Food and Chemical Toxicology 181 (2023) 114068

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Antiemetic effects of sclareol, possibly through 5-HT_3 and D_2 receptor interaction pathways: *In-vivo* and *in-silico* studies

Mehedi Hasan Bappi [a,1], Abdullah Al Shamsh Prottay [a,1], Khattab Al-Khafaji [b], Md Showkoth Akbor [a], Muhammad Kamal Hossain [c,d], Md Shahazul Islam [a], Afia Ibnath Asha [e], Cassio Rocha Medeiros [f], Catarina Martins Tahim [f], Elaine Cristina Pereira Lucetti [f], Henrique Douglas Melo Coutinho [g,*], Hossam Kamli [h], Muhammad Torequl Islam [a,**]

[a] Department of Pharmacy, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, 8100, Bangladesh
[b] Department of Environmental Science, College of Energy and Environmental Science, Al-Karkh University of Science, Baghdad, 10081, Iraq
[c] School of Pharmacy, Jeonbuk National University, Jeonju, 54896, Republic of Korea
[d] Department of Pharmacy, University of Science & Technology Chittagong, Chittagong, 4202, Bangladesh
[e] Department of Biochemistry and Molecular Biology, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, 8100, Bangladesh
[f] CECAPE College, Av. Padre Cícero, 3917 - São José, Juazeiro Do Norte, CE, 63024-015, Brazil
[g] Department of Biological Chemistry, Laboratory of Microbiology and Molecular Biology, Regional University of Cariri, Crato, CE, 63105-000, Brazil
[h] Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Khalid University, Abha, 61421, Saudi Arabia

ARTICLE INFO

Handling Editor: Dr. Bryan Delaney

Keywords:
Emesis
Sclareol
Diterpene
Gallus gallus domesticus
Molecular interactions

ABSTRACT

Background: Emesis is a complex physiological phenomenon that serves as a defense against numerous toxins, stressful situations, adverse medication responses, chemotherapy, and movement. Nevertheless, preventing emesis during chemotherapy or other situations is a significant issue for researchers. Hence, the majority view contends that successfully combining therapy is the best course of action. *In-vivo* analysis offers a more comprehensive grasp of how compounds behave within a complex biological environment, whereas *in-silico* evaluation refers to the use of computational models to forecast biological interactions.

Objectives: The objectives of the present study were to evaluate the effects of Sclareol (SCL) on copper sulphate-induced emetic chicks and to investigate the combined effects of these compounds using a conventional co-treatment approach and *in-silico* study.

Methods: SCL (5, 10, and 15 mg/kg) administered orally with or without pre-treatment with anti-emetic drugs (Ondansetron (ODN): 24 mg/kg, Domperidone (DOM): 80 mg/kg, Hyoscine butylbromide (HYS): 100 mg/kg, and Promethazine hydrochloride (PRO): 100 mg/kg) to illustrate the effects and the potential involvement with $5HT_3$, D_2, M_3/ACh_M, H_1, or NK_1 receptors by SCL. Furthermore, an *in-silico* analysis was conducted to forecast the role of these receptors in the emetic process.

Results: The results suggest that SCL exerted a dose-dependent anti-emetic effect on the chicks. Pretreatment with SCL-10 significantly minimized the number of retches and lengthened the emesis tendency of the experimental animals. SCL-10 significantly increased the anti-emetic effects of ODN and DOM. However, compared to the ODN-treated group, (SCL-10 + ODN) group considerably ($p < 0.0001$) extended the latency duration (109.40 ± 1.03 s) and significantly ($p < 0.01$) decreased the number of retches (20.00 ± 0.70), indicating an anti-emetic effect on the test animals. In *in-silico* analysis, SCL exhibited promising binding affinities with suggesting receptors.

Conclusion: SCL-10 exerted an inhibitory-like effect on emetic chicks, probably through the interaction of the $5HT_3$ and D_2 receptors. Further studies are highly appreciated to validate this study and determine the precise mechanism(s) behind the anti-emetic effects of SCL. We expect that SCL-10 may be utilized as an antiemetic treatment in a single dosage form or that it may function as a synergist with other traditional medicines.

* Corresponding author.
** Corresponding author.
E-mail addresses: hdmcoutinho@gmail.com, hdmcoutinho@urca.br (H.D.M. Coutinho), dmt.islam@bsmrstu.edu.bd, mti031124@gmail.com (M.T. Islam).
[1] Authors contributed equally.

https://doi.org/10.1016/j.fct.2023.114068
Received 5 June 2023; Received in revised form 12 September 2023; Accepted 27 September 2023
Available online 18 October 2023

1. Introduction

Emesis, commonly termed "vomiting", is a fairly frequent symptom that is specifically linked to gastrointestinal motor movements. It is often thought of as a defensive mechanism wherein unwanted items are promptly expelled from the stomach through the mouth (Stern et al., 2011). A broad range of gastrointestinal and non-gastrointestinal triggers can induce emesis - the phenomenon where noxious contents are ejected with force (Lang, 1999). These include ingesting an external toxin, digestive issues, infections, foodborne illness, dizziness, extreme pain, and hormonal and metabolic disturbances (Mohammad et al., 2018; Gallo et al., 2020). Additionally, it is the most frequent adverse reaction to several drugs, radiation therapy, chemotherapy for cancer, brain tumors, pregnancy, and other medical conditions (Bonfield and Engh, 2012). This means that while vomiting may be a physiologically homeostatic reaction to ingested toxins, proximity to damaged foodstuffs, or movements, it can also be the consequence of gastrointestinal (GI) illnesses, organs nearby the gastrointestinal system malfunctioning, or conditions of the central nervous system (Reintam Blaser et al., 2012). Emesis is now treated using a range of antiemetic medicines, which can be divided into categories such as anti-dopaminergic medications, corticosteroids, serotonin antagonists, butyrophenones, antihistamines, anticholinergic treatments, benzamides, NK_1-receptor blockers, cannabinoids, and agonist anti-emetics (Ahmed et al., 2013; Hauser et al., 2018). Anti-emetic drugs are often well tolerated, but they can come with a range of adverse effects. It might be more frequent, such as moderate headaches or vertigo, chills, fatigue, hypersensitive reaction, malaise, sleepiness, hypotension, or unusual, anaphylactic shock, hepatic failure, hypokalemia, constipation, serotonin syndrome, CNS depression, xerostomia, aggravation of narrow-angle glaucoma and other unusual conditions including extrapyramidal responses like dystonia, dyskinesia, and akathisia might occur (Leung and Robson, 2007; Hendren et al., 2015; Schaefer and Zito, 2018; Theriot et al., 2020; Bhuia et al., 2023a,b).

When activated by diverse stimuli, 5-HT produced from the GIT stimulates 5-HT_3 receptors on the vagal afferent system that trigger the vomiting center (Naylor and Inall, 1994). A vomiting area within the brain stem that is isolated from the blood by the blood-brain barrier must be stimulated to cause emesis (Andrews and Horn, 2006). Through a number of central neurologic routes, it is stimulated by convergent afferents. A number of receptors are expressed in the vomiting center and have significance in the regulation of emesis, including muscarinic acetylcholine (AChM), dopamine (D_2), neurokinin (NK_1), selective serotonin receptor ($5HT_3$), and histamine 1 (H_1) (Ahmed et al., 2013; Bhuia et al., 2023a,b). The vomiting center receives emesis impulses from the cerebral cortex, while signals from the vestibular regions are sent to CTZ and the vomiting center (Oman and Cullen, 2014). Therefore, anti-emetic effects are present in receptor antagonists implicated in the modulation of nausea and vomiting. Physical, pharmacological, and peripheral GI receptors are excited by external agents and internal components that build up during infection, ischemia, and irritation. For an emetic stimulus, serotonergic (5-HT_3 and 5-HT_4), D_2, ACh_M, and NK_1 are largely preserved, whilst gamma amino butyric acid ($GABA_B$), serotonergic (5-HT_{1A}), cannabinoids (CB_1), and opioids (μ_2) have anti-emetic actions (Sanger and Andrews, 2006; Drewes et al., 2020). Additionally, the gastrointestinal mucosa has 5-HT_3 receptors, which are also present in the area postrema of the brain, as well as D_2, 5-HT_3, 5-HT_4, ACh_M, NK_1, and μ_2. When substance P binds to NK_1 receptors, it can cause nausea, but the 5-HT_4 receptors in the myenteric plexus need ACh as a mediator (Fleming et al., 2020).

The purpose of antiemetic research is to develop methods and drugs that help prevent or lessen nausea and vomiting, which can be brought on by a variety of conditions, including chemotherapy, surgery, motion sickness, and pregnancy (Nunes et al., 2020). Many nations in the world use anti-dopaminergic medications e.g., domperidone extensively as an antiemetic treatment. In addition to affecting upper GI motility, they are efficient at preventing stimuli that activate the CTZ (Glare et al., 2008). Numerous findings to date indicate that 5-HT_3 receptor antagonists such as ondansetron do give a much-improved result in battling emesis after chemotherapy and there is also a paucity of thorough research on antihistamines' (e.g., promethazine) ability to cure emesis (Kris et al., 2005; Patanwala et al., 2010). Antihistamines are not the first line of therapy for emesis due to a lack of information and their overall sedative action (Kassel et al., 2018). At muscarinic receptors in the gut and CNS, anticholinergic agents interact with Ach (Moulton and Fryer, 2011). The drug in this family that has both anti-emetic and anti-spasmodic activity in the gut wall is scopolamine, which is the most often used (Sharkey and Wallace, 2011). The chemoreceptor trigger zone (CTZ), nucleus tractus solitarii (NTS), and GI tract all have a significant number of NK_1 receptors. The drugs in this family, which target the NK_1 receptor to block the release of substance-P, a key regulator of vomiting, are highlighted by the aprepitant and fosaprepitant (Martinez and Philipp, 2016; Diemunsch and Grélot, 2000). Despite their psychoactive side effects, synthetic cannabinoids like dronabinol and nabilone have been investigated for Chemotherapy-induced nausea and vomiting (CINV) and exhibit potential activities (Ward et al., 2021). For better results, particularly for CINV, dexamethasone and other corticosteroids have been used with other antiemetics but also has side effects (Celio et al., 2019). Acupressure and acupuncture may be helpful alternative treatments for treating nausea and vomiting, according to some research, but they may have some drawbacks, such as the possibility of fever, infection, skin issues, bleeding, bruising, and discomfort at the insertion sites (Zheng et al., 2023; Berger et al., 2021). These medications have a history of causing side effects, which should be managed to provide optimal antiemetic therapy for people.

The experimental gap in the earlier finding of an emetic treatment signifies the absence of a complete understanding or inadequate knowledge about certain parts of the impacts, mechanisms, or prospective uses of the medication. It's unclear how exactly the medication interacts with the body to cause emesis since the molecular pathways and receptors involved may not have been fully explained. It is possible that the link between the emetic medication's dosage and the degree of emesis it causes has not been fully described, leaving questions regarding the right dosing regimens for various situations. There may be some doubt about the recurrent use of emetic medication because the long-term effects of exposure to it have not been fully analyzed. It's possible that the medication's selectivity for particular body targets is not completely known, which might have unwanted effects on systems other than the targets. Its therapeutic usage may be constrained by incomplete research on its side effects, possible toxicity, and interactions with other medications or substances, which might endanger patient safety. The effectiveness of the drug in actual clinical settings could not match what was anticipated based on preliminary experimental results, underscoring the need for more research. For improving our comprehension of the features, possible uses, and safety concerns of the emetic drug, overcoming these gaps through continuous research and evaluation is essential. Our goal is to find a novel antiemetic medication with a lower toxicity profile, which will eventually result in better patient care and more informed medical decisions.

In order to find novel anti-emetic medicines, researchers are continuing to focus on mechanism-based strategies that include specific molecular and cellular targets. Numerous phytochemicals discovered via ethnomedical data have so far been demonstrated to have antiemetic properties in various animal models (Ashraf et al., 2017). Naturally derived secondary metabolites are trusted sources of novel medications as they frequently exhibit significant structural diversity (H. Wang et al., 2022; Freitas et al., 2021; Şahin et al., 2023). There are many different phytochemicals with a wide range of structural variations and intriguing pharmacological effects in nature (Kocyigit et al., 2023; Ferrarini et al., 2022; Fernández et al., 2021; Rahaman et al., 2023). Numerous studies indicate that phytochemicals produced from plants have a wide range of biological activities, including those that are anticancer, antidiabetic,

anti-nociceptive, anti-inflammatory, and beneficial for treating allergy and cardiac conditions as well as cancer. Antioxidant, antibacterial, and many others (Chy et al., 2021; Bachheti et al., 2022; Mitra et al., 2022; Akpoveso et al., 2023; Cerqua et al., 2022; D'Avino et al., 2023). As a result, raw natural products with antiemetic efficacy are frequently submitted to analytical research to identify the active ingredients. Flavonoids, cannabinoids, polysaccharides, hydroxycinnamic acids, chalcones, lignans, diarylheptanoids, glucosides, phenylpropanoids, saponins, and terpenes are among the chemicals in this family that have been shown to have antiemetic properties thus far (Ahmed, 2014). According to some studies, many natural compounds with antiemetic characteristics have not yet passed thorough scientific examinations and rigorous evaluation of their side effects (Houghton, 1995). To create effective anti-emetics with no toxicity and minimal side effects, it is crucial to comprehend the physiology and pharmacology of emesis (Pleuvry, 2012). Additional clinical studies are required to confirm the therapeutic potential of the discovered anti-emetic natural compounds because the evidence of activity in an animal model cannot be regarded as an unequivocal confirmation of efficacy in people. While the underlying mechanisms for certain potential natural compounds have been clarified, further research is needed for the overwhelming majority of natural anti-emetic drugs.

