employed was the muscarinic acetylcholine M3 receptor in complex with a selective antagonist (PDB ID: 5ZHP) [8], [12].

The test ligands analyzed in this study comprised secondary metabolites previously reported to be present in *E. indica* based on LC-MS/MS-QTOF [10]. The three-dimensional structures of each compound were computationally constructed, including 3-indolylacrylic acid, schaftoside, orientin, vitexin, L-phenylalaninosecologanin, tricin, coumarin, loliolide, pana, 2-{[(2E)-1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-ylidene]amino}benzoic acid, 3,4-dihydroxy-1-{[3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]oxy}-5-{[3-(4-hydroxyphenyl)prop-2-enoyl]oxy}cyclohexane-1-carboxylic acid, 4-(1-hydroxyethyl)-2,6-bis(3-methylbut-2-en-1-yl)phenol, octadecatetraenoic acid, andrographolide, dihydroactinidiolide, 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)tetradecan-3-one, ergostine, ergobalansine, and methyl (11E,13E)-octadeca-9,11,13,15-tetraenoate [10]. Loperamide was employed as the reference ligand and was prepared in three-dimensional structural format for comparative purposes in the molecular interaction analysis [12], [13].

2.2. Instruments

The molecular docking simulation was performed on an ASUS TUF Gaming A15 laptop equipped with an AMD Ryzen 7 7435HS processor with a base clock speed of 3.1 GHz, running a 64-bit Windows operating system. The software utilized included Molegro Virtual Docker (MVD) version 5.0 as the primary docking simulation platform, ChemDraw version 22.0.0 for two-dimensional molecular structure drawing, and Chem3D version 22.0.0 for three-dimensional structural modeling of the analyzed compounds. The target protein structure was retrieved from the official RCSB Protein Data Bank portal.

2.3. Drug-likeness Prediction

The simplified molecular-input line-entry system (SMILES) codes for each compound were obtained from the PubChem online web tool (https://pubchem.ncbi.nlm.nih.gov/) [14]. The SMILES codes were subsequently submitted to the SwissADME web tool (http://www.swissadme.ch/) to predict druglikeness properties, encompassing molecular weight ≤500 g/mol, number of hydrogen bond acceptors (HBA) ≤10, number of hydrogen bond donors (HBD) ≤5, LogP value ≤5, and compliance with Lipinski's Rule of Five [15].

2.4. Molecular Docking

2.4.1. Protein and Ligand Preparation

The three-dimensional crystal structure of the receptor used in the molecular docking analysis was retrieved from the Protein Data Bank (PDB) via the RCSB Protein Data Bank website (https://www.rcsb.org/). Receptor preparation was performed by isolating the macromolecular protein from other molecular components, retaining only the receptor protein and the native ligand, this preparation was carried out directly within MVD.

The three-dimensional structures of *E. indica* secondary metabolite compounds were initially downloaded from the PubChem database in 3D format. Each compound subsequently underwent geometric optimization to obtain the most energetically stable molecular conformation. Optimization was performed using the MMFF94 (Merck Molecular Force Field) method, aimed at minimizing the total energy of the molecular system. Optimized compounds were saved in ".mol2" file format in preparation for the docking simulation.

2.4.2. Method Validation

Validation of the molecular docking method was performed on the native ligand using a redocking approach to ensure that the docking parameters could accurately reproduce the position and conformation of the ligand within the protein active site. This process involved redocking the native ligand into the binding pocket of the protein and comparing the resulting conformation with the crystallographically determined conformation using the root mean square deviation (RMSD) value. Grid box settings were configured to define the ligand binding space within the active site, referenced to the position of the native ligand bound to the protein macromolecule in the downloaded crystal structure, thereby providing coordinates for subsequent test ligand docking. The RMSD value served as an indicator of docking accuracy, a smaller RMSD indicates a closer agreement between the redocked ligand conformation and the original crystallographic conformation, reflecting greater validity and experimental representativeness of the docking method [16], [17].

2.4.3. Visualization

Ligand–protein interaction analyses were conducted using Molegro Virtual Docker. The target protein structure was first imported into the software, after which water molecules and cofactors present in the structure were removed to focus the analysis specifically on the protein active site region. Binding pocket identification on the receptor was subsequently performed using the detect cavities function, which determines potential binding areas on the protein surface. Following cavity identification, test ligands were introduced into the system and docking simulations were executed to predict binding orientation and interaction strength between the ligands and the target receptor.

