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ORIGINAL RESEARCH ARTICLE

Antidiarrheal activity assessment of *Operculina turpethum* stem extract and *in silico* analysis of its compounds

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Abstract

Operculina turpethum is widely known for its use in traditional treatment practices to heal several diseases, such as bronchitis, pectoralgia, arthralgia, diarrhea, obesity, helminthiasis, gastropathy, ascites, sporadic fever, leucoderma, inflammation, pruritis, ulcers, erysipelas, tumors, jaundice, hemorrhoids, and ophthalmia. In this study, the antidiarrheal potential of the ethanolic extract of *O. turpethum* stem was assessed in an animal model, and molecular docking of previously reported stem-derived compounds was performed to determine the possible mechanism of action. The *in vivo* antidiarrheal effect was assessed using a castor oil-induced mouse model. *In silico* molecular docking analysis was performed using the 'Vina Wizard' program in PyRx 0.8. The stem extracts at 250 and 500 mg/kg produced significant, dose-dependent antidiarrheal activity. Among the four previously isolated compounds, three (22,23-dihydro-α-spinosterol-ß-D-glucoside, paprazine, and sitogluside) showed satisfactory binding affinity against the target M3 muscarinic acetylcholine receptor, comparable to the reference standard drug loperamide. These findings suggest that the *O. turpethum* stem has significant potential to prevent diarrhea that warrants further phytochemical, pharmacological, and mechanistic investigations.

Keywords: Anti-diarrheal; Plant extract; Molecular docking; ADME

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1. Background

Diarrheal diseases pose a significant challenge in developing nations and are responsible for millions of deaths each year.[1] Diarrhea can be described as a change in typical bowel movement and is attributed to an increase in water content, volume, or stool frequency.[2] Globally, one in ten deaths among children under the age of five is attributed to diarrhea, making up to approximately 8,00,000 deaths per year.[3] There are a total of 2.8 billion incidents of diarrhea in children (>5 years of age), adults, and adolescents.[4] Multiple influential factors are implicated in its pathophysiology, including poor intestinal absorption, altered gut motility, gastric hypersensitivity, microbial infection, metabolic insufficiency, genetic pre-disposition, chemical irritation, a weak immune system, and a plethora of secretory stimuli, such as bacterial enterotoxins, hormones, dihydroxy bile acids, hydroxylated fatty acids, and inflammatory cytokines.[5] Effective strategies for treatment and prevention include continued breastfeeding, oral rehydration therapy, improved hygiene, zinc supplementation, immunization programs, and the use of antibiotics.[6]

Plant-derived substances have long been used as a reliable source of biologically active metabolites with diverse pharmacological activity. Nearly 80% of the population, especially those from developing and underdeveloped countries, rely directly or indirectly on plant-based medications. Natural compounds have advantages over synthetic molecules, such as better drug-likeness and compatibility with the body's biological system, making them attractive candidates for research and drug development. A myriad of plant species are still being investigated for their medicinal constituents and therapeutic plants continue to represent a promising source of antidiarrheal agents.[7] Plant-derived compounds, such as 1,8-cineole, friedelin, Stachysrosane (1), Stachysrosane (2), and apigenin have potent antidiarrheal activity.[8] For this reason, the World Health Organization (WHO) has encouraged studies on the prevention and treatment of diarrheal diseases using traditional medical practices.[9,10]

Operculina turpethum (L.) Silva Manso, a member of the Convolvulaceae family, is an important therapeutic plant widely used in both Unani and Ayurvedic practices. This indigenous Asian plant resides in Bangladesh, India, Nepal, Sri Lanka, Pakistan, China, Taiwan, and Myanmar.[11] In traditional Unani practice, *O. turpethum* roots are prescribed for conditions, such as colic, constipation, paralysis, helminthiasis, gastropathy, ascites, leucoderma, pruritis, ulcers, and hemorrhoids.[12] The stems are rich in phenols, flavonoids, phytosterols, terpenoids, and cardiac glycosides.[13] To date, four natural metabolites have been isolated from the chloroform extract of the stem, namely, β-sitosterol-β-D-glucoside, 22,23-dihydro-α-spinosterol-β-D-glucoside, salicylic acid,[14,15] and (2E)-3-(4-Hydroxyphenyl)-N-^{2}-(4-hydroxyphenyl)-ethyl]-acrylamide.[16] Although originally identified from chloroform fractions, the structural and pharmacological relevance of these compounds, particularly sterol glucosides and phenolic derivatives, supports their presence in other solvent extracts, including ethanol. According to our literature review, no study investigated the antidiarrheal activity of *O. turpethum* stems until recently. This knowledge gap provides the rationale for conducting the present study, involving *in vivo* and *in silico* assessments of the ethanolic extract of the stem.