The herbal and flavoring plant *Salvia sclarea* L. produces inflorescences that contain the diterpene alcohol sclareol (SCL, Labd-14-ene-8, 13-diol), which is enriched in capitate oil glands of the calyx (Schmiderer et al., 2008; Caissard et al., 2012). Because of its therapeutic potential and long history of usage in herbal remedies, this labdane class phytochemical has garnered notable interest (Qadirifard et al., 2021). *S. sclarea*'s empirical medicine concentrated on treating a variety of illnesses, including rheumatoid arthritis, oral inflammation, and digestive tract disorders (Peana and Moretti, 2002; Kostić et al., 2017). However, SCL had more comprehensive and remarkable biological properties, such as anti-tumor, anti-inflammation, and anti-pathogenic microbe effects, as well as effects against diabetes and high blood pressure (Zhou et al., 2022). The use of SCL may be thought of as a key strategy in the prevention of cancer in light of the numerous effects that have been shown in recent studies, mostly through testing on human and animal models (Dimas et al., 2007; Paradissis et al., 2007; Afshari et al., 2020). SCL is currently being studied as a possible treatment for Parkinson's disease and novel coronavirus disease 2019 (Covid-19) (P. Wang et al., 2022; Zhou et al., 2022). In this research, we highlighted the therapeutic potential of the bioactive phytochemical SCL and its molecular mechanism(s) of anti-emetic effects for further examining its involvement in emetic therapy using *in-vitro* and *in-silico* approaches.

2. Materials and methods

2.1. In-vivo study

2.1.1. Chemicals and reagents

Sclareol 98% (SCL) [Cas No. 515-03-7] was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany), while ondansetron (ODN) and domperidone (DOM), hyoscine butylbromide (HYS) and promethazine hydrochloride (PRO) were collected from Incepta Pharmaceuticals Ltd. (Dhaka, Bangladesh) and Opsonin Pharmaceuticals Ltd. (Barishal, Bangladesh), respectively. The emesis inducer copper sulphate pentahydrate ($CuSO_4.5H_2O$), and emulsifying agent tween-80 were purchased from Loba Chemie Pvt. Ltd., Mumbai, Maharashtra, India (Fig. 1.).

2.1.2. Experimental animals

We purchased young chickens (*Gallus domesticus*) from Nourish Grand Parent Ltd. In Rangpur, Bangladesh. The chicks were of either sex, 2 days old, and weighed between 40 and 45 g (Grade-A). Before the experiment, all chickens were kept for an extra two days in stainless steel cages which were opened in the top hood at room temperature with a 12-h light/dark period and were free access to eat (standard food) and drink (water *ad libitum*). This study was performed between 10:00 a.m. to 2:00 p.m. after a 2-h fasting period. This project was approved by the Department of Pharmacy and Animal and Human Ethical Committee of Bangabandhu Sheikh Mujibur Rahman Science and Technology University (BSMRSTU), Gopalganj, Bangladesh [#bsmrstu/phr17-50/21].

2.1.3. Study design

(see Table 1)

2.1.4. Copper sulphate-induced emesis in chicks

With a small modification, the procedures of (Akita et al., 1998) were followed in the conduct of this research. Twelve groups of five birds each were created from the entire flock. Each chick spent 10 min in a large transparent plastic container before the treatments. The test sample (SCL) was produced in three dosages of 5, 10, and 15 mg/kg and supplied orally using the syringe feeding method after being dissolved in a 0.9% NaCl solution and 1% tween 80. Ondansetron (ODN), domperidone (DOM), hyoscine butylbromide (HYS), and promethazine hydrochloride (PRO) were given orally as positive controls at dosages of 24, 80, 100, and 100 mg/kg per body weight, respectively. Additionally, to see possible agonistic/antagonistic or modulatory effects of SCL it was combined with the reference drugs at 10 mg/kg. The control (NC) consisted of the vehicle as mentioned above. For inducing emesis, $CuSO_4.5H_2O$ was given orally to each chick at a dosage of 50 mg/kg per body weight, after 30 min of all treatments. Then, the first retching and a total number of retches of each animal were noted within 10 min after receiving the emetic inducer ($CuSO_4.5H_2O$) (Fig. 2.). According to the following formulae, the percentage increases in latency and decreases in retches in comparison to the NC group were determined.

$$\%\text{Increase in retches} = \frac{A - B}{A} \times 100$$

$$\%\text{Decrease in latency} = \frac{C - D}{C} \times 100$$

Where, A and B mean number of retches observed in NC and test and/or standard groups, respectively. C and D mean the latency observed in NC and test and/or standard groups, respectively.

2.1.5. Statistical analysis

Antiemetic activity values are displayed as mean ± SEM (standard error of the mean). Applying the statistical program GraphPad Prism (version 9.5) accessed on May 1, 2022, we conducted an analysis of variance (ANOVA) and a t-Student-Newman-Keuls posthoc test to compare the experimental groups with the vehicle (control) group. P values ($p < 0.05$) were considered significant at 95% confidence intervals, while P values ($p < 0.0001$) were considered the most significant (Bappi et al., 2023).

2.2. In-silico study

2.2.1. Homology model of selected receptors and macromolecules

SWISS-MODEL, an excellent online tool (https://swissmodel.expasy.org/interactive), was used to undertake homology modeling of the serotonin ($5HT_{3A}$), dopamine-2 (D_2), muscarinic-3 (M_3), histamine-1 (H_1), and neurokinin-1 (NK_1) receptors (Biasini et al., 2014). Prior to modeling, sequences from UniProt (https://www.uniprot.org/) (Consortium, 2015) were obtained, and the NCBI BLAST tool (Ye et al., 2012) was used to do a BLAST analysis to determine the best templates. The Ramachandran plot was used to evaluate the homology models. PROCHECK and ProSA were used to verify the model quality. (Mia et al., 2023; Sobolev et al., 2020). To clarify the binding method of these receptors, molecular docking of SCL and standard medicines was carried out.

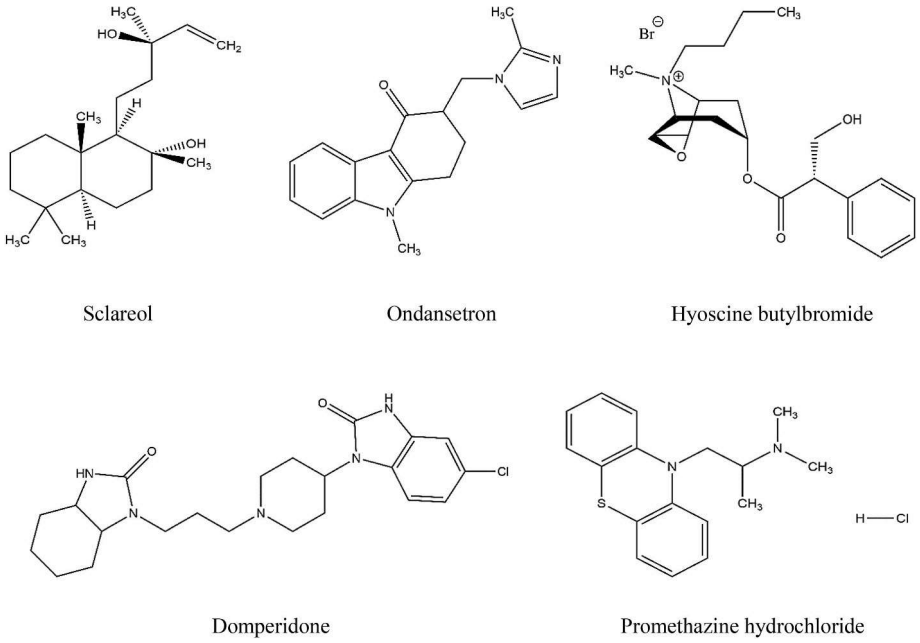

Fig. 1. Chemical structures of sclareol and conventional standards.

Table 1
Groups designed for the emetic experiment.

Treatment Groups	Description	Dose (p.o.)
Gr.-I: NC	Vehicle (0.05% Tween 80 + 0.9% NaCl solution)	10 mL/kg
Gr.-II: ODN	Standard 1: Ondansetron [Serotonin ($5HT_{3A}$) antagonist]	24 mg/kg
Gr.-III: DOM	Standard 2: Domperidone [Dopamine-2 (D_2) antagonist]	80 mg/kg
Gr.-IV: HYS	Standard 3: Hyoscine butylbromide [Muscarinic-3 (M_3) antagonist]	100 mg/kg
Gr.-V: PRO	Standard 4: Promethazine Hydrochloride [Histamine-1 (H_1) antagonist]	100 mg/kg
Gr.-VI: SCL-5	Test sample: Sclareol-5 (Lower dose)	5 mg/kg
Gr.-VII: SCL-10	Test sample: Sclareol-10 (Middle dose)	10 mg/kg
Gr.-VIII: SCL-15	Test sample: Sclareol-15 (Upper dose)	15 mg/kg
Gr.-IX: (Gr.-VII + Gr.-II)	Sclareol + Ondansetron	10 mg/kg + 24 mg/kg
Gr.-X: (Gr.-VII + Gr.-III)	Sclareol + Domperidone	10 mg/kg + 80 mg/kg
Gr.-XI: (Gr.-VII + Gr.-IV)	Sclareol + Hyoscine butylbromide	10 mg/kg + 100 mg/kg
Gr.-XII: (Gr.-VII + Gr.-V)	Sclareol + Promethazine	10 mg/kg + 100 mg/kg
*Vomiting inducer agents	Copper sulphate pentahydrate ($CuSO_4.5H_2O$)	50 mg/kg

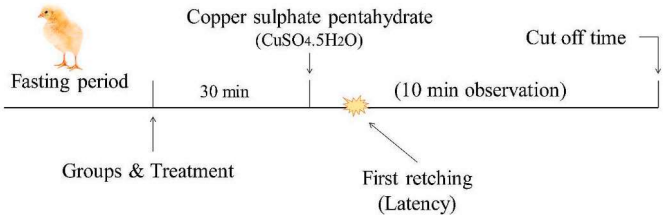

Fig. 2. Work outline of anti-emetic test in chicks.

2.2.1.1. Ligand preparation. Before docking, we used the Swiss-PDB Viewer software program (version 4.1.0, accessed on July 16, 2022) to minimize the energy of the crystal structure (Islam et al., 2022). Additionally, the 3D chemical structure of sclareol (SCL) (PubChem ID: 163, 263), as well as conventional medications (PubChem ID: 4595, 3151, 6, 852,391, 6014, and 135,413,536), was retrieved from the PubChem database in the 'sdf' format. Chem3D Pro 20.1.1 software packages (PerkinElmer Informatics, Inc., accessed on April 7, 2021) were used to optimize all of the ligands' internal energies (Kamli et al., 2023; Maljurić et al., 2018).

2.2.1.2. Docking protocol. Molecular docking simulation is a computational strategy for drug design used in pharmaceutical research. By analyzing and aligning molecules to target binding sites using the PyRx (version 0.8, Scripps research institute, accessed on July 22, 2022) virtual screening tool (Meshram et al., 2021), this method is being utilized to evaluate the pharmacodynamic properties of an active substance. The outcome of docking determines the degree of ligand binding with the particular receptor's active site. Receptor's active binding regions are identified using PyMol Edu (version 1.7.4.5, accessed on December 11, 2020) (Manochitra and Parija, 2017) as the ligand's coordinates in the original target receptor grids, and they are examined using BIOVIA Discovery Studio (version 21.1.0, Dassault Systems, accessed on December 15, 2021) (Gholam et al., 2022).

2.2.2. Pharmacokinetics and drug-likeness properties

Pharmacokinetics is the statistical study of how the body responds to drugs provided throughout exposure (ADMET properties). Initial pharmacokinetic property evaluation is essential in the *in-silico* strategy since it aids in the development of a compound to become a successful medication (Shaker et al., 2021). A qualitative criterion known as "drug-likeness" is used in the research and development of medications to evaluate how closely a chemical molecule resembles a pharmaceutical in various contexts (Ursu et al., 2011). Thus, using the SwissADME database (http://www.swissadme.ch/) (Daina et al., 2017), the pkCSM online application (Pires et al., 2015), and the openly available ProTox-II servers (https://tox-new.charite.de/protox_II/) (Banerjee et al., 2018), the pharmacokinetic functionalities and drug-likeness properties of SCL and the standards were evaluated.

2.2.3. Molecular dynamic (MD) simulation study

MD simulation can be more useful for identifying and characterizing the impact of ligand-receptor complexes which obtained from molecular docking. It provides a visual representation of the behavior or

conformational changes over time, that are utilized to assess the stability of the receptor-ligand complex. The chosen receptor-SCL and receptor-standards complexes were simulated using MD in the current study. All-atoms MD simulation for a 20 ns timespan was performed. The three-dimensional structures of five receptors were modeled using the Charmm 27 force field (Bjelkmar et al., 2010), and the SwissParam online (https://www.swissparam.ch/) server (Zoete et al., 2011) was employed to generate the topology of sclareol and antagonist drugs. The receptor–ligand systems were solvated using the TIP3P water model and immersed in a truncated octahedron box. The required number of sodium and chloride ions was added to neutralize the system. The physiological pH was retained by maintaining the ionic strength of 0.15 M. The temperature for the whole simulation time was maintained at 300 K through the Langevin thermo-stat (Liu et al., 2016). The collision frequency was set to 2 ps?1 at 1 atm using Monte Carlo barostat (Fernández-Pendás et al., 2014) with volume conversation attempts every 100 fs? The integration step was kept with a 2fs step. The SHAKE algorithm (Hess et al., 2008) further was implemented for the covalent bond constrained associated with hydrogens. The particle mesh Ewald method was considered for -range electrostatics. The equilibration of the system was performed for a 10 ns time span consisting of rounds of Canonical ensemble (NVT, where three parameters including the number of atoms (N), the volume (V), and the absolute temperature (T) are fixed throughout the simulation) and Isothermal–isobaric ensemble (NPT, where the number of atoms (N), the pressure (P), and the absolute temperature (T) are fixed) (Zheng et al., 2019). In order to assess the stability and behavior of the system, several parameters including root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), solvent-accessible surface area (SASA), the radius of gyration (gyrate), and PCA analysis were explored.