Docking results were evaluated based on binding energy values expressed as the Rerank Score (RS). A lower or more negative RS value indicates a more stable ligand–receptor complex. On this basis, the predicted biological activity of each test ligand was assessed by comparing its Rerank Score against that of the reference compound. Test ligands yielding RS values equal to or lower than that of the reference ligand were predicted to possess comparable or superior biological activity potential [18].

3. Result and Discussion

3.1. Druglikeness Prediction

The in silico approach plays a crucial role in accelerating drug discovery, particularly in identifying the activity of traditional medicinal compounds whose mechanisms of action remain poorly understood. One widely adopted method is druglikeness evaluation based on Lipinski's Rule of Five, which involves virtual screening of the physicochemical properties and molecular structures of potential compounds [19], [20]. This rule is applied to predict the pharmacokinetic characteristics and suitability of compounds as oral drug candidates [21], and can be integrated with in vitro and in vivo studies in the development of new therapeutic agents [22]. However, this approach has inherent limitations in covering a broader chemical space beyond previously characterized drug compounds [20].

Table 1. Physicochemical Analysis of *E. Indica* Compounds

Compounds name	Code	Formula	Molecular weight (g/mol)	Num. H-bond acceptors	Num. H-bond donors	log P	Lipinski
3-indolylacrylic acid	M1	$C_{11}H_9NO_2$	187.19	2	2	1.32	Yes; 0 violation
Schaftoside	M2	$C_{26}H_{28}O_{14}$	564.49	14	10	-3.97	No; 3 violations: MW>500, NorO>10, NHorOH>5
Orientin	M3	$C_{21}H_{20}O_{11}$	448.38	11	8	-2.51	No; 2 violations: NorO>10, NHorOH>5
Vitexin	M4	$C_{21}H_{20}O_{10}$	432.38	10	7	-2.02	Yes; 1 violation: NHorOH>5
L-Phenylalaninosecologanin	M5	$C_{26}H_{35}NO_{11}$	537.56	12	6	-3.14	No; 3 violations: MW>500, NorO>10, NHorOH>5
Tricin	M6	$C_{17}H_{14}O_7$	330.29	7	3	-0.07	Yes; 0 violation
Coumarin	M7	$C_9H_6O_2$	146.14	2	0	1.65	Yes; 0 violation
Loliolide	M8	$C_{11}H_{16}O_3$	196.24	3	1	1.49	Yes; 0 violation
Pana	M9	$C_{16}H_{13}N$	219.28	0	1	4.19	Yes; 1 violation: MLOGP>4.15
2-{[(2e)-1-hydroxy-3-(4hydroxy-3methoxyphenyl)prop-2en-1ylidene]amino}benzoic acid	M10	$C_{17}H_{15}NO_5$	313.30	6	3	2.25	Yes; 0 violation
3,4-dihydroxy-1-{[3-(4hydroxy-3methoxyphenyl)prop-2enoyl]oxy}-5-{[3-(4hydroxyphenyl)prop-2enoyl]oxy}cyclohexane1-carboxylic acid	M11	$C_{26}H_{26}O_{11}$	514.48	11	5	0.34	No; 2 violations: MW>500, NorO>10
Tricin	M12	$C_{17}H_{14}O_7$	330.29	7	3	-0.07	Yes; 0 violation
4-(1-hydroxyethyl)-2,6-bis(3-methylbut-2-en-1-yl)phenol	M13	$C_{18}H_{26}O_2$	274.40	2	2	3.75	Yes; 0 violation
Octadecatetraenoic acid	M14	$C_{18}H_{28}O_2$	276.41	2	1	4.29	Yes; 1 violation: MLOGP>4.15
Andrographolide	M15	$C_{20}H_{30}O_5$	350.45	5	3	1.98	Yes; 0 violation
Dihydroactinidiolide	M16	$C_{11}H_{16}O_2$	180.24	2	0	2.37	Yes; 0 violation
5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)tetradecan-3-one	M17	$C_{21}H_{34}O_4$	350.49	4	2	3.06	Yes; 0 violation
Ergostine	M18	$C_{34}H_{37}N_5O_5$	595.69	6	3	1.62	Yes; 1 violation: MW>500
Ergobalansine	M19	$C_{28}H_{35}N_5O_5$	521.61	6	4	0.30	Yes; 1 violation: MW>500
methyl (11E,13E)octadeca-9,11,13,15tetraenoate	M20	$C_{19}H_{30}O_2$	290.44	2	0	4.53	Yes; 1 violation: MLOGP>4.15

Note: Red text indicates that it does not meet Lipinski's rules.