2. Materials and methods

2.1. Drugs and chemicals

Ethanol was acquired from Merck (Germany), and Tween 80 from BDH Chemicals (UK). The standard drug loperamide (Square Pharmaceuticals, Bangladesh) was obtained from a local pharmacy, and castor oil was procured from a neighboring chemical supplier (WELL'S Castor Oil, Spain). All the chemicals and drugs employed in this study were of analytical grade.

2.2. Plant collection, identification, and preparation of the ethanolic extract

O. turpethum stems were collected from Khulna University and its surrounding area. The plant was identified by experts at the Bangladesh National Herbarium, Mirpur, Dhaka, and a voucher specimen (no. 46484 DACB) was deposited for future reference. A total of 210 g of dried powdered stem was placed in a properly cleaned flat-bottomed glass container and soaked in 800 mL of 96% ethanol. The container was then sealed and stored for 14 days with occasional shaking and stirring. The extract was first filtered through clean cotton cloth and then through Whatman no. 1 filter paper (Whatman™, UK). Eventually, the filtrate was evaporated through a rotary evaporator (LabTech, Italy) to yield the ethanolic extract of the *O. turpethum* stem, with a yield of 3.58%. The extract was stored at 4°C for further analysis.

2.3. Experimental animals

Swiss albino mice, which were aged approximately 4–6 weeks old and weighed approximately 22–28 g, were procured from the animal research branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDRB). Mice were acclimatized under standard laboratory conditions for 1 week. All the experimental animals were given standard laboratory food and clean tap water and maintained under a natural light–dark cycle. All experiments were performed in a noise-free and isolated pharmacological laboratory at the Pharmacy Discipline, Khulna University. The experiment was approved by the Animal Ethics Committee of Khulna University [Ref: KUAEC-2018-01-08].

2.4. Antidiarrheal activity assessment in castor oil-induced diarrhea

The antidiarrheal activity was evaluated using the castor oil-induced diarrhea model, as previously described.[1] Initially, all the mice were monitored by administering 0.5 mL of castor oil; only those exhibiting diarrhea were included in the subsequent experiment. The animals were divided into four groups (n = 5 per group) receiving different interventions orally: Negative control (1% Tween 80 in water, 10 mL/kg body weight), positive control (loperamide, 3 mg/kg body weight), test-I (*O. turpethum* extract, 250 mg/kg body weight), and test-II (*O. turpethum* extract, 500 mg/kg body weight). Each mouse was housed individually in a cage lined with blotting paper, which was replaced hourly. Thirty minutes after treatment, diarrhea

was induced by oral administration of 0.5 mL of castor oil. The animals were then observed for 4 h, during which the total number of fecal outputs and diarrheal feces were recorded. The percentage inhibition of diarrhea was calculated using the following equation: [(TD control – TD test groups)/TD control] × 100, where TD control represents the total number of diarrheal feces in the negative control group, and TD test groups represents the total number of diarrheal feces of the positive control or test groups.

2.5. Molecular docking study

Four compounds were selected for molecular docking studies: paprazine [(E)-3-(4-hydroxyphenyl)-N-^{2}-(4-hydroxyphenyl)ethyl]prop-2-enamide; PubChem CID: 5372945], loperamide (PubChem CID: 3955), salicylic acid (PubChem CID: 338), and sitogluside [β-sitosterol-β-D-glucoside; PubChem CID: 5742590]. The chemical structures of the compounds were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Figure 1).[17] Since the 3D structure of 22,23-dihydro-a-spinosterol-β-D-glucoside (designated as "spinosterol" in this paper for simplicity) was unavailable, it was manually drawn in Avogadro (version 1.2.0; http://avogadro.cc/), based on its reported 2D structure (Figure 1).[15] All ligands were optimized using Avogadro with the Universal Force Field (UFF), and then saved in Protein Data Bank (.pdb) format for docking.

The M3 muscarinic acetylcholine receptor (MAChR; PDB ID: 4U14)[18] was obtained from the Protein Data Bank (http://www.rcsb.org/).[19] The structure was cleaned using PyMOL Molecular Graphics System (version 2.0, Schrödinger, US) and optimized with Swiss-PdbViewer (version 4.1; Swiss-PdbViewer/DeepView, by Nicolas Guex, Alexandre Diemand, Manuel C. Peitsch, and Torsten Schwede, https://spdbv.unil.ch/). Ramachandran plots were generated using the VADAR server to validate the predicted protein structures, based on criteria, such as preferred, allowed, and disallowed amino acid residue locations.