3. Results

3.1. In-vivo study

In the *in-vivo* antiemetic activity test, the administration of ODN (24 mg/kg), DOM (80 mg/kg), HYS (100 mg/kg), and PRO (100 mg/kg) reduced the number of retches, while enhancing the onset of retching (latency) in chicks compared to the control vehicle group. Furthermore, in contrast to the NC, the administered doses of SCL-10 remarkably decreased the number of retches and increased the latent period. However, pretreatment of SCL-10 in the combined groups caused significant changes. SCL-10 alone (each dose) or in its combination (SCL-10 + ODN) significantly ($p < 0.0001$) increased the latency period and also exhibited a similar/reduced number of retches significantly ($p < 0.01$) to ODN treated group. Fig. 3 Suggests that chicks pretreated with (SCL-10 + ODN) group took the time to retch (109.40 ± 1.03 s), whereas the lowest time (12.00 ± 0.83 s) observed for the first retch is for the NC group.

In comparison to the NC, HYS, and PRO groups in the chicks, which had values of the number of retches (75.40 ± 0.51, 29.80 ± 1.28, and 38.20 ± 1.15, respectively) for the intermediate dose SCL-10 group (26.20 ± 0.66) demonstrated superior activity. The combined groups showed fewer retches than the NC group, and the group with the fewest retches was the (SCL-10 + ODN) group (20.00 ± 0.70), according to Fig. 4.

For the intermediate dosage of SCL-10, a percentage increase in latency inspection of NC of 84.17% was noted. The highest percentage increase in latency (89.03%) for combined groups was observed in the (SCL-10 + ODN) group. The same group, however, also experienced the highest %decrease in NC retches inspection. The SCL-10 group has the best value of 65.25% for the %decrease in retches of the sample group's inspection of NC, as shown in Table 2 (see Table 3).

3.2. In-silico study

3.2.1. Homology model of selected receptors

By significantly reducing the disparity between experimentally observed receptor molecules and known receptor sequences, homology modeling has developed into an important structural biology approach (Waterhouse et al., 2018). Establishing proper receptor mappings and having quick and clear access to modeling innovations, simulation, and evaluation is made possible by the use of fully automated platforms and databases, which simplify and standardize the homology modeling process (Muhammed and Aki-Yalcin, 2019). The Uniprot database contains the amino acid sequences for the serotonin ($5HT_{3A}$), dopamine-2 (D_2), muscarinic-3 (M_3), histamine-1 (H_1), and neurokinin-1 (NK_1) receptors (Uniprot accession ID: P46098, P14416, P20309, P35367, P25103; and PDB ID: 6np0, 7jvr, 7dfl, 4daj, 7rmg respectively). The nearest homologous templates of ($5HT_{3A}$, D_2, M_3, H_1, and NK_1), produced by the Swiss model, were then chosen using the NCBI Blast Program. The 3D homology models of chosen receptors are depicted in Fig. 5. The Swiss-PDB Viewer software program (v4.1.0) was used to optimize the emetic models prior to docking. The PROCHECK server submitted PDB data (Khare et al., 2022) to the PDBsum (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) online site to validate the homology models and which produced the most favored regions of the selected receptors (Bappi et al., 2023). Which is shown in Suppl. Fig. 1.

A rapid method to observe the distribution of torsion angles in a

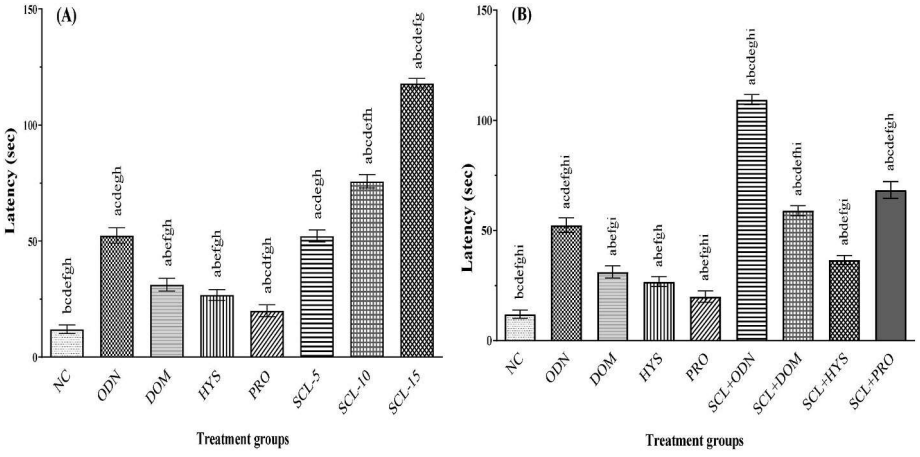

Fig. 3. Latency of retching observed in test samples, controls, and combinations [(**A**) $^{de}p<0.01$, $^{ae}p<0.001$, $^{abcdefgh}p<0.0001$; (**B**) $^{bg}p<0.05$, $^{de}p<0.01$, $^{ae}p<0.001$, $^{abcdefghi}p<0.0001$; ^{a}NC (vehicle), ^{b}ODN (positive control); ^{c}DOM; ^{d}HYS; ^{e}PRO; ^{f}SCL + ODN; ^{g}SCL + DOM; ^{h}SCL + HYS; ^{i}SCL + PRO; Values are mean ± S.E.M. (n = 5); (one-way ANOVA and *t*-Student-Neuman-Keuls *post hoc* test with multiple comparisons)].

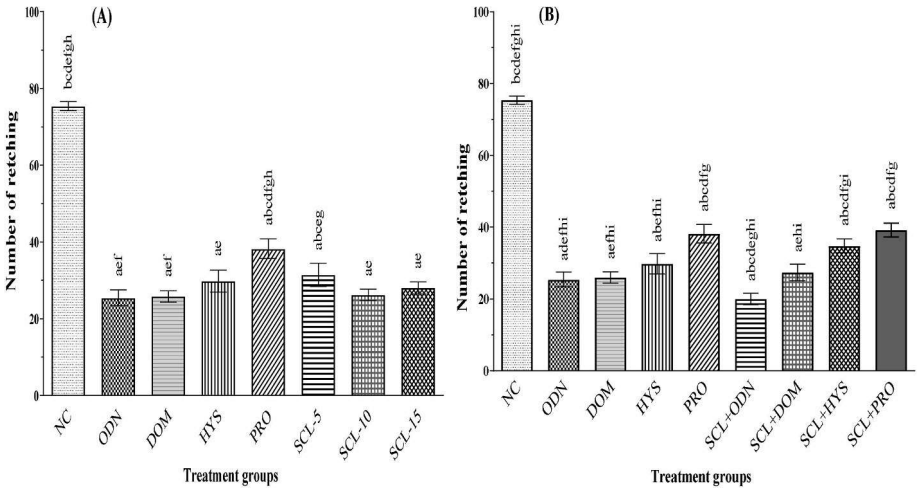

Fig. 4. Number of retching observed in test and/or control groups [(**A**) $^{bdfg}p<0.05$, $^{bcf}p<0.01$, $^{abcdefgh}p<0.0001$; (**B**) $^{bdhi}p<0.05$, $^{bcf}p<0.01$, $^{abcdefghi}p<0.0001$; ^{a}NC (vehicle), ^{b}ODN (positive control); ^{c}DOM; ^{d}HYS; ^{e}PRO; ^{f}SCL + ODN; ^{g}SCL + DOM; ^{h}SCL + HYS; ^{i}SCL + PRO; Values are mean ± S.E.M. (n = 5); (one-way ANOVA and *t*-Student-Neuman-Keuls *post hoc* test with multiple comparisons)].

Table 2
Comparison of latency and retches among the test controlled groups.

Treatment Groups	%Increase in latency	%Decrease in retches
NC	-	–
ODN	77.10	66.31
DOM	61.53	65.78
HYS	55.22	60.48
PRO	40.00	49.34
SCL-5	77.01	58.36
SCL-10	84.17	65.25
SCL-15	89.83	62.86
SCL + ODN	89.03	73.47
SCL + DOM	79.66	64.20
SCL + HYS	67.21	53.85
SCL + PRO	82.46	48.1

receptor structure is the Ramachandran plot. For assessing the caliber of three-dimensional receptor structures, it also specifies the boundaries of torsion angle values that are allowed and prohibited. The Ramachandran plot represents the Φ-Ψ torsion angles for each residue in the structure (except those at the chain termini). Triangles are used to indicate glycine residues since they are not limited to the plot areas designated for the other side chain classes. The map's color and shade represent the many portions that have been mentioned; the "core" areas, or darkest areas, correlate to the most advantageous Φ-Ψ value combinations. The most favored residues are displayed in red, while those that are authorized are displayed in yellow and those that are widely permitted are displayed in a fainter yellow. Remains in the restricted area are indicated by the white color. In an ideal scenario, these "core" areas would include over 90% of the leftovers. The percentage of residues located in the "core" areas is considered one of the most consistent predictors of stereochemical integrity (Suppl. Fig. 1).

In order to assess the validity of the receptor model, the ProSA quality measurement online tool (https://prosa.services.came.sbg.ac.at/prosa.php/) was used (Kwofie et al., 2018). The ProSA program compares the receptor's total model quality score to that of empirically addressed receptor structure models in the PDB and the results are plotted (Suppl. Fig. 2). A more successful receptor model is implied by a higher negative z-score (Yakubu et al., 2017). According to Ramachandran plot statistics, the residues in the most favored regions and Rama-Z score for $5HT_{3A}$, D_2, M_3, H_1, and NK_1 receptors were approximately 90.03 % and −4.49; 92.30 % and −2.06; 89.60 % and −3.0; 89.70 % and −4.39; 94.90 % and −3.06, respectively.

3.2.1.1. Interaction of sclareol (SCL) with selected receptors. SCL had a strong affinity for the $5HT_{3A}$, D_2, M_3, H_1, and NK_1 receptors. The corresponding binding affinities were −7.6, −8.7, −8.1, −7.6, and −8.2 kcal/mol, respectively. Through one carbon H-bond with TRP472, nine alkyl bonds, and five pi-alkyls, SCL is attached to the $5HT_{3A}$. SCL confirmed a binding affinity with D_2 through two H-bonds (conventional and carbon) with TYR408, and THR412 respectively, one alkyl bond with VAL115, and ten pi-alkyl bonds. Through one H-bonds (conventional), with ILE223, one alkyl bond with LEU226, seven pi-alkyl bonds, and two pi-sigma bonds, SCL showed binding interactions with M_3. Furthermore, SCL exhibited a binding affinity for H_1 through two alkyl bonds with LEU104 and ILE454 and nine pi-alkyl bonds. Moreover, SCL displayed binding interactions with NK_1 through three alkyl bonds, eight pi-alkyl bonds, one pi-sigma bond with TYR287, and no H-bond. Fig. 6 Depicts the 2D and 3D structures of the non-bond interactions between SCL and chosen receptors.

3.2.1.2. Interaction of antagonist drugs with their specific receptors. The Suppl. Table 1. Shows that Ondansetron (ODN), Domperidone (DOM), Hyoscine butylbromide (HYS), Promethazine Hydrochloride (PRO), and Aprepitant (APT) had a strong affinity with their specific receptors $5HT_{3A}$, D_2, M_3, H_1, and NK_1. The corresponding binding affinities were −8.5, −9.5, −8.3, −6.6, and −10.0 kcal/mol, respectively. Through one conventional H-bond, one pi-pi T-shaped bond, five pi-alkyls, and one pi-cation, ODN is attached to the $5HT_{3A}$. DOM confirmed a binding affinity with D_2 through two carbon H-bonds, two alkyl bonds, five pi-pi bonds, and five pi-alkyl bonds. Through two H-bonds (conventional and carbon), one pi-alkyl, and one pi-pi stacked bond, HYS showed binding interactions with M_3. Furthermore, PRO exhibited a binding affinity for H_1 through one alkyl bond, one pi-alkyl bond, four pi-pi bonds as well as one pi-sulfur bond. Moreover, APT displayed binding interactions with NK_1 through five H-bonds, one pi-pi bond, two alkyl bonds, two pi-alkyl bonds, and four halogen bonds. Fig. 7 Depicts the 2D and 3D structures of the non-bond interactions between antagonist drugs and chosen receptors.

3.2.2. Pharmacokinetics and drug-likeness properties

The precise investigation and collection of pharmacokinetic data are crucial for the therapeutic use of medicines since it is a key factor in drug development. Currently, building biological models, data analysis, and other techniques are the major ways that researchers and developers of novel medications gather their pharmacokinetic characteristics. On the basis of prior research, we have proof that SCL has substantial

Table 3
Best binding affinity results and non-bond interactions of sclareol with emetic receptors.