Druglikeness prediction in this study was performed using the SwissADME web tool, which is capable of analyzing multiple molecules simultaneously and presenting results in both individual and interactive graphical formats. This platform is integrated with SwissDrugDesign and was developed by the Swiss Institute of Bioinformatics (SIB) to support drug discovery research [23], [24], [25].

Lipinski's Rule of Five defines the primary parameters for oral drug candidacy, including molecular weight ≤500 g/mol, number of hydrogen bond acceptors (HBA) ≤10, number of hydrogen bond donors (HBD) ≤5, and LogP value ≤5. Compounds with lower molecular weights generally exhibit superior permeability across biological membranes [26]. Furthermore, adherence to Lipinski's rule and blood–brain barrier permeability (BBB permeant: yes) serve as important indicators in evaluating the pharmacokinetic potential of compounds [27].

As presented in Table 1, the majority of the tested compounds demonstrated favorable druglikeness profiles and met the criteria for oral drug candidates. Compounds M1, M6, M7, M8, M10, M12, M13, M15, M16, and M17 fully satisfied all Lipinski parameters without any violations, suggesting good predicted oral bioavailability. Compounds M4, M9, M14, M18, M19, and M20 also complied with Lipinski's rule despite exhibiting one tolerable violation. In contrast, compounds M2, M3, M5, and M11 displayed more than one violation, primarily concerning molecular weight and the number of hydrogen bond donors and acceptors, suggesting reduced membrane permeability and oral absorption. Overall, the majority of the evaluated compounds exhibited favorable pharmacokinetic characteristics and hold considerable potential for further development.

3.2. Molecular Docking Validation

Validation of the molecular docking method is an essential step to ensure that the applied parameters can accurately reproduce the binding position of the ligand within the active site of the target protein [16], [17]. In this study, validation was performed using grid box parameters centered at coordinates X = −21.10, Y = −48.22, and Z = 196.56, with a radius of 7 Å, number of runs 10. These parameters were defined to confine the ligand search space to the active binding pocket of the protein, thereby improving the specificity and efficiency of the docking process. The accuracy of the grid box dimensions directly influences the ability of the ligand to explore optimal conformations within the active site [16], [28].

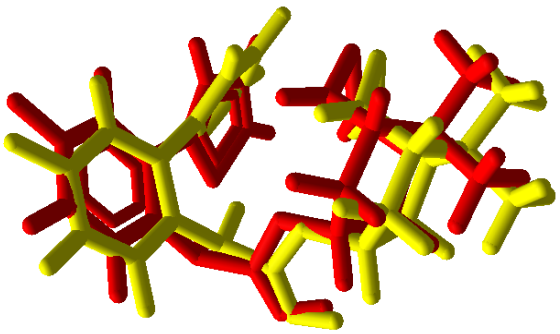

Figure 1. Redocking Validation Of The Ligand In The Receptor Binding Site (Red = Initial Conformation; Yellow = Redocked Conformation)

As illustrated in Figure 1, the redocked ligand conformation (yellow) closely overlapped with the original crystallographic conformation (red), indicating a high degree of structural agreement. Validation results yielded a Root Mean Square Deviation (RMSD) value of 0.777437 Å, which falls below the generally accepted tolerance threshold of ≤2.0 Å. This value indicates that the molecular docking method employed demonstrated good accuracy in predicting the position and orientation of the ligand relative to the target protein [16], [17]. The low RMSD value confirms that the redocked native ligand conformation closely approximated its crystallographic position, thus validating the reliability of the docking parameters [26], [29]. Accordingly, the docking method applied in this study met the required validation criteria and is considered suitable for analyzing test ligand interactions with the target protein, including binding energy evaluation and amino acid residue interaction analysis, thereby providing a high degree of confidence for subsequent investigations.

3.3. Molecular Docking Results

In molecular docking analysis, the Rerank Score serves as the primary parameter for evaluating the stability of ligand–protein interactions. This parameter was selected because its calculation encompasses a more detailed evaluation of interaction energies compared to the MolDock Score, including contributions from steric, electrostatic, and hydrogen bonding interactions, rendering it more representative in predicting the stability of the ligand–receptor complex. A lower Rerank Score indicates more stable ligand–protein interactions and superior binding affinity.

As summarized in Table 2, the native ligand 9EC_1203 [A] exhibited the lowest Rerank Score of −129.912, indicating the highest interaction stability with the target protein. Among all test compounds, M15 recorded the lowest Rerank Score of −116.686, followed by M13 at −114.300 and M17 at −107.273. These values suggest that all three compounds possess favorable binding affinities and are capable of forming relatively stable complexes with the target protein.