Molecular docking of the receptor with each ligand was performed using the Vina Wizard program in PyRx – Python Prescription 0.8.[20] The ligands and the receptor were loaded into the program with the appropriate designation as ligand or macromolecule. The docked complexes were subsequently visualized in PyMOL. For further interaction analysis, the complexes were imported into Discovery Studio Visualizer (version 4.5.0.15071, BIOVIA, Dassault Systèmes, France). The ligand–receptor interactions were observed, and snapshots of the best docking poses were obtained.

2.6. Pharmacokinetic parameters

Basic pharmacokinetic parameters were analyzed using the SwissADME web server (http://www.swissadme.ch/).[21] The input was provided in the form of SMILES or chemical structures.

2.7. Statistical analysis

GraphPad Prism software (version 8, GraphPad Software Inc., USA) was used to analyze all the experimental data. Results are presented as the mean ± standard error of the mean (SEM). A $p<0.05$ was considered statistically significant.

3. Results

3.1. Castor oil-induced diarrhea

The ethanolic extract of *O. turpethum* stem demonstrated dose-dependently antidiarrheal activity, with the most

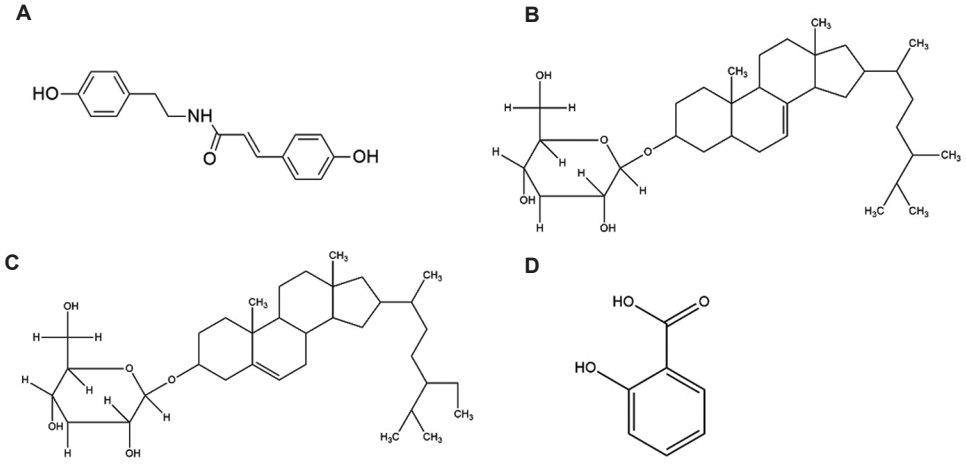

Figure 1. Previously isolated compounds from *O. turpethum*. (A) Paprazine. (B) Spinosterol. (C) Sitogluside. (D) Salicylic acid.

pronounced effect observed at 500 mg/kg (Table 1). At this dose, the mean latency period was 134 ± 3.71 min (Figure 2A), while defecation was inhibited by approximately 51% (Figure 2B). These results were comparable to those of the standard drug loperamide, which resulted in 89% inhibition with a mean latency period of 153 ± 3.11 min. Similarly, a 250 mg/kg dose demonstrated approximately 31% inhibition, with a mean latency period of 80 ± 2.07 min.

3.2. Molecular docking study

Loperamide was used as the standard compound for the docking study. The Ramachandran plot (Figure 3A) of the M3 MAChR (PDB ID: 4U14) showed 86% amino acids in the favored region, 11% in the allowed region, and 3% in the generously allowed or disallowed region (Figure 3B), indicating the suitability of the protein for molecular docking studies. The docking was carried out with a maximized grid box dimension (Table 2).

Table 1. Effect of *Operculina turpethum* stem extract on fecal output of castor oil-induced diarrhea mouse models

Treatment	Mean latent period (min)	Mean number of feces	Inhibition of defecation (%)
Negative control	35.2±2.63	18.6±1.86	-
Positive control	153±3.11	2±0.32*	89.24
Extract (250 mg/kg)	80±2.07*	12.8±0.37*	31.18
Extract (500 mg/kg)	134±3.70*	9.2±0.37*	50.53

Notes: Values are expressed as mean±standard error of the mean (n=5). *p<0.05 compared with the negative control.

