



Original Research

## Anti-diarrheal activities of phytol along with its possible mechanism of action through *in-vivo* and *in-silico* models

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**Abstract:** Phytol (PHY), a chlorophyll-derived diterpenoid, exhibits numerous pharmacological properties, including antioxidant, antimicrobial, and anticancer activities. This study evaluates the anti-diarrheal effect of phytol (PHY) along with its possible mechanism of action through *in-vivo* and *in-silico* models. The effect of PHY was investigated on castor oil-induced diarrhea in Swiss mice by using prazosin, propranolol, loperamide, and nifedipine as standards with or without PHY. PHY at 50 mg/kg (p.o.) and all other standards exhibit significant ( $p < 0.05$ ) anti-diarrheal effect in mice. The effect was prominent in the loperamide and propranolol groups. PHY co-treated with prazosin and propranolol was found to increase in latent periods along with a significant reduction in diarrheal section during the observation period than other individual or combined groups. Furthermore, molecular docking studies also suggested that PHY showed better interactions with the  $\alpha$ - and  $\beta$ -adrenergic receptors, especially with  $\alpha$ -ADR1a and  $\beta$ -ADR1. In the former case, PHY showed interaction with hydroxyl group of Ser192 at a distance of 2.91Å, while in the latter it showed hydrogen bond interactions with Thr170 and Lys297 with a distance of 2.65 and 2.72Å, respectively. PHY exerted significant anti-diarrheal effect in Swiss mice, possibly through blocking  $\alpha$ - and  $\beta$ -adrenergic receptors.

**Key words:** Phytol; Diterpenoid; Diarrhea; *Mus musculus*; Molecular docking.

### Introduction

Diarrhea is generally defined as the passage of three or more unformed stools per day (often along with other enteric symptoms) or the passage of more than 250 g of unformed stool per day. On the basis of its duration, diarrhea can be classified as acute (<14 days), persistent (14–29 days), or chronic ( $\geq 30$  days) (1). It often lasts for a few days and can result in dehydration due to fluid loss. Additionally, it can range from a mild, temporary condition, to a potentially life-threatening. Approximately, 2 billion cases of diarrheal disease occur each year, and 1.9 million children under the age of 5 years, mostly in developing countries, die from diarrhea. There are many overlapping features in diarrheal diseases that can vary in severity, duration, clinical manifestations, and sequelae according to the causal facts, which in turn can

impact the economic burden on patients and their families (2). Diarrhea may also cause nausea, vomiting, and abdominal cramps, which may force affected individuals into bed rest until the hydro electrolytic balance is restored (3). The most common cause of diarrhea is an infection of the intestines due to a virus, bacterium, or parasite, or to gastroenteritis. These infections are often acquired from food or water that has been contaminated by feces, or directly from another infected person. Diarrhea produces changes in the transport of water and electrolytes that result in a hyper-secretory response and generation of giant contraction of the intestine. Thus, a potential anti-diarrheal agent may exhibit its effect by inhibiting gut motility and/or electrolyte outflux (4).

Despite the significant effect of synthetic anti-diarrheal drugs, side effects remain that necessitate the search for other treatments; medicinal plants may pro-

vide such valuable therapeutic alternatives(5-7). Along this line, use of traditional medicinal plants and their derived compounds to treat gastrointestinal disorders such as constipation and diarrhea has gained popularity worldwide because of their natural origin, availability, and fewer side effects (8). Accordingly, there has been an increased global interest in traditional medicines as alternative therapeutic tools (9). Furthermore, commonly used anti-diarrheal agents can cause a number of mild to severe side effects, such as dizziness, drowsiness, tiredness, constipation, nausea, vomiting, stomach/abdominal pain, uncomfortable fullness of the stomach/abdomen, fast/irregular heartbeat, and fainting. Diterpenes, a family of essential oils, are generally known for their antioxidant activity, and can exert protective effect in biological systems (10-12). Scientific reports suggest that diterpenes and their modified derivatives have potential anti-diarrheal effects due to their anti-microbial and anti-protozoal effects, and can act as antioxidant and anti-inflammatory agents (13, 14). Phytol (PHY), a chlorophyll-derived diterpenoid, exerts several important biological activities (15, 16). However, its anti-diarrheal effect has not been fully investigated. In this study, we have evaluated PHY's anti-diarrheal effect in castor oil-induced diarrheal mice. Additionally, we have also investigated the possible mechanism of action by using a number of standard anti-diarrheal agents in addition to an *in-silico* study.