Table 2. Molecular Docking Results of *E. Indica* Compounds Against The 5ZHP Protein

Ligand	MolDock Score	Rerank Score	Hbond
9EC_1203 [A]	-162.566	-129.9120	-4.22271
M15	-139.835	-116.6860	-7.69612
M13	-138.108	-114.3000	-4.20853
M17	-146.900	-107.2730	-2.84554
M6	-114.832	-99.3905	-3.07811
M12	-117.103	-98.6939	-2.87857
M20	-132.562	-97.9616	0.00000
M10	-123.420	-97.0527	-5.07185
M14	-131.837	-96.9898	-0.00600499
M9	-113.812	-96.8215	-1.70478
M4	-128.028	-94.5188	-8.35512
M1	-103.044	-85.8598	0.00000
M16	-96.0985	-75.6862	0.00000
M8	-85.5311	-68.8634	-3.21714
M7	-71.1843	-62.8534	-2.50000
M18	-142.432	-42.5319	0.308021
Loperamide	-138.184	-41.9287	-3.731620
M19	-132.111	147.3740	1.501660

Compounds M6, M12, M20, M10, M14, and M9 also demonstrated notably low Rerank Scores ranging from approximately −96 to −99, and are therefore classified as exhibiting good receptor interactions. Additionally, M4 yielded a Rerank Score of −94.5188, while M1, M16, M8, and M7 showed progressively higher values, suggesting comparatively lower interaction stability relative to the aforementioned compounds.

Notably, compound M18 displayed a low MolDock Score yet produced a Rerank Score of −42.5319, whereas M19 yielded a positive Rerank Score of 147.374. These values suggest that although both compounds are capable of entering the protein active site, the resulting complexes exhibit relatively low stability, indicating suboptimal interactions. Furthermore, the reference compound loperamide produced a Rerank Score of −41.9287, which was higher than that of the majority of the test compounds.

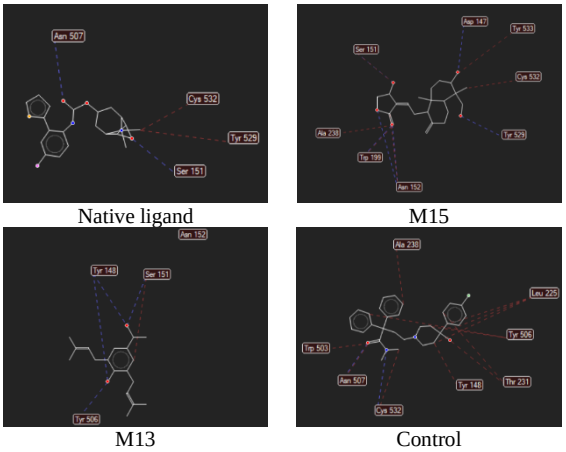

Figure 2. Visualization of Amino Acid Residue Interactions from Molecular Docking of *E. Indica* Compounds Against The 5ZHP Protein

As illustrated in Figure 2, the interaction patterns of M15 and M13 within the binding pocket of the 5ZHP receptor were comparable to those of the native ligand and the reference compound. Based on these findings, compounds M15 and M13 demonstrated the most favorable interaction profiles, exhibiting the lowest Rerank Scores among all test compounds. These results suggest that both compounds possess superior binding affinity and complex stability toward the target protein, warranting further investigation as potential antidiarrheal candidates.

4. Conclusion

The findings of this in silico study indicate that several of the tested compounds possess physicochemical properties that align with Lipinski's Rule of Five and display binding affinity profiles toward the target protein that may support further development. Among the screened compounds, M15 and M13 stood out as the most viable candidates, given that they met all the druglikeness criteria while recording the lowest Rerank Scores, a preliminary indicator of relatively favorable ligand–receptor interactions. It should be noted, however, that these observations are derived exclusively from computational prediction and have not been corroborated by molecular dynamics simulations or empirical experimental data. As this study was limited in scope, encompassing druglikeness prediction rather than a full ADMET assessment, and lacking evaluation within a complex biological context. Accordingly, M15 and M13 are proposed as priority compounds for subsequent in vitro and in vivo validation to assess their actual therapeutic efficacy and safety profiles.

Acknowledgments

The authors would like to thank Hang Tuah University for its financial support and institutional assistance in the conduct of this research. The support provided by the university is greatly appreciated and contributed significantly to the successful completion of this study.

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