Sclareol (SCL) & Protein (Receptor)	Binding affinity (Kcal/mol)	H-bond			Hydrophobic bond			Other bonds [Electrostatic (E) or, Others (O)]		
		Residues	Bond length (Å)	Bond Types	Residues	Bond length (Å)	Bond Types	Residues	Bond length (Å)	Bond Types
(A) SCL and Serotonin ($5HT_{3A}$)	−7.6	TRP472	2.56	Carbon Hydrogen	LEU304	5.43	Alkyl	–		
					ILE305	5.01				
					VAL246	4.77				
					LEU250	3.64				
					LEU468	4.70				
					LEU471	4.90				
					LEU250	5.35				
					ILE305	5.04				
					LEU468	4.57				
					PHE166	4.22	Pi-Alkyl			
					PHE166	4.91				
					PHE166	4.71				
					TYR308	4.91				
					TRP475	4.60				
(B) SCL and Dopamine-2 (D_2)	−8.7	TYR408	2.18	Conventional	VAL115	3.85	Alkyl	–		
					TRP386	3.99	Pi-Alkyl			
					TRP386	4.22				
					PHE389	4.02				
					PHE389	4.68				
		THR412	2.53	Carbon Hydrogen	PHE389	5.43				
					PHE389	4.76				
					PHE390	5.26				
					PHE390	4.78				
					HIS393	5.11				
					TYR416	4.51				
(C) SCL and Muscarinic-3 (M_3/Ach_M)	−8.1	ILE223	2.27	Conventional	LEU226	4.72	Alkyl	–		
					PHE222	2.49	Pi-sigma			
					TYR530	2.86				
					PHE125	4.94	Pi-Alkyl			
					PHE125	4.90				
					TRP144	4.92				
					PHE222	4.73				
					PHE222	4.80				
					TYR530	4.54				
					TYR530	3.88				
(D) SCL and Histamine-1 (H_1)	−7.6	–			LEU104	5.12	Alkyl	–		
					ILE454	3.90				
					TRP103	4.53	Pi-Alkyl			
					TYR108	4.67				
					TYR108	3.97				
					TYR108	5.38				
					TYR108	5.27				
					TRP158	4.46				
					TRP428	5.10				
					PHE432	5.08				
					PHE432	4.42				
(E) SCL and Neurokinin-1 (NK_1)	−8.2	–			TYR287	3.76	Pi-Sigma	–		
					ALA93	4.01	Alkyl			
					ILE283	5.26				
					PRO271	3.75				
					TYR92	4.39	Pi-Alkyl			
					PHE267	4.72				
					PHE267	4.93				
					PHE268	4.83				
					PHE268	4.50				
					TYR278	4.15				
					TYR287	4.47				
					TYR287	4.31				

antiemetic properties. Additionally, we performed *in-silico* simulation to forecast the physicochemical properties, biological functions, and hazardous risk of the chosen phytochemical using the standards since a substance needs to satisfy specific requirements in order to be classified as a medication. These characteristics may be an early warning sign that a product won't fail in clinical trials for potential causes that may have been identified and ignored. All computed variables for SCL compared to the standards, including those for its physiochemical characteristics, lipophilicity, water solubility, GI absorption, potential CYP family metabolism, and permeability during synthesis, were within the acceptable range, as shown in Table 4. SCL's toxicity profile has also been deemed to be outstanding in terms of organ toxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity (see Table 5).

The term "drug-likeness" denoted the consistency between structural characteristics and other molecular attributes that influence the development of new drugs. The major factor in the development of pharmaceuticals is the five Lipinski guidelines. Since SCL doesn't break these criteria, it is considered to be a drug-like substance (Fig. 8.).

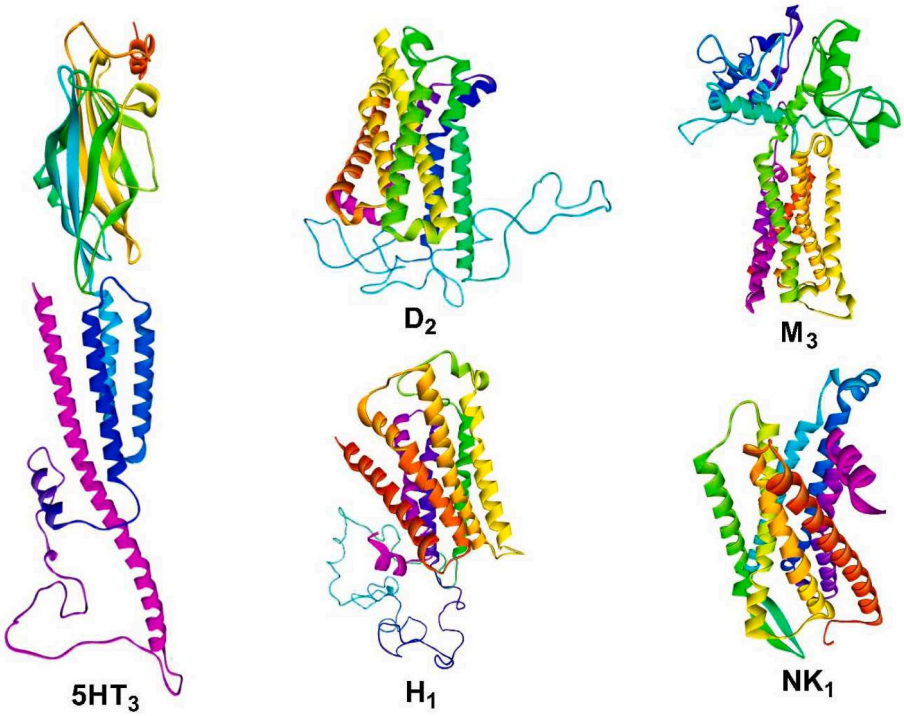

Fig. 5. Homology model of $5HT_{3A}$, D_2, M_3, H_1, and NK_1 receptors through the SWISS-MODEL.

3.2.3. Molecular dynamic (MD) simulation study

Fig. 9. A shows the Root Mean Square Deviation (RMSD) values over time of the $5HT_{3A}$ receptor's backbone when it bound to ODN and SCL were almost less than 1 nm, For SCL and DOM with D_2 receptor (Fig. 9. B), the RMSD values have fluctuated less than 0.8 nm. The average fluctuation of the position is calculated by Root Mean Square Fluctuation (RMSF) values of each residue to check the mobility or flexibility of the residues of a receptor during a simulation. The RMSF values of the antagonist drugs and their specific receptors and compared with SCL-bound targeted receptors (Fig. 9. C&D). In order to investigate the overall effect of SCL on selected targets and compare it with standards and with their specific receptors, Radius of Gyration (Rg) values were calculated for each complex. Rg values exhibited identical behavior for SCL and standards and with their specific receptors except Rg for SCL-bound and ODN-bound $5HT_{3A}$ receptors where SCL decreased the compactness of $5HT_{3A}$ receptors (Fig. 9. E&F). Furthermore, Solvent Accessible Surface Area (SASA) for the complexes was analyzed and shown in Fig. 9. G&H. We can observe all SCL-bound receptors and antagonist-receptors are similar to the standard drugs.

4. Discussion

The brain's fourth ventricle and a section of it known as the CTZ serve as the site of the vomiting center. It is also known as the area postrema, and when the CTZ is triggered, vomiting can occur (Gan, 2007). The CTZ is important in emesis, however, there are additional locations that also communicate with the vomiting center to cause emesis, such as the GI system, the vestibular system, and the higher centers in the brain and thalamus (Becker, 2010). The CTZ causes vomiting to start when its receptors find emetogenic chemicals in the blood and cerebrospinal fluid (CSF) and transmit this data to the adjacent NTS. This is also where abdominal vagal afferents detect possibly emetogenic compounds in the lumen end (Hornby, 2001). The NTS is the start of the ultimate common channel via which all emetic stimuli cause vomiting (Miller and Leslie, 1994). CTZ can be activated by exogenous stimuli to cause emesis, and receptors including 5-HT_3, ACh_M, D_2, NK_1, and H_1, etc. Have an impact on the regulation of emesis generation. Many of these receptors are found both in the periphery, such as the gastrointestinal tract, and vagal afferents, and in the brainstem dorsal vagal complex, such as the area postrema (Navari, 2014; Zhong et al., 2021).

The gastrointestinal system is thought to be a susceptible target of toxicity, according to existing human and animal studies. Many cases of nausea, vomiting, and stomach discomfort in persons who consume copper-tainted drinks or water have been documented (Dorsey and Ingerman, 2004). Intragastric infusion of $CuSO_4$) an irritant to the stomach lining and corrosive to the mucosal surfaces of the gastrointestinal tract (Wang and Borison, 1951), is the standard stimulus for the GI vagal afferent component of emesis (Horn et al., 2014). Peripheral activities influence emesis by stimulating visceral afferent nerve fibers in the stomach, which then convey the impulses to the vomiting center (Andrews and Lawes, 2020).

Instead of frogs, the novel assay technique was invented to test anti-emetic substances of natural origin using young chicks. The benefits of the new approach over earlier ones were entirely parallel outcomes as well as lowering standard errors (Akita et al., 1998). Other techniques need lengthy experiment courses (the frog method, for instance, takes 90 min) and are only capable of screening anti-emetic action on a limited scale. However, the new approach that used young chicks had advantages including being simple to handle, affordable, requiring only a 30-min trial, and making it simple to count the retching action.

The process of discovering and developing new drugs is complex, protracted, and multidisciplinary. *In-silico* molecular modeling has been increasingly popular in recent years as part of CADD (Computer Aided Drug Design). The fundamental advantage of *in-silico* drug design is that it makes pharmaceutical research and development more affordable (Terstappen and Reggiani, 2001). From the preclinical phase through the late stage, this technique can contribute significantly to all aspects of drug development. The diverse facets of fundamental study and application are merged and motivate one another in the large field of *in-silico* approach (Rao and Srinivas, 2011). The discipline makes use of contemporary methods including homology modeling, Ramachandran plot, molecular docking, structure-based design, molecular dynamic simulation, and the rising amount of chemical and biological databases

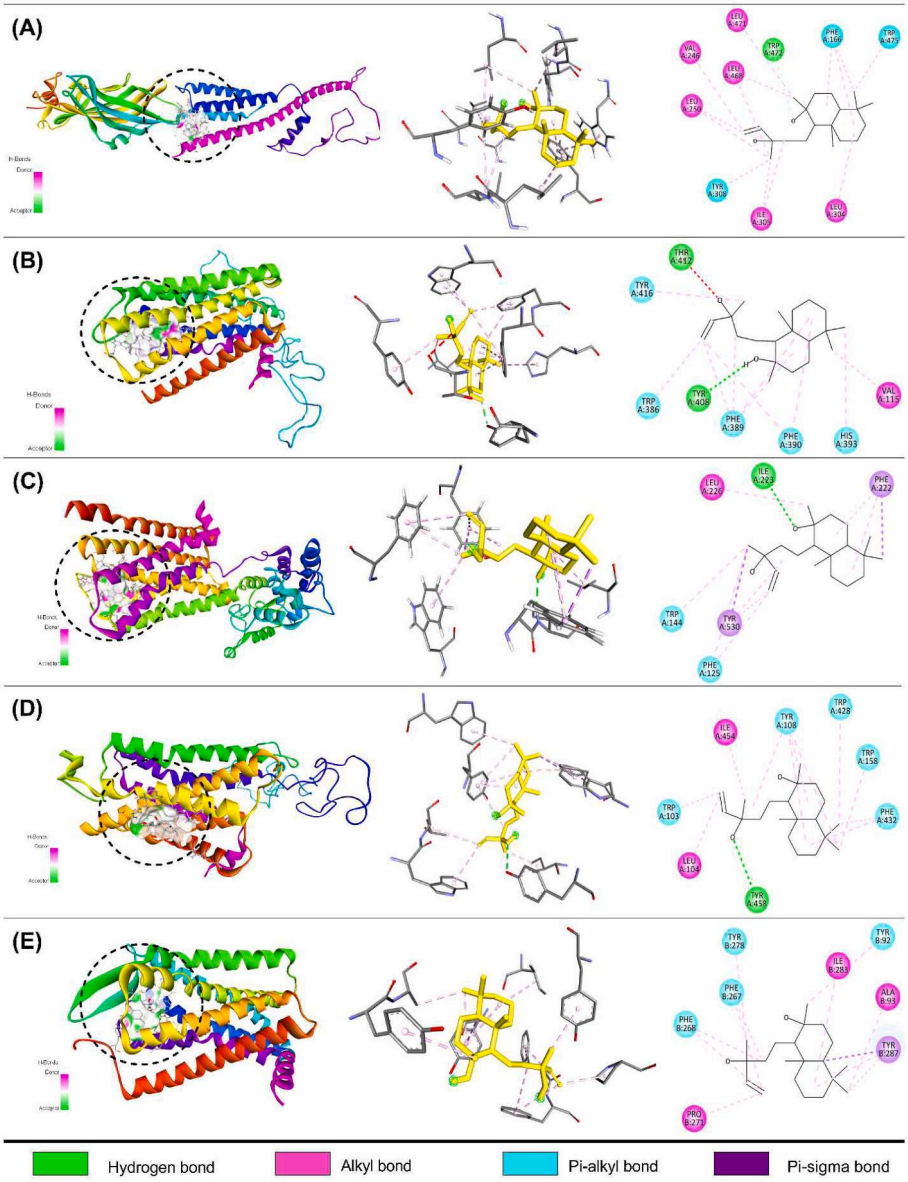

Fig. 6. The 2D and 3D structure of molecular docking interactions of (A) $5HT_{3A}$, (B) D_2, (C) M_3, (D) H_1, and (E) NK_1 receptors with sclareol (SCL).

(Wadood et al., 2013).

By using a similar homologous receptor's experimental 3D structure and its amino acid pattern, a technique known as homology modeling may be used to create an atomic-resolution model of the target receptor (Bertoni et al., 2017). The Ramachandran plot, which illustrates the primary chain conformation angles (Φ and Ψ) of the polypeptide chain of a receptor molecule, is vital in establishing the stereochemical integrity of the receptor model (Gopalakrishnan et al., 2007). In order to find and enhance treatment prospects, molecular docking examines and simulates the interactions between the ligands and the target macromolecules. Docking studies generate and select the best-suited ligand conformations and orientations (Jakhar et al., 2020). The statistical assessment procedure that converts the interaction energy into numerical values called docking scores, determines how well a molecule docking experiment is performed (Kumar et al., 2019). The score simulates the possible energy shift that might occur when the receptor and ligand interact with one another. In other words, a very negative score indicates a strong connection, whereas a less negative or even positive value indicates a minimal or nonexistent interaction (Korb et al., 2009). The physical underpinnings of the structure and operation of biological macromolecules can be better understood with the help of molecular dynamics simulations. It is used to determine the stability of ligand-receptor complexes (Shen et al., 2021). Thus, *in-silico* approaches have proved crucial for identifying targets and for making predictions about new medications.