Table 2. Grid box parameters used for molecular docking

Parameter	X	Y	Z
Centre coordinates	7.9825	20.6062	367.0162
Dimensions (Å)	60.9444	60.2098	86.2207

The binding energies of the best-docked complexes and their interacting amino acids are summarized in Table 3. Loperamide exhibited a binding energy of −8.3 kcal/mol with the receptor. All ligands, except salicylic acid, demonstrated satisfactory binding interactions with the receptor and thus were predicted to inhibit M3 MAChR activity. Ranking the ligands by binding affinity revealed the following order: spinosterol >paprazine >loperamide >sitogluside >salicylic acid.

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Among the tested ligands, 22,23-dihydro-α-spinosterol-ß-D-glucoside showed the strongest binding and emerged as the best candidate. Paprazine exhibited slightly lower affinity but outperformed the standard drug loperamide. β-Sitosterol-β-D-glucoside performed marginally less than the standard, although the binding affinities of both compounds were similar. In contrast, salicylic acid exhibited considerably lower affinity and is unlikely to serve as a potential antidiarrheal agent. Figures 4 and 5 show the 2D and 3D interactions between the ligands and the receptor, respectively. Table 4 explains the bonding categories and subcategories.

3.3. Pharmacokinetic analysis

The drug-like potential of the three ligand molecules was assessed based on absorption, distribution, metabolism, and elimination (ADME) parameters (Table 5). The evaluated

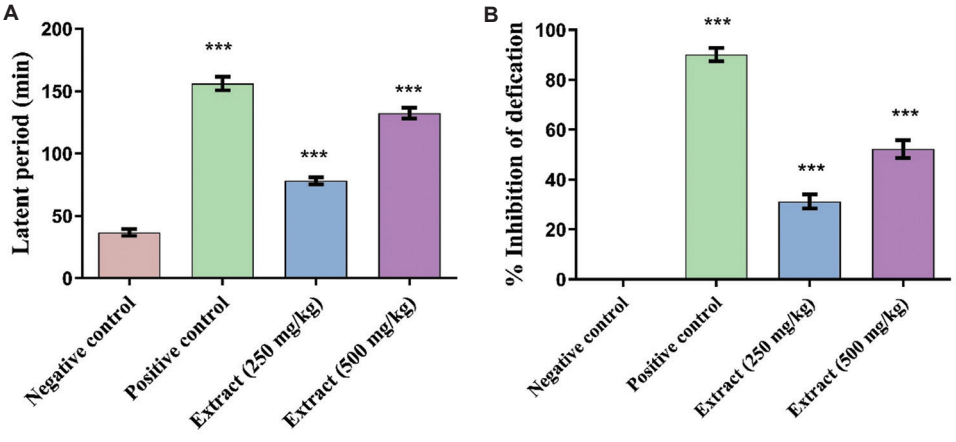

Figure 2. *In vivo* antidiarrheal activity of *O. turpethum* stem extract. (A) Effect of stem extract on prolongation of the latent period in castor oil-induced diarrheal episodes in mice. (B) Effect of stem extract based on inhibition of defecation in castor oil-induced diarrheal episodes in mice.

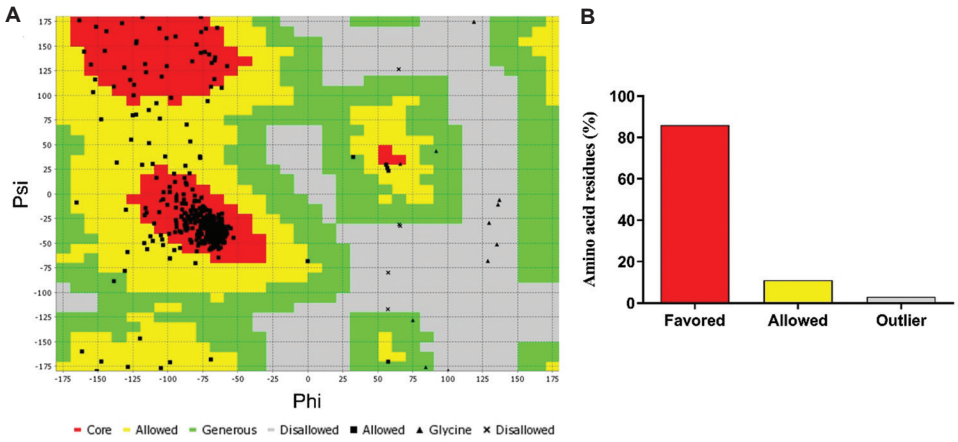

Figure 3. Structural validation of M3 muscarinic acetylcholine receptor. (A) Ramachandran plot of the receptor showing amino acids in the favored and allowed regions. (B) Percentage of amino acids in different regions.