## Materials and Methods

### Reagents and chemicals

Castor oil was purchased from a local market of Bangladesh. LOP and PRA were kindly supplied by the Square Pharmaceuticals Ltd., while PRO and NIF were provided by the ACI Ltd. and Drug International Ltd. Bangladesh, respectively. Phytol and tween 80 were purchased from Sigma Aldrich, USA.

### Animals

Adult male albino mice (22–30 g), purchased from the animal resource branch of Jahangir Nagar University (JU), Dhaka, were used throughout this investigation. These animals were housed under standard environmental conditions (temperature:  $25 \pm 2$  °C, humidity:  $50 \pm 5\%$ , and 12-hour light/dark cycles) in sanitized polypro-

pylene cages containing sterile paddy husk as bedding. They were kept under standard conditions mentioned in the Animals By-Laws 2008 of the University of Malakand (Scientific Procedures Issue-1). They were given free access to standard pellets as basal diet and water *ad libitum*. All animals were acclimatized for seven days before the study. Animals were randomized into experimental and control groups and were starved 12 hours before the experiment, test compounds were orally administered by gavage. Test protocol (#PHR07/2019) was approved by the Committee on Animal Research at the Department of Pharmacy, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, Bangladesh. After the study, all animals were euthanized with sodium pentobarbital (135 mg/kg, i.p.).

### Grouping and Treatment (Castor oil-induced diarrhea in mice)

The procedure we followed in this investigation is the one outlined by Awouters et al. (29) with slight modifications. Animals were treated with 0.5 mL castor oil 30 minutes after the sample (Gr-II) and controls (Gr-I & Gr-VI) treatment. Similarly, PHY (Gr-II) was given 15 minutes before Gr-III to Gr-VI (Gr-VII to Gr-X) (Table 1). Animals were then observed for latency and total defecation up to 4 h in each group.

### In-silico studies

#### Docking setup

In this study, 10 docked poses were generated for each ligand using default MOE-Dock parameters, and the top-ranked docked pose of each ligand was analyzed via MOE-Dock program.

#### $\alpha$ -ADRIa homology model

Homology modeling of Human  $\alpha$ -1a Adrenergic Receptor ( $\alpha$ -ADRIa) was performed by Swiss-model (30). Prior to modeling, sequence was retrieved from UniProt (31) followed by BLAST Analysis using NCBI BLAST (32) program to choose the template. Top-ranked template with optimized E-value was subjected to multiple sequence alignment with the aid of Clustal Omega (33). PROCHECK (26) was employed for the validation of the Homology Model. Binding Site exploration of

**Table 1.** Animals fasting overnight were treated with the following substances at 10 mL/kg.

Treatment group	Description	Activity pathway
Gr-I: VEH (i.p.)	0.05% Tween 80 dissolved in 0.9% NaCl solution	-
Gr-II: PHY (i.p.)	50 mg/kg (emulsified in VEH)	Under investigation
Gr-III: LOP (p.o.)	3 mg/kg (dissolved in VEH)	$\mu$ -opoid receptor agonist
Gr-IV: PRA (i.p.)	1 mg/kg (dissolved in VEH)	$\alpha$ -adrenergic receptor blocker
Gr-V: PRO (i.p.)	10 mg/kg (dissolved in VEH)	$\beta$ -adrenergic receptor blocker
Gr-VI: NIF (i.p.)	2.5 mg/kg (dissolved in VEH)	Ca <sup>+2</sup> channel blocker
Gr-VII: (Gr-II + Gr-III)		Under investigation
Gr-VIII: (Gr-II + Gr-IV)	Gr-III to Gr-VI were administered	Under investigation
Gr-IX: (Gr-II + Gr-V)	15 minutes after the PHY 50 mg/kg administration.	Under investigation
Gr-X: (Gr-II + Gr-VI)		Under investigation

Values are mean  $\pm$  SD (n = 5)

$\alpha$ -ADRIa was performed by MOE Site finder module implemented in the MOE Suite (27). Molecular docking of PRA and PHY compounds were performed to shed light on the binding mode of  $\alpha$ -ADRIa.

### Receptors and ligand preparation

Receptors such as  $\alpha$ -ADRIa,  $\beta$ -ADRI,  $\mu$ -OR, and VGIC