In this study, all the standards enhanced the latency duration and decreased the number of retches in the chick groups in comparison to the NC group. Among all of the standards in chicks, the ODN ingested group had the fewest retches (25.40 ± 0.92) in comparison to the NC group (75.40 ± 0.51). In terms of the latency period, the ODN group also has the highest average latency in comparison to the NC group. Interestingly, SCL-10 reduced the number of retches (26.20 ± 0.66) and augmented the latent period (75.80 ± 1.28 s) in comparison to the NC group (12.00 ± 0.83 s). And the value is similar to all of the standard groups. Additionally, to comprehend the synergistic activity, we mixed the median SCL dosage (SCL-10) with all the standards. A synergistic effect, also known as synergism, is a pharmacological phenomenon in which the combined effects of two or more drugs are larger than the outcomes of the drug delivered individually (García-Fuente et al., 2018). As evidence of the synergistic impact, the combined drug therapy in this

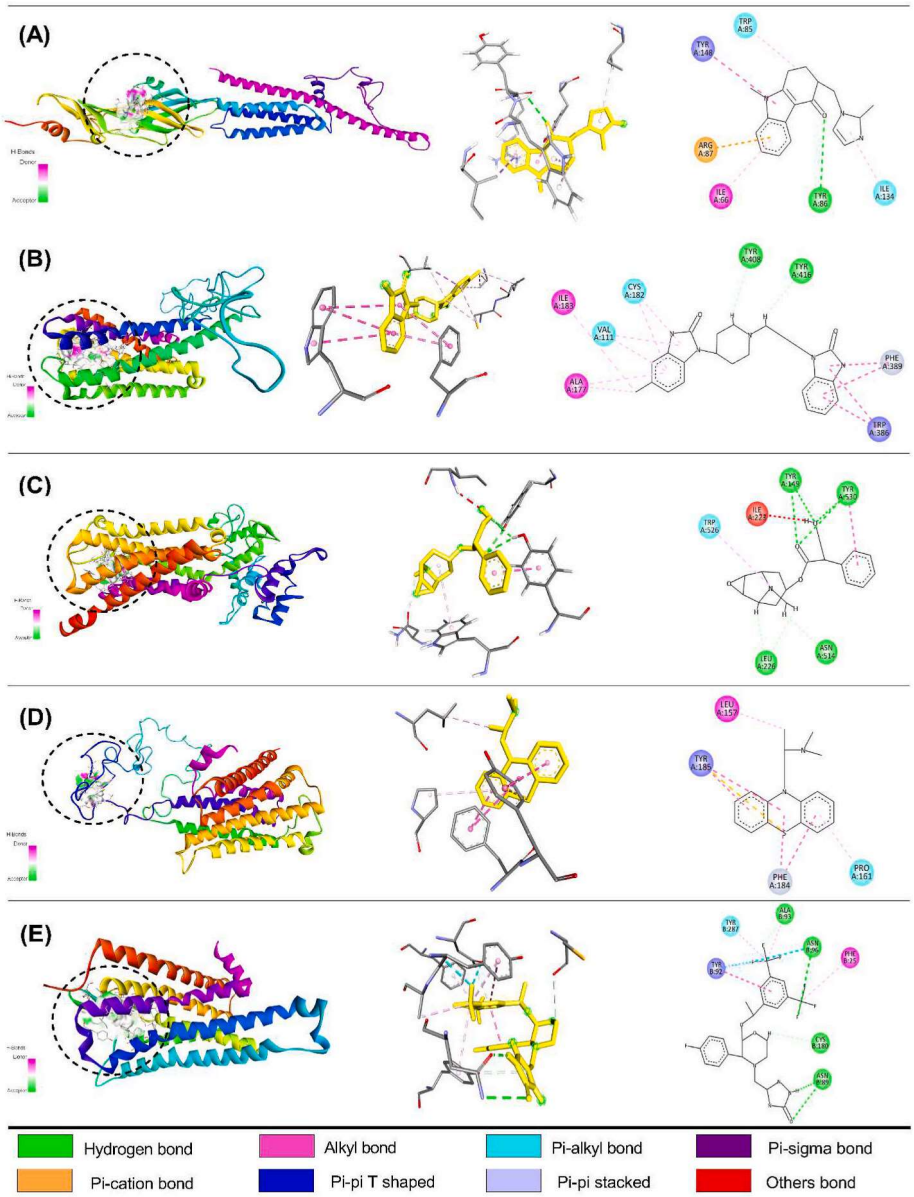

Fig. 7. The 2D and 3D structure of molecular docking interactions of (A) $5HT_{3A}$, (B) D_2, (C) M_3, (D) H_1, and (E) NK_1 receptors and with their specific antagonist drugs.

experiment produced better results in the chick groups for both scenarios (e.g., the number of retches and the latency duration). According to the results of our *in-vivo* investigation, the group of chicks pretreatment with (SCL-10 + ODN) exhibited the longest delay before the first retching (109.40 ± 1.03 s) and the fewest retches (20.00 ± 0.70). SCL-10 or its combination (SCL-10 + ODN) significantly ($p < 0.0001$) increased the latency period and also exhibited a similar/reduced number of retches significantly ($p < 0.01$) to the ODN-treated group. Additionally, the highest percentage increase in latency (89.03%) for combined groups was observed in the (SCL-10 + ODN) group. The same group, however, also experienced the highest %decrease (73.47%) in NC retches inspection. The SCL-15 group has the best %increase (89.83%) in latency duration, on the other hand, the SCL-10 group has the best value of 65.25% for the %decrease in retches of the sample group's inspection of NC.

Our *in-silico* research indicates that the optimal receptors for molecular docking simulation have been extracted using the homology modeling approach. SCL demonstrated outstanding docking performance against $5HT_{3A}$, D_2, M_3, H_1, and NK_1 with docking scores of −7.2, −8.7, −8.1, −7.6, and −8.2 kcal/mol, respectively, which are comparable to and/or sometimes even better to the standards. According to the analysis of drug-receptor interaction, D_2 and SCL have more similar binding sites as DOM. TRP386 and PHE389 are two examples of amino acid residues in D_2 that have hydrophobic bonds and are similar to those in ligands. We also evaluate SCL's pharmacokinetic and drug-likeness properties to all the standards since these two factors are essential for a chemical to qualify as a medication. When compared to standards, SCL's physicochemical and pharmacokinetic characteristics were favorable. According to Table 4, all estimated characteristics including molecular weight, the number of H bonds acceptors and donors (Cavalli et al., 2002), the octanol/water partition coefficient (QPlogPo/w), and aqueous solubility (QPlogS) (Jorgensen and Duffy, 2002) were within a desirable range. The BBB is a sophisticated structure that isolates the peripheral tissue from the CNS. It helps to maintain homeostasis and contributes to the removal of cellular waste products and toxins from the brain into circulation (Małkiewicz et al., 2019). BBB permeability values for SCL were estimated to be 0.073. The principal cytochromes (CYP) of the P450 family were inhibited in order to determine metabolism. The

Table 4
Best binding affinity results and non-bond interactions of antagonist drugs and with their specific emetic receptors.

Antagonist drug & Protein (Receptor)	Binding affinity (Kcal/mol)	H-bond			Hydrophobic bond			Other bonds [Electrostatic (E) or, Others (O)]		
		Residues	Bond length (Å)	Bond Types	Residues	Bond length (Å)	Bond Types	Residues	Bond length (Å)	Bond Types
(A) Ondansetron and Serotonin ($5HT_{3A}$)	−8.5	TYR86	2.99	Conventional	TYR148	5.81	Pi-pi T-shaped	ARG87	4.91	Pi-cation (E)
					TRP85	4.81	Pi-Alkyl			
					TRP85	4.09				
					ILE134	4.95				
					ARG87	4.49				
					ILE66	4.86	Pi-sigma			
(B) Domperidone and Dopamine-2 (D_2)	−9.5	TYR408	3.01	Carbon Hydrogen	PHE389	4.16	Pi-Pi			
					PHE389	4.71	Stacked			
					TRP386	5.23	Pi-Pi T-shaped			
					TRP386	4.95				
					TRP386	5.21				
					ALA177	3.63	Alkyl			
		THR416	2.35		ILE183	5.25				
					VAL111	4.52	Pi-Alkyl			
					ALA177	5.36				
					CYS182	4.79				
					VAL111	4.61				
					CYS182	4.56				
(C) Hyoscine butylbromide and Muscarinic-3 (M_3/Ach_M)	−8.3	TYR149	2.54	Conventional	TRP526	5.28	Pi-Alkyl			
		TYR149	2.18							
		TYR530	2.60							
		TYR530	2.50							
		LEU226	3.01	Carbon Hydrogen	TYR530	3.96	Pi-pi stacked			
		LEU226	2.86							
		ASN514	2.70							
(D) Promethazine Hydrochloride and Histamine-1 (H_1)	−6.6				PHE184	4.27	Pi-Pi	TYR185	5.80	Pi-sulfur (O)
					PHE184	5.33	Stacked			
					TYR185	5.37	Pi-Pi T-shaped			
					TYR185	5.23				
					LEU157	4.60	Alkyl			
					PRO161	5.35	Pi-Alkyl			
(E) Aprepitant and Neurokinin-1 (NK_1)	−10.0	ASN89	2.08	Conventional	TYR92	5.72	Pi-Pi T-shaped	ASN96	2.90	Halogen (Fluorine)
		ASN96	2.90		ALA93	4.41	Alkyl	ALA93	2.26	
		ASN89	2.06					TYR92	2.94	
		ALA93	2.26	Carbon Hydrogen	PHE25	4.32				
		CYS180	2.70		TYR92	4.33	Pi-Alkyl	ASN96	3.01	
					TYR287	4.99				

main enzyme responsible for metabolizing medications having a limited therapeutic index is CYP2C9 (Daly et al., 2017). As with other conventional inhibitors, SCL was expected to probably inhibit CYP2C9.

A drug's bioavailability is connected to its excretion rate, which typically happens as a mixture of hepatic and renal clearance. Excretion is crucial for figuring out dosage levels to reach steady-state concentrations (Pires et al., 2015). Applying the total clearance feature of pkCSM for SCL, excretion values (0.914 mL/min/kg) were calculated. We also forecast acute oral rat toxicity and hepatotoxicity (LD_{50} values) (Domínguez-Villa et al., 2021). The estimated LD_{50} for SCL was 1.848 mol/kg. Xenobiotics and medicines are bio-transformed by the liver, an essential organ that is critical for energy exchanges. It always interferes with proper metabolism when injured and may cause liver failure (Daina et al., 2017). ODN, DOM, HYS, and APT were predicted to be hepatotoxic by the hepatotoxicity feature, whereas our phytochemical SCL has not proven toxic. The categorization and prediction models for hepatotoxicity, cytotoxicity, mutagenicity, and carcinogenicity are built using the Random Forest (RF) method (Breiman, 2001). In comparison to all the standards, SCL's toxicity profile has again been considered to be exceptional. SwissADME was used to determine the drug-likeness characteristics (MW < 500 Da, HBDs <5, HBAs <10, and LogP <5) chosen according to the Lipinski guidelines, which indicate a compound's excellent absorption (Daina et al., 2017). Analysis of the SCL revealed no violations of these guidelines, demonstrating that it will exhibit good absorption.

Molecular dynamic simulation (MDs) is one of the most popular computational techniques employed for the research of biological systems. Further, it serves as one of the best techniques for comprehending interactions' stability and dynamic activity. It is complex at different periods due to a variety of factors, including fast internal movement and delayed structural changes (Ponnulakshmi et al., 2019). Our investigation of the MDs data demonstrates that (Fig. 9.) SCL effectively attaches to the active regions of $5HT_{3A}$ and D_2 receptors. RMSD, RMSF, Rg, and SASA revealed that SCL has effects on the dynamic properties of target receptors that are almost identical to those of conventional medicines. It has been conclusively proven that SCL may be employed in copper sulphate-induced emetic chicks by confirming the overall analysis of *in-silico* findings and showing that the *in-vivo* experiments are comparable with the *in-silico* statistics.

The inflammatory signaling system is triggered by a variety of stimuli and chemotherapy medicines, which also upregulate the production of cytokines to cause gastrointestinal mucositis, leading to emesis (Zhao et al., 2022). They could stimulate cytokine storm syndrome by activating monocytes, macrophages, and dendritic cells with excessive levels of pro-inflammatory cytokines (such as IL-6, IL-1β, IL-1, IFN-γ, TNF-α, COX-2, iNOS, NF-κB, IκBα, and T reg cells) (Zhang et al., 2021). Numerous bodily organs along with the digestive system might be harmed by the systemic inflammatory response. Thus, these cytokines activated the vomiting center ($5\text{-}HT_3$, D_2, NK_1, M_3/ACh_M, H_1 receptors) (Zhang et al., 2021). Meanwhile, SCL has potential activity in

Table 5
Pharmacokinetic profiles and Drug-likeness properties of SCL and standards.