Table 3. Docking results along with interacting amino acids

Ligand name	Binding affinity (kcal/mol)	RMSD/ub	RMSD/lb	Interacting amino acids
Paprazine	−8.9	0	0	TYR254, THR257, GLU258, LEU482, LYS487, ALA1093, VAL1094, and TRP1158
Spinosterol	−9.4	0	0	PHE124, TYR127, TRP143, LEU144, TYR148, ILE222, LEU225, ASN513, ASP517, LYS522, TRP525, and TYR529
Salicylic acid	−5.7	0	0	TYR148 and CYS532
Sitogluside	−8	0	0	LEU198, TRP199, PRO201, ALA202, PHE205, TRP206, PHE209, PHE224, ILE230, ALA237, and ALA238
Loperamide	−8.3	0	0	TYR104, SER108, CYS111, ILE146, VAL149, ILE188, TRP192, PHE196, and ALA200

Abbreviations: Rmsd/lb: Root mean square deviation lower bound; Rmsd/ub: Root mean square deviation upper bound.

Table 4. Types of bonds observed in the 2D diagram

Interaction category	Interaction type	Sub-type	Specific interaction
Favorable	Hydrogen bonds	Classical	Conventional hydrogen bond
		Non-classical	Carbon–hydrogen bond
	Hydrophobic	Pi hydrophobic (same color)	Pi–pi stacked
			Pi–pi T-shaped
			Amide-pi stacked
		Alkyl Hydrophobic	Alkyl[a]
		Mixed pi/alkyl hydrophobic	Pi-alkyl[a]
			Pi-sigma
	Miscellaneous	Sulfur	Pi-sulfur
Unfavorable	Acceptor/Donor clash	-	Unfavorable donor–donor

Note: [a]Both alkyl groups are represented in the same color.

parameters influence the cell permeation, bioavailability, and metabolic properties of the ligands. Notably, paprazine satisfied Lipinski's rule of five, suggesting it as a potentially drug-like compound.

4. Discussion

The ethanolic stem extract of *O. turpethum* was evaluated for its possible antidiarrheal effect in mice, and the results showed that the extract significantly attenuated castor oil-induced diarrhea in comparison with the standard drug loperamide. The phytochemicals that are present in *O. turpethum* stems, such as phenols, flavonoids, phytosterols, terpenoids, and cardiac glycosides,[13] are well known for their antidiarrheal properties.[25,26] Flavonoids, tannins, and saponins have been implicated in calcium channel-blocking activity, which might explain the therapeutic effect of *O. turpethum*.[27,28] Although our study focused on the stem extract, further comparative phytochemical analyses across different plant parts (i.e., root, stem, leaves) are warranted to better understand variations in active compound concentrations and their potential pharmacological implications.

Hydrolysis of castor oil results in the formation of ricinolic acid in the gastrointestinal tract, which induces

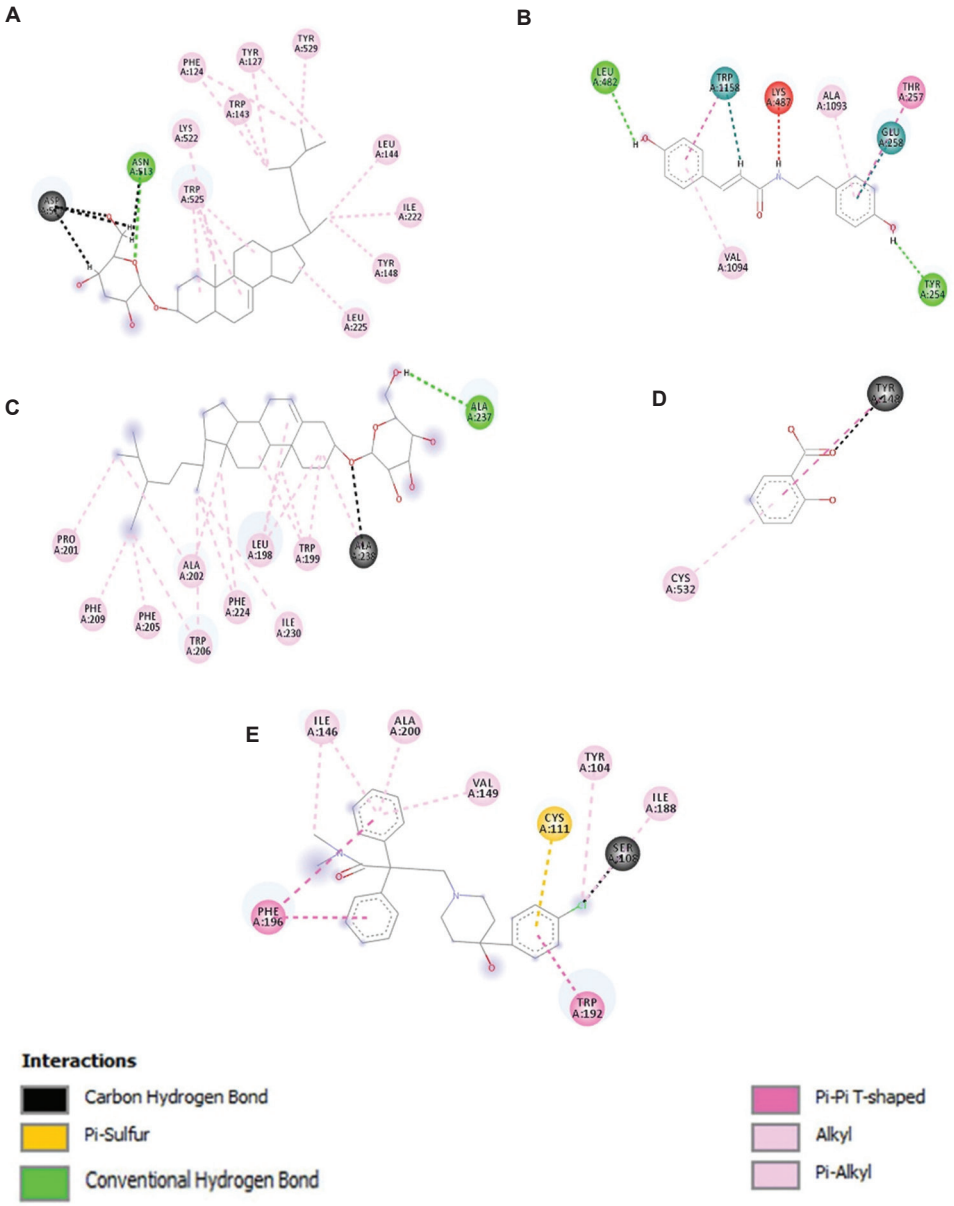

Figure 4. 2D interaction diagrams of the best binding poses for ligands docked to the M3 muscarinic acetylcholine receptor (PDB ID: 4U14). (A) Spinosterol. (B) Paprazine. (C) Sitogluside. (D) Salicylic acid. (E) Loperamide. Different bond types are represented in distinct colors and illustrated as dotted lines, showing the interactions between ligands and protein residues. Detailed interaction information is summarized in Table 4.

diarrhea by altering water and electrolyte transport, triggering a hypersecretory response, and stimulating massive contraction of the intestine.[29] Thus, phytochemicals present in *O. turpethum* likely counteracted these effects by hindering gut motility and electrolyte outflux. While the efficacy of *O. turpethum* was somewhat lower than that of loperamide, the results suggest that the extract possessed an acceptable inhibitory effect on gut motility and electrolyte outflux. To further confirm the possible inhibitory effect of the extract on gut motility, four previously isolated compounds—spinosterol, paprazine, sitogluside, and salicylic acid—were subjected to *in silico* molecular docking.

Gut motility is strongly associated with the activity of the M3 MAChR,[30] which is primarily regulated by the parasympathetic nervous system. Anticholinergic drugs, which antagonize M3 receptors, reduce smooth muscle spasms and relieve gastrointestinal disorders.[31] As a G protein–coupled receptor, the M3 MAChR mediates acetylcholine-induced increases in intracellular calcium,