Properties	Factors	ODN	DOM	HYS	PRO	APT	SCL
Physico-chemical Properties	**Formula**	$C_{18}H_{19}N_3O$	$C_{22}H_{24}C_lN_5O_2$	$C_{21}H_{30}BrNO_4$	$C_{17}H_{21}C_lN_2S$	$C_{23}H_{21}F_7N_4O_3$	$C_{20}H_{36}O_2$
	MW (g mol^{-1})	293.36	425.91	440.37	320.88	534.43	308.50
	Heavy atoms	22	30	27	21	37	22
	Arom. Heavy atoms	14	18	6	12	17	0
	H-Bond acceptor (HBAs)	2	3	4	1	12	2
	H-Bond donor (HBDs)	0	2	1	0	2	2
	Molar refractivity	87.39	124.08	112.69	97.03	118.82	95.43
Lipophilicity	**Log Po/w (XLOGP3)**	2.29	3.90	3.22	5.61	4.20	4.93
Water solubility	**Log S (ESOL)**	Soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble
Drug likeness	**Lipinski**	Yes	Yes	Yes	Yes	Yes	Yes
Absorption (A)	**GI absorption**	High	High	High	High	Low	High
	Aqueous solubility (Log mol/L)	(−) 3.175	(−) 2.892	(−) 1.627	(−) 4.019	(−) 3.821	(−) 4.988
	Caco-2 permeability (Log Papp in 10–6 cm/s)	(+) 1.685	(+) 0.81	(+) 1.33	(+) 1.379	(+) 1.146	(+) 1.60
	Intestinal absorption (% Absorbed)	99.93	77.42	82.03	88.28	87.23	90.824
Distribution (D)	**Blood brain barrier (log BB)**	(+) 0.213	(−) 0.836	(+) 0.146	(+) 1.119	(−) 2.076	(−) 0.073
	CNS Permeability (Log PS)	(−) 1.737	(−) 2.264	(−) 3.05	(−) 1.687	(−) 2.389	(−) 2.517
Metabolism (M)	**CYP1A2 inhibitor**	Yes	Yes	No	Yes	No	Yes
	CYP2C9 inhibitor	No	No	No	No	Yes	No
Excretion (E)	**Total clearance (Log ml/min/kg)**	0.817	0.946	1.609	1.063	0.379	0.914
Organ Toxicity (T)	**Hepatotoxicity**	Yes	Yes	Yes	No	Yes	No
Other Toxicity (T)	**Carcinogenicity**	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
	Immunotoxicity	Inactive	Active	Inactive	Inactive	Inactive	Inactive
	Mutagenicity	Active	Inactive	Inactive	Inactive	Inactive	Inactive
	Cytotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
	Oral Rat Acute Toxicity (LD_{50}, mol/kg)	2.321	2.477	2.310	3.430	2.714	1.848
	Toxicity class	III	IV	IV	III	IV	V

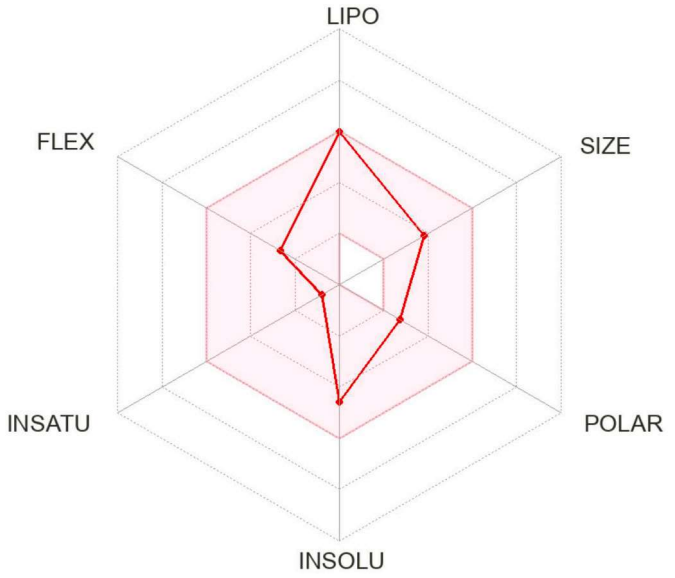

Fig. 8. ADMET properties of Sclareol generated by SwissADME [The colored zone is the suitable physicochemical space for oral bioavailability. LIPO (Lipophilicity): −0.7 < XLOGP3 < +5.0; SIZE: 150 g/mol < MV < 500 g/mol; POLAR (Polarity): 20 Å^2 < TPSA <130 Å^2; INSOLU (Insolubility): −6 < Log S (ESOL) < 0; INSATU (Insaturation): 0.25 < Fraction Csp 3 < 1; FLEX (Flexibility): 0 < NUM. rotatable bonds <9].

the regulation of the inflammatory response by reducing inflammatory cytokines which express anti-emetic action (Huang et al., 2012; Hsieh et al., 2017). In our study, animal models and computational data showed excellent antiemetic activity of SCL. We hypothesize that SCL could have a possible antiemetic function on inflammatory signaling using data from our *in-vivo* and *in-silico* study. Additional investigation is required to confirm our experimental data and determine the precise role played by SCL in emetic action. Fig. 10 depicts a possible anti-emetic mechanism of SCL based on *in-vivo* and *in-silico* investigations.

Studies using certain laboratory animals provide necessary and important data regarding the beneficial and harmful effects of new drug candidates and their possible biopharmaceutical considerations (White, 1997). Thus, each pre-clinical study helps medicinal scientists evaluate biologically active molecule's potential for clinical studies. These studies allow for the determination of test dose and dosage frequency, the right administration routes, the drug metabolism profile, and the development of error correction machines during clinical trials. In this study, we have seen that all standard anti-emetic drugs inhibit the emesis tendency in animals. The test sample's SCL also exerted dose-dependent anti-emetic effects in animals. In comparison to the control and standard groups, SCL exerted strong anti-emetic effects in animals, which opens a platform to expand the possibility of testing this hopeful bioactive compound in larger animal models (i.e., those that are closely related to human physiology, such as mice, guinea pigs, rats, rabbits, etc.) prior to starting clinical investigations. Although this new drug candidate has yet to undergo extensive toxicological studies in animal models, in this study SCL treatment did not show any toxicological phenomena, nor did it cause the death of any chicks, which demonstrates its safety in this animal model. Moreover, our *in-vivo* findings also corroborate the outcomes of *in-silico* studies, demonstrating the possibility of considering this bioactive in the emesis of laboratory animals.

5. Conclusion

In the Young Chick model, SCL substantially decreased the number of retches and increased delay time. Our *in-vivo* findings suggest that

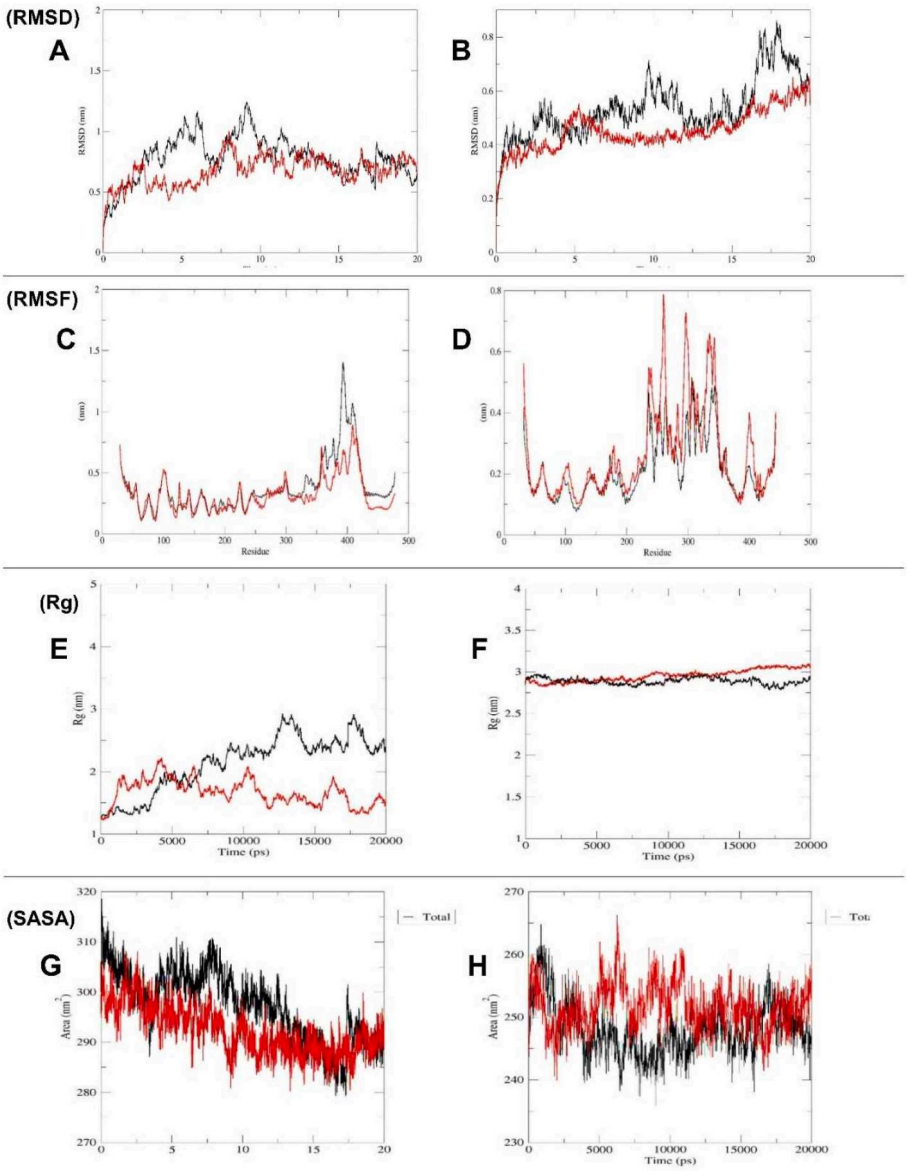

Fig. 9. RMSD values over time: (A) SCL (black line) and ODN (red line) with $5HT_{3A}$ receptor; (B) SCL (black line) and DOM (red line) with D_2 receptor. RMSF values per residue: (C) SCL (black line) and ODN (red line) with $5HT_{3A}$ receptor; (D) SCL (black line) and DOM (red line) with D_2 receptor. Rg values over time: (E) SCL (black line) and ODN (red line) with $5HT_{3A}$ receptor; (F) SCL (black line) and DOM (red line) with D_2 receptor. SASA values over time: (G) SCL (black line) and ODN (red line) with $5HT_{3A}$ receptor; (H) SCL (black line) and DOM (red line) with D_2 receptor. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

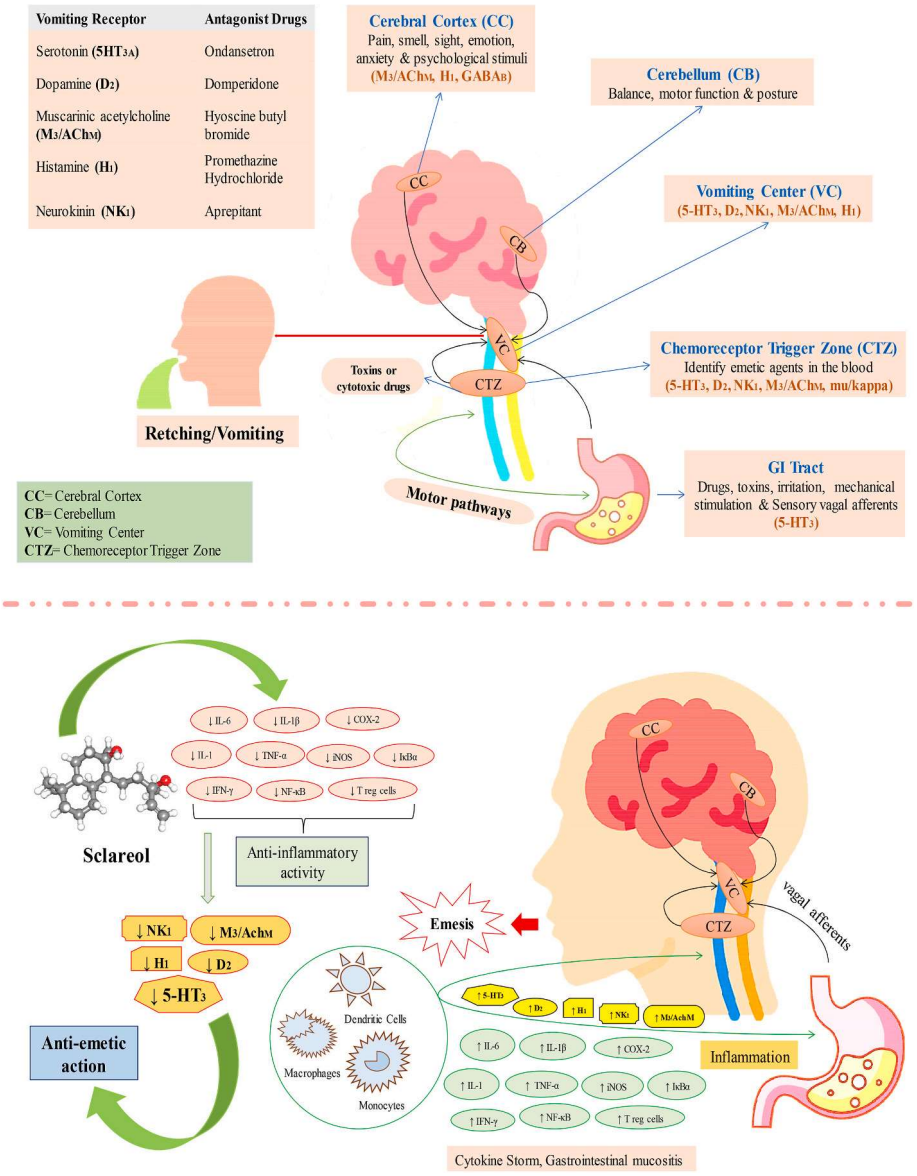

Fig. 10. A possible anti-emetic mechanism of sclareol.

acute SCL administration has an antiemetic-like effect on $CuSO_4.5H_2O$-induced emetic chicks. SCL alone or in combination such as the (SCL + ODN) group considerably ($p < 0.0001$) extended the latency duration and significantly ($p < 0.01$) decreased the number of retches. Furthermore, the *in-silico* results exhibited by SCL were similar to traditional medications. When combined with standards that target specific receptors, the compound also exhibits synergistic activity. The observations provide additional support for the hypothesis that SCL has an outstanding ability to interact with the $5HT_3$ and D_2 receptors to produce inhibitory-like effects. With other receptors including M_3, H_1, and NK_1, SCL also binds to them satisfactorily. Analysis of the pharmacokinetics and drug-like properties further demonstrates an excellent ADMET profile of SCL. Additional research is required to validate this effectiveness and determine the precise mechanism(s) of action. Our investigations may be used in clinical practice using this strategy.

CRediT authorship contribution statement

Mehedi Hasan Bappi: Conceptualization. **Abdullah Al Shamsh Prottay:** Conceptualization. **Khattab Al-Khafaji:** Methodology. **Md Showkoth Akbor:** Methodology. **Muhammad Kamal Hossain:** Methodology. **Md Shahazul Islam:** Software. **Afia Ibnath Asha:** Software. **Cassio Rocha Medeiros:** First Draft of the Manuscript, Methodology, Software. **Catarina Martins Tahim:** First Draft of the Manuscript, Investigation, Software. **Elaine Cristina Pereira Lucetti:** First Draft of the Manuscript, Resources, Supervision. **Henrique Douglas Melo Coutinho:** Project administration. **Hossam Kamli:** Supervision. **Muhammad Torequl Islam:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific

Research at King Khalid University, Abha, Saudi Arabia, for funding this work through a large group research project under grant number RGP2/362/44.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2023.114068.

References

Afshari, H., Nourbakhsh, M., Salehi, N., Mahboubi-Rabbani, M., Zarghi, A., Noori, S., 2020. STAT3-mediated apoptotic-enhancing function of sclareol against breast cancer cells and cell sensitization to cyclophosphamide. Iran. J. Pharm. Res. (IJPR): Iran. J. Pharm. Res. (IJPR) 19 (1), 398.

Ahmed, S.M., 2014. Natural antiemetics: an overview. Pak. J. Pharm. Sci. 27.

Ahmed, S., Hasan, M.M., Ahmed, S.W., Mahmood, Z.A., Azhar, I., Habtemariam, S., 2013. Anti-emetic effects of bioactive natural products. Phytopharmacology 4 (2), 390–433.

Akita, Y., Yang, Y., Kawai, T., Kinoshita, K., Koyama, K., Takahashi, K., Watanabe, K., 1998. New assay method for surveying anti-emetic compounds from natural sources. Nat. Prod. Sci. 4 (2), 72–77.

Akpoveso, O.O.P., Ubah, E.E., Obasanmi, G., 2023. Antioxidant phytochemicals as a potential therapy for diabetic complications. Antioxidants 12 (1), 123.

Andrews, P.L.R., Lawes, I.N.C., 2020. A Protective Role for Vagal Afferents: an Hypothesis. *Neuroanatomy And Physiology of Abdominal Vagal Afferents*, pp. 280–302.

Andrews, P.L., Horn, C.C., 2006. Signals for nausea and emesis: implications for models of upper gastrointestinal diseases. Auton. Neurosci. 125 (1–2), 100–115.

Ashraf, K., Sultan, S., Shah, S., 2017. Phychemistry, phytochemical, pharmacological and molecular study of Zingiber officinale Roscoe: a review. Int. J. Pharm. Pharmaceut. Sci. 9 (11), 8–16.

Bachheti, R.K., Worku, L.A., Gonfa, Y.H., Zebeaman, M., Pandey, D.P., Bachheti, A., 2022. Prevention and treatment of cardiovascular diseases with plant phytochemicals: a review. Evid. base Compl. Alternative Med. 2022, 5741198.

Banerjee, P., Eckert, A.O., Schrey, A.K., Preissner, R., 2018. ProTox-II: a web server for the prediction of toxicity of chemicals. Nucleic Acids Res. 46 (W1), W257–W263.

Bappi, M.H., Prottay, A.A.S., Kamli, H., Sonia, F.A., Mia, M.N., Akbor, M.S., et al., 2023. Quercetin antagonizes the sedative effects of linalool, possibly through the GABAergic interaction pathway. Molecules 28 (14), 5616.

Becker, D.E., 2010. Nausea, vomiting, and hiccups: a review of mechanisms and treatment. Anesth. Prog. 57 (4), 150–157.

Berger, A.A., Liu, Y., Jin, K., Kaneb, A., Welschmeyer, A., Cornett, E.M., et al., 2021. Efficacy of acupuncture in the treatment of chronic abdominal pain. Anesthesiol. Pain Med. 11 (2).

Bertoni, M., Kiefer, F., Biasini, M., Bordoli, L., Schwede, T., 2017. Modeling receptor quaternary structure of homo-and hetero-oligomers beyond binary interactions by homology. Sci. Rep. 7 (1), 1–15.

Bhuia, M.S., Kamli, H., Islam, T., Sonia, F.A., Kazi, M.A., Siam, M.S.H., et al., 2023a. Antiemetic activity of trans-ferulic acid possibly through muscarinic receptors interaction pathway: *In vivo* and *in silico* study. Result. Chem. 6, 101014.

Bhuia, M.S., Rahaman, M.M., Islam, T., Bappi, M.H., Sikder, M.I., Hossain, K.N., et al., 2023b. Neurobiological effects of gallic acid: current perspectives. Chin. Med. 18 (1), 27.

Biasini, M., Bienert, S., Waterhouse, A., Arnold, K., Studer, G., Schmidt, T., et al., 2014. SWISS-MODEL: modeling receptor tertiary and quaternary structure using evolutionary information. Nucleic Acids Res. 42 (W1), W252–W258.

Bjelkmar, P., Larsson, P., Cuendet, M.A., Hess, B., Lindahl, E., 2010. Implementation of the CHARMM force field in GROMACS: analysis of receptor stability effects from correction maps, virtual interaction sites, and water models. J. Chem. Theor. Comput. 6 (2), 459–466.

Bonfield, C.M., Engh, J.A., 2012. Pregnancy and brain tumors. Neurol. Clin. 30 (3), 937–946.

Breiman, L., 2001. Random forests. Mach. Learn. 45 (1), 5–32.

Caissard, J.C., Olivier, T., Delbecque, C., Palle, S., Garry, P.P., Audran, A., et al., 2012. Extracellular localization of the diterpene sclareol in clary sage (Salvia sclarea L., Lamiaceae). PLoS One 7 (10), e48253.

Cavalli, A., Poluzzi, E., De Ponti, F., Recanatini, M., 2002. Toward a pharmacophore for drugs inducing the long QT syndrome: insights from a CoMFA study of HERG K+ channel blockers. J. Med. Chem. 45 (18), 3844–3853.

Celio, L., Bonizzoni, E., Zattarin, E., Codega, P., de Braud, F., Aapro, M., 2019. Impact of dexamethasone-sparing regimens on delayed nausea caused by moderately or highly emetogenic chemotherapy: a meta-analysis of randomised evidence. BMC Cancer 19, 1–13.

Cerqua, I., Neukirch, K., Terlizzi, M., Granato, E., Caiazzo, E., Cicala, C., et al., 2022. A vitamin E long-chain metabolite and the inspired drug candidate α-amplexichromanol relieve asthma features in an experimental model of allergen sensitization. Pharmacol. Res. 181, 106250.

Chy, M.N.U., Adnan, M., Chowdhury, M.R., Pagano, E., Kamal, A.M., Oh, K.K., et al., 2021. Central and peripheral pain intervention by Ophiorrhiza rugosa leaves: potential underlying mechanisms and insight into the role of pain modulators. J. Ethnopharmacol. 276, 114182.

Consortium, U., 2015. UniProt: a hub for receptor information. Nucleic Acids Res. 43 (D1), D204–D212.

D'Avino, D., Cerqua, I., Ullah, H., Spinelli, M., Di Matteo, R., Granato, E., et al., 2023. Beneficial effects of Astragalus membranaceus (fisch.) bunge extract in controlling inflammatory response and preventing asthma features. Int. J. Mol. Sci. 24 (13), 10954.

Daina, A., Michielin, O., Zoete, V., 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 7 (1), 1–13.

Daly, A.K., Rettie, A.E., Fowler, D.M., Miners, J.O., 2017. Pharmacogenomics of CYP2C9: functional and clinical considerations. J. Personalized Med. 8 (1), 1.

Diemunsch, P., Grélot, L., 2000. Potential of substance P antagonists as antiemetics. Drugs 60 (3), 533–546.

Dimas, K., Hatziantoniou, S., Tseleni, S., Khan, H., Georgopoulos, A., Alevizopoulos, K., et al., 2007. Sclareol induces apoptosis in human HCT116 colon cancer cells *in vitro* and suppression of HCT116 tumor growth in immunodeficient mice. Apoptosis 12 (4), 685–694.

Domínguez-Villa, F.X., Durán-Iturbide, N.A., Ávila-Zárraga, J.G., 2021. Synthesis, molecular docking, and *in-silico* ADME/Tox profiling studies of new 1-aryl-5-(3-azidopropyl) indol-4-ones: potential inhibitors of SARS CoV-2 main protease. Bioorg. Chem. 106, 104497.

Dorsey, A., Ingerman, L., 2004. Toxicological Profile for Copper.

Drewes, A.M., Olesen, A.E., Farmer, A.D., Szigethy, E., Rebours, V., Olesen, S.S., 2020. Gastrointestinal pain. Nat. Rev. Dis. Prim. 6 (1), 1–16.

Fernández, J., Silván, B., Entrialgo-Cadierno, R., Villar, C.J., Capasso, R., Uranga, J.A., et al., 2021. Antiproliferative and palliative activity of flavonoids in colorectal cancer. Biomed. Pharmacother. 143, 112241.

Fernández-Pendás, M., Escribano, B., Radivojević, T., Akhmatskaya, E., 2014. Constant pressure hybrid Monte Carlo simulations in GROMACS. J. Mol. Model. 20, 1–10.

Ferrarini, E.G., Paes, R.S., Baldasso, G.M., de Assis, P.M., Gouvêa, M.C., De Cicco, P., et al., 2022. Broad-spectrum cannabis oil ameliorates reserpine-induced fibromyalgia model in mice. Biomed. Pharmacother. 154, 113552.

Fleming, M.A., Ehsan, L., Moore, S.R., Levin, D.E., 2020. The enteric nervous system and its emerging role as a therapeutic target. Gastroenterol. Res. Pract. 2020, 8024171.

Freitas, M.A., Vasconcelos, A., Gonçalves, E.C., Ferrarini, E.G., Vieira, G.B., Cicia, D., et al., 2021. Involvement of opioid system and TRPM8/TRPA1 channels in the antinociceptive effect of spirulina platensis. Biomolecules 11 (4), 592.

Gallo, M., Ferrara, L., Calogero, A., Montesano, D., Naviglio, D., 2020. Relationships between food and diseases: what to know to ensure food safety. Food Res. Int. 137, 109414.

Gan, T.J., 2007. Mechanisms underlying postoperative nausea and vomiting and neurotransmitter receptor antagonist-based pharmacotherapy. CNS Drugs 21 (10), 813–833.

García-Fuente, A., Vázquez, F., Viéitez, J.M., Garcia Alonso, F.J., Martín, J.I., Ferrer, J., 2018. CISNE: an accurate description of dose-effect and synergism in combination therapies. Sci. Rep. 8 (1), 1–9.

Gholam, G.M., Firdausy, I.A., Artika, I.M., Abdillah, R.M., Firmansyah, R.P., 2022. Molecular Docking: Bioactive Compounds of Mimosa Pudica as an Inhibitor of Candida Albicans Sap 3. *bioRxiv*.

Glare, P.A., Dunwoodie, D., Clark, K., Ward, A., Yates, P., Ryan, S., Hardy, J.R., 2008. Treatment of nausea and vomiting in terminally I11 cancer patients. Drugs 68 (18), 2575–2590.

Gopalakrishnan, K., Sowmiya, G., Sheik, S.S., Sekar, K., 2007. Ramachandran plot on the web (2.0). Receptor Peptide Lett. 14 (7), 669–671.

Hauser, J.M., Azzam, J.S., Kasi, A., 2018. Antiemetic Medications.

Hendren, G., Aponte-Feliciano, A., Kovac, A., 2015. Safety and efficacy of commonly used antiemetics. Expet Opin. Drug Metabol. Toxicol. 11 (11), 1753–1767.

Hess, B., Kutzner, C., Van Der Spoel, D., Lindahl, E., 2008. Gromacs 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. J. Chem. Theor. Comput. 4 (3), 435–447.

Horn, C.C., Meyers, K., Lim, A., Dye, M., Pak, D., Rinaman, L., Yates, B.J., 2014. Delineation of vagal emetic pathways: intragastric copper sulfate-induced emesis and viral tract tracing in musk shrews. Am. J. Physiol. Regul. Integr. Comp. Physiol. 306 (5), R341–R351.

Hornby, P.J., 2001. Central neurocircuitry associated with emesis. Am. J. Med. 111 (8), 106–112.

Houghton, P.J., 1995. The role of plants in traditional medicine and current therapy. J. Alternative Compl. Med. 1 (2), 131–143.

Hsieh, Y.H., Deng, J.S., Pan, H.P., Liao, J.C., Huang, S.S., Huang, G.J., 2017. Sclareol ameliorate lipopolysaccharide-induced acute lung injury through inhibition of MAPK and induction of HO-1 signaling. Int. Immunopharm. 44, 16–25.

Huang, G.J., Pan, C.H., Wu, C.H., 2012. Sclareol exhibits anti-inflammatory activity in both lipopolysaccharide-stimulated macrophages and the λ-carrageenan-induced paw edema model. J. Nat. Prod. 75 (1), 54–59.

Islam, M.S., Hossain, R., Ahmed, T., Rahaman, M.M., Al-Khafaji, K., Khan, R.A., et al., 2022. Anxiolytic-like effect of quercetin possibly through GABA receptor interaction pathway: *In vivo* and *in silico* studies. Molecules 27 (21), 7149.

Jakhar, R., Dangi, M., Khichi, A., Chhillar, A.K., 2020. Relevance of molecular docking studies in drug designing. Curr. Bioinf. 15 (4), 270–278.

Jorgensen, W.L., Duffy, E.M., 2002. Prediction of drug solubility from structure. Adv. Drug Deliv. Rev. 54 (3), 355–366.

Kamli, H., Bappi, M.H., Islam, T., Hossain, R., Hossen, M.M., Al Faruq, et al., 2023. Antioxidant, anti-inflammatory, and anxiolytic-like effects of *Urena sinuata* L: *in vitro*, *in vivo*, and *in silico* studies. J. Biol. Regul. Homeost. Agents 37 (9), 5035–5052.

Kassel, L., Nelson, M., Shine, J., Jones, L.R., Kassel, C., 2018. Scopolamine use in the perioperative patient: a systematic review. AORN J. 108 (3), 287–295.