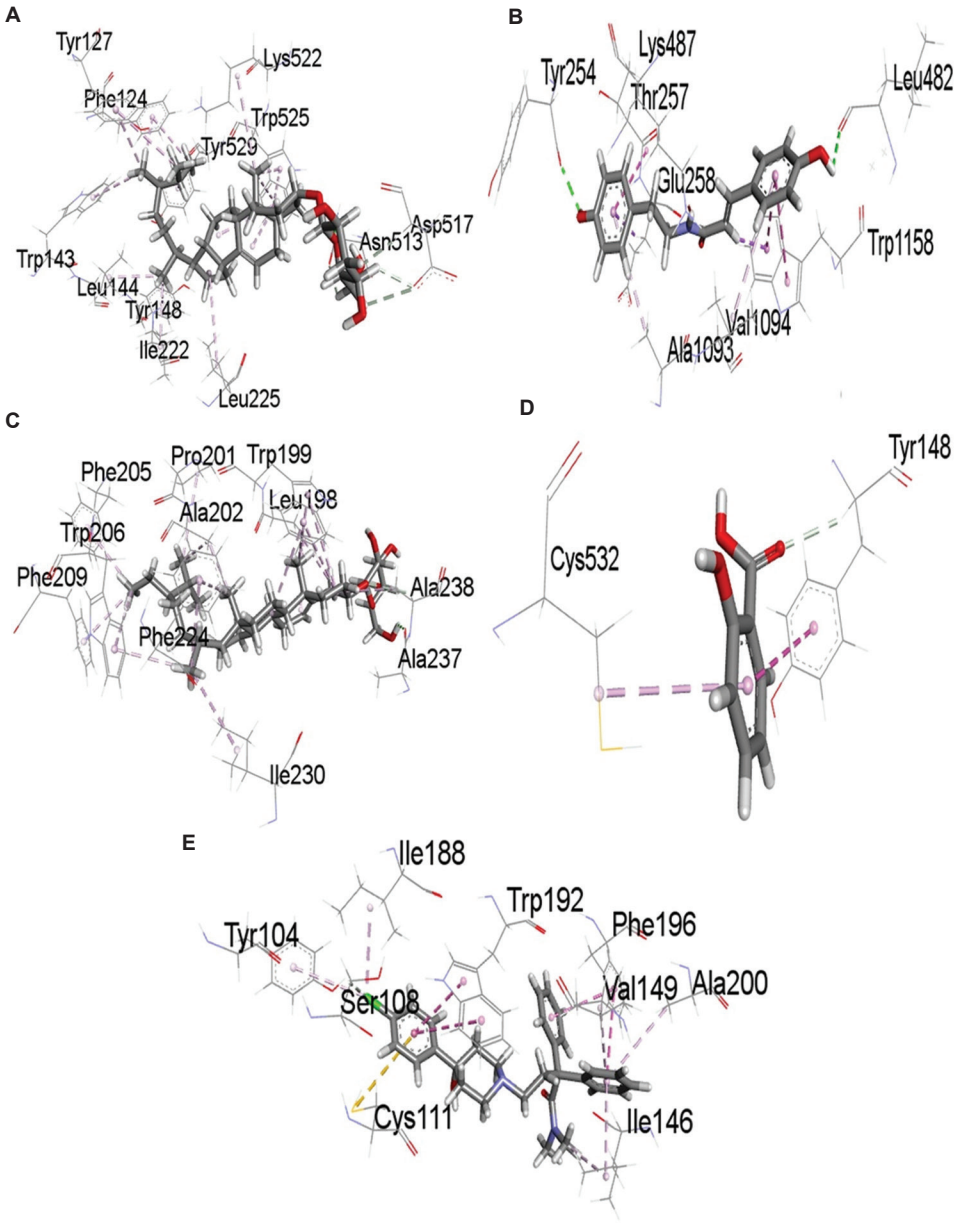

Figure 5. 3D interaction diagrams of the best-ranked binding poses for ligands docked to the M3 muscarinic acetylcholine receptor (PDB ID: 4U14). (A) Spinosterol. (B) Paprazine (C) Sitogluside. (D) Salicylic acid. (E) Loperamide.

leading to smooth muscle contraction.[32] Blocking this receptor reduces calcium influx, thereby decreasing gut constriction and providing antidiarrheal effects.[33] With this rationale, the four compounds and the standard drug loperamide were docked to the M3 MAChR. Among them, spinosterol, paprazine, and sitogluside exhibited excellent binding affinity with M3 MAChR, suggesting their potential to inhibit the receptor and block free calcium ion reflux in the cytoplasm, thereby contributing to the antidiarrheal effect of *O. turpethum*. Conversely, salicylic acid may not be a potential inhibitor of the receptor, but its presence in the stem may play a synergistic role because it can competitively inhibit prostaglandin formation.[34] Prostaglandins promote intestinal contractions and cause a range of gastrointestinal disorders, including diarrhea.[35]

ADME analysis of the four compounds further revealed that only paprazine satisfied Lipinski's rule of five as well as the Ghose, Veber, Egan, and Muegge criteria, suggesting that it is a potential drug-like molecule with favorable GI absorption and relatively easier synthesis.