Khare, N., Maheshwari, S.K., Rizvi, S.M.D., Albadrani, H.M., Alsagaby, S.A., Alturaiki, W., et al., 2022. Homology modelling, molecular docking and molecular

dynamics simulation studies of CALMH1 against secondary metabolites of Bauhinia variegata to treat alzheimer's disease. Brain Sci. 12 (6), 770.
Kocyigit, E., Kocaadam-Bozkurt, B., Bozkurt, O., Ağagündüz, D., Capasso, R., 2023. Plant toxic receptors: their biological activities, mechanism of action and removal strategies. Toxins 15 (6), 356.
Korb, O., Stutzle, T., Exner, T.E., 2009. Empirical scoring functions for advanced receptor– ligand docking with PLANTS. J. Chem. Inf. Model. 49 (1), 84–96.
Kostić, M., Kitić, D., Petrović, M.B., Jevtović-Stoimenov, T., Jović, M., Petrović, A., Živanović, S., 2017. Anti-inflammatory effect of the Salvia sclarea L. ethanolic extract on lipopolysaccharide-induced periodontitis in rats. J. Ethnopharmacol. 199, 52–59.
Kris, M.G., Hesketh, P.J., Herrstedt, J., Rittenberg, C., Einhorn, L.H., Grunberg, S., et al., 2005. Consensus proposals for the prevention of acute and delayed vomiting and nausea following high-emetic-risk chemotherapy. Support. Care Cancer 13 (2), 85–96.
Kumar, S., Kaushik, A., Narasimhan, B., Shah, S.A.A., Lim, S.M., Ramasamy, K., Mani, V., 2019. Molecular docking, synthesis and biological significance of pyrimidine analogues as prospective antimicrobial and antiproliferative agents. BMC Chem. 13 (1), 1–17.
Kwofie, S.K., Dankwa, B., Odame, E.A., Agamah, F.E., Doe, L.P., Teye, J., et al., 2018. *In silico* screening of isocitrate lyase for novel anti-buruli ulcer natural products originating from Africa. Molecules 23 (7), 1550.
Lang, I.M., 1999. Noxious stimulation of emesis. Dig. Dis. Sci. 44 (8 Suppl. l), 58S–63S.
Leung, A.K., Robson, W.L.M., 2007. Acute gastroenteritis in children. Pediatr. Drugs 9 (3), 175–184.
Liu, J., Li, D., Liu, X., 2016. A simple and accurate algorithm for path integral molecular dynamics with the Langevin thermostat. J. Chem. Phys. 145 (2), 024103.
Maljurić, N., Golubović, J., Otašević, B., Zečević, M., Protić, A., 2018. Quantitative structure–retention relationship modeling of selected antipsychotics and their impurities in green liquid chromatography using cyclodextrin mobile phases. Anal. Bioanal. Chem. 410 (10), 2533–2550.
Małkiewicz, M.A., Szarmach, A., Sabisz, A., Cubała, W.J., Szurowska, E., Winklewski, P. J., 2019. Blood-brain barrier permeability and physical exercise. J. Neuroinflammation 16 (1), 1–16.
Manochitra, K., Parija, S.C., 2017. *In-silico* prediction and modeling of the Entamoeba histolytica receptors: serine-rich Entamoeba histolytica receptor and 29 kDa Cysteine-rich protease. PeerJ 5, e3160.
Martinez, A.N., Philipp, M.T., 2016. Substance P and antagonists of the neurokinin-1 receptor in neuroinflammation associated with infectious and neurodegenerative diseases of the central nervous system. J. Neurol. & Neuromed. 1 (2), 29.
Meshram, R.J., Bagul, K.T., Aouti, S.U., Shirsath, A.M., Duggal, H., Gacche, R.N., 2021. Modeling and simulation study to identify threonine synthase as possible drug target in Leishmania major. Mol. Divers. 25 (3), 1679–1700.
Mia, M.N., Smrity, S.Z., Bappi, M.H., Kamli, H., Islam, T., Prottay, A.A.S., et al., 2023. Anxiolytic-like effect of succinic acid: a possible GABAergic intervention. Food Biosci. 55, 103044.
Miller, A.D., Leslie, R.A., 1994. The area postrema and vomiting. Front. Neuroendocrinol. 15 (4), 301–320.
Mitra, S., Das, R., Emran, T.B., Labib, R.K., Noor-E-Tabassum, Islam, F., Sharma, R., Ahmad, I., Nainu, F., Chidambaram, K., Alhumaydhi, F.A., Chandran, D., Capasso, R., Wilairatana, P., 2022. Diallyl disulfide: a bioactive garlic compound with anticancer potential. Biol. Aspects Targeted Drug Discov.: Dev. Novel Targets and/or Chemother. Drug Repurposing 16648714, 128.
Mohammad, A.M., Chowdhury, T., Biswas, B., Absar, N., 2018. Food poisoning and intoxication: a global leading concern for human health. In: Food Safety and Preservation. Academic Press, pp. 307–352.
Moulton, B.C., Fryer, A.D., 2011. Muscarinic receptor antagonists, from folklore to pharmacology; finding drugs that actually work in asthma and COPD. Br. J. Pharmacol. 163 (1), 44–52.
Muhammed, M.T., Aki-Yalcin, E., 2019. Homology modeling in drug discovery: overview, current applications, and future perspectives. Chem. Biol. Drug Des. 93 (1), 12–20.
Navari, R.M., 2014. Olanzapine for the prevention and treatment of chronic nausea and chemotherapy-induced nausea and vomiting. Eur. J. Pharmacol. 722, 180–186.
Naylor, R.J., Inall, F.C., 1994. The physiology and pharmacology of postoperative nausea and vomiting. Anaesthesia 49, 2–5.
Nunes, C.P., de Campos Rodrigues, C., Cardoso, C.A.F., Cytrynbaum, N., Kaufman, R., Rzetelna, H., et al., 2020. Clinical evaluation of the use of ginger extract in the preventive management of motion sickness. Curr. Ther. Res. 92, 100591.
Oman, C.M., Cullen, K.E., 2014. Brainstem processing of vestibular sensory exafference: implications for motion sickness etiology. Exp. Brain Res. 232 (8), 2483–2492.
Paradissis, A., Hatziantoniou, S., Georgopoulos, A., Psarra, A.M.G., Dimas, K., Demetzos, C., 2007. Liposomes modify the subcellular distribution of sclareol uptake by HCT-116 cancer cell lines. Biomed. Pharmacother. 61 (2–3), 120–124.
Patanwala, A.E., Warholak, T.L., Sanders, A.B., Erstad, B.L., 2010. A prospective observational study of medication errors in a tertiary care emergency department. Ann. Emerg. Med. 55 (6), 522–526.
Peana, A.T., Moretti, M.D., 2002. Pharmacological activities and applications of Salvia sclarea and Salvia desoleana essential oils. Stud. Nat. Prod. Chem. 26, 391–423.
Pires, D.E., Blundell, T.L., Ascher, D.B., 2015. pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. J. Med. Chem. 58 (9), 4066–4072.
Pleuvry, B.J., 2012. Physiology and pharmacology of nausea and vomiting. Anaesth. Intensive Care Med. 13 (12), 598–602.
Ponnulakshmi, R., Shyamaladevi, B., Vijayalakshmi, P., Selvaraj, J., 2019. *In silico* and *in vivo* analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. Toxicol. Mech. Methods 29 (4), 276–290.
Qadirifard, M.S., Arabpour, Z., Afsahi, S., Sorkheh, F., Hamidpour, S.N., Ahangarzadeh, N., et al., 2021. Sclareol and cancer prevention: a mini-review, 4 OncoReview 11 (44), 112–119.
Rahaman, M.M., Wilairatana, P., Bappi, M.H., Islam, T., Mia, M.N., Islam, M.T., et al., 2023. Anticancer effect of herbal and marine products: a systematic review. J. King Saud Univ. Sci. 35 (8), 102919.
Rao, V.S., Srinivas, K., 2011. Modern drug discovery process: an *in-silico* approach. J. Bioinf. Sequence Anal. 2 (5), 89–94.
Reintam Blaser, A., Malbrain, M.L., Starkopf, J., Fruhwald, S., Jakob, S.M., De Waele, J., et al., 2012. Gastrointestinal function in intensive care patients: terminology, definitions and management. Recommendations of the ESICM Working Group on Abdominal Problems. Intensive Care Med. 38 (3), 384–394.
Şahin, T.Ö., Yılmaz, B., Yeşilyurt, N., Cicia, D., Szymanowska, A., Amero, P., et al., 2023. Recent insights into the nutritional immunomodulation of cancer-related microRNAs. Phytother Res. 1–23.
Sanger, G.J., Andrews, P.L., 2006. Treatment of nausea and vomiting: gaps in our knowledge. Auton. Neurosci. 129 (1–2), 3–16.
Schaefer, T.S., Zito, P.M., 2018. Antiemetic Histamine H_1 Receptor Blockers.
Schmiderer, C., Grassi, P., Novak, J., Weber, M., Franz, C., 2008. Diversity of essential oil glands of clary sage (Salvia sclarea L., Lamiaceae). Plant Biol. 10 (4), 433–440.
Shaker, B., Ahmad, S., Lee, J., Jung, C., Na, D., 2021. *In-silico* methods and tools for drug discovery. Comput. Biol. Med. 137, 104851.
Sharkey, K.A., Wallace, J.L., 2011. Treatment of disorders of bowel motility and water flux; anti-emetics; agents used in biliary and pancreatic disease. Goodman & Gilman's the Pharmacol. Basis Therapeutics 12, 1323–1349.
Shen, K.H., Fan, M., Hall, L.M., 2021. Molecular dynamics simulations of ion-containing polymers using generic coarse-grained models. Macromolecules 54 (5), 2031–2052.
Sobolev, O.V., Afonine, P.V., Moriarty, N.W., Hekkelman, M.L., Joosten, R.P., Perrakis, A., Adams, P.D., 2020. A global Ramachandran score identifies receptor structures with unlikely stereochemistry. Structure 28 (11), 1249–1258.
Stern, R.M., Stern, R.M., Koch, K.L., Andrews, P., 2011. Nausea: Mechanisms and Management. OUP, USA.
SWISS-MODEL: Homology modelling of receptor structures and complexes. Nucleic Acids Res., 46(W1), W296-W303..
Terstappen, G.C., Reggiani, A., 2001. *In-silico* research in drug discovery. Trends Pharmacol. Sci. 22 (1), 23–26.
Theriot, J., Wermuth, H.R., Ashurst, J.V., 2020. Antiemetic serotonin-5-HT3 receptor blockers. In: StatPearls [Internet]. StatPearls Publishing.
Ursu, O., Rayan, A., Goldblum, A., Oprea, T.I., 2011. Understanding drug-likeness. Wiley Interdiscip. Rev. Comput. Mol. Sci. 1 (5), 760–781.
Wadood, A., Ahmed, N., Shah, L., Ahmad, A., Hassan, H., Shams, S., 2013. *In-silico* drug design: an approach which revolutionarised the drug discovery process. OA Drug des Deliv 1 (1), 3.
Wang, H., Xie, M., Rizzi, G., Li, X., Tan, K., Fussenegger, M., 2022. Identification of sclareol as a natural neuroprotective Cav 1. 3-Antagonist using synthetic Parkinson-mimetic gene circuits and computer-aided drug discovery. Adv. Sci. 9 (7), 2102855.
Wang, P., Ma, X.M., Geng, K., Jiang, Z.Z., Yan, P.Y., Xu, Y., 2022. Effects of Camellia tea and herbal tea on cardiometabolic risk in patients with type 2 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. Phytother Res. 36 (11), 4051–4062.
Wang, S.C., Borison, H.L., 1951. Copper sulphate emesis: a study of afferent pathways from the gastrointestinal tract. Am. J. Physiol.-Legacy Content 164 (2), 520–526.
Ward, S.J., Lichtman, A.H., Piomelli, D., Parker, L.A., 2021. Cannabinoids and cancer chemotherapy-associated adverse effects. JNCI Monographs 2021 (58), 78–85.
Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., et al. (2018) .
White, H.S., 1997. Clinical significance of animal seizure models and mechanism of action studies of potential antiepileptic drugs. Epilepsia 38 (Suppl. 1), S9–S17.
Yakubu, A., De Donato, M., Imumorin, I.G., 2017. Modelling functional and structural impact of non-synonymous single nucleotide polymorphisms of the DQA1 gene of three Nigerian goat breeds. S. Afr. J. Anim. Sci. 47 (2), 146–156.
Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., Madden, T.L., 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinf. 13 (1), 1–11.
Zhang, T., Liu, D., Tian, D., Xia, L., 2021. The roles of nausea and vomiting in COVID-19: did we miss something? J. Microbiol. Immunol. Infect. 54 (4), 541–546.
Zhao, Y.Z., Dai, Y.Z., Nie, K., 2022. Research progress on the antiemetic effect of traditional Chinese medicine against chemotherapy-induced nausea and vomiting: a review. Front. Pharmacol. 12, 4131.
Zheng, L., Alhossary, A.A., Kwoh, C.K., Mu, Y., 2019. Molecular Dynamics and Simulation.
Zheng, Z., Stelmach, W.S., Ma, J., Briedis, J., Hau, R., Tacey, M., et al., 2023. Health professionals' attitudes towards acupuncture/acupressure for post-operative nausea and vomiting: a survey and implications for implementation. Acupunct. Med. 41 (1), 16–26.
Zhong, W., Shahbaz, O., Teskey, G., Beever, A., Kachour, N., Venketaraman, V., Darmani, N.A., 2021. Mechanisms of nausea and vomiting: current knowledge and recent advances in intracellular emetic signaling systems. Int. J. Mol. Sci. 22 (11), 5797.
Zhou, J., Xie, X., Tang, H., Peng, C., Peng, F., 2022. The bioactivities of sclareol: a mini review. Front. Pharmacol. 13.
Zoete, V., Cuendet, M.A., Grosdidier, A., Michielin, O., 2011. SwissParam: a fast force field generation tool for small organic molecules. J. Comput. Chem. 32 (11), 2359–2368.