Table 5. Pharmacokinetic properties of the ligands with good binding affinity

Properties	Paprazine	Sitogluside	Spinosterol
Molecular formula	$C_{17}H_{17}NO_3$	$C_{35}H_{60}O_6$	$C_{33}H_{56}O_5$
MW (g/mol)	283.32	576.85	532.79
TPSA (Å²)	69.56	99.38	79.15
Consensus Log $P_{o/w}$ (Lipophilicity)	2.46	5.51	5.65
Water solubility	Soluble or moderately soluble	Poorly soluble or moderately soluble	Poorly soluble or moderately soluble
GI absorption	High	Low	High
BBB permeant	Yes	No	No
P-gp substrate	No	No	No
CYP2D6 inhibitor	Yes	No	No
CYP3A4 inhibitor	Yes	No	No
Log Kp (skin permeation)	−6.10 cm/s	−4.32 cm/s	−4.21 cm/s
Drug-likeness			
Lipinski	Yes; no violations	Yes; 1 violation: MW > 500	No; 2 violations: MW > 500, MLOGP > 4.15
Ghose	Yes	No; 4 violations: MW > 480, WLOGP > 5.6, MR > 130, #atoms > 70	No; 4 violations: MW > 480, WLOGP > 5.6, MR > 130, #atom s > 70
Veber	Yes	Yes	Yes
Egan	Yes	Yes	No; 1 violation: WLOGP > 5.88
Muegge	Yes	No; 1 violation: XLOGP3 > 5	No; 1 violation: XLOGP3 > 5
Bioavailability score	0.55	0.55	0.17
Lead-likeness	Yes	No; 3 violations: MW > 350, Rotors > 7, XLOGP3 > 3.5	No; 3 violations: MW > 350, Rotors > 7, XLOGP3 > 3.5
Synthetic accessibility	2.28 (easy)	8.02 (difficult)	7.49 (difficult)

Notes: XLOGP3: Atomistic and knowledge-based method for calculating Log $P_{o/w}$ (XLOGP version 3.2.2, CCBG, Shanghai Institute of Organic Chemistry). WLOGP: Atomistic method for calculating Log $P_{o/w}$ (Wildman and Crippen).[22] MLOGP: Topological method for calculating Log $P_{o/w}$ (Moriguchi *et al*. and Lipinski *et al.*).[23,24]
Abbreviations: MR: Molar refractivity, MW: Molecular weight; TPSA: Topological Polar Surface Area.

This work has certain limitations. Although the extract was stored at 4°C to preserve its constituents, no formal stability analysis was performed to confirm the chemical integrity of the bioactive compounds during storage. In addition, while higher doses of the extract may improve efficacy, toxicity profiling will be essential before exploring dose escalation. Future investigations should address these limitations through stability studies, toxicity assessments, and mechanistic validation.

5. Conclusion

The ethanolic extract of *O. turpethum* stem demonstrated significant dose-dependent antidiarrheal activity in a castor oil-induced mouse model, although its efficacy was lower than that of the standard drug loperamide. *In silico* molecular docking studies revealed that three of the four previously reported stem-derived compounds—spinosterol, paprazine, and sitogluside—exhibited favorable binding affinities toward the M3 MAChR, supporting their potential contribution to the observed pharmacological effects of *O. turpethum*. While these findings suggest that *O. turpethum* contains promising bioactive constituents with antidiarrheal potential, future studies are necessary to perform detailed phytochemical profiling of the ethanolic extract using liquid chromatography with tandem mass spectrometry or nuclear magnetic resonance, evaluate the toxicity and safety of higher extract doses, assess synergistic interactions among isolated compounds, and investigate the mechanisms of action through *in vitro* and *ex vivo* models.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Md. Abdullah Al Fahad, Iqbal Ahmed
Investigation: Md. Abdullah Al Fahad, Iqbal Ahmed
Methodology: Md. Abdullah Al Fahad, Md. Fahim Hasan, Iqbal Ahmed
Writing – original draft: Md. Abdullah Al Fahad, Nusrat Jahan, Md. Fahim Hasan, Md. Arman Islam
Writing – review & editing: Iqbal Ahmed, Md. Abdullah Al Fahad

Ethics approval and consent to participate

The experiment was approved by the Animal Ethics Committee of Khulna University (Ref: KUAEC-2018-01-08).

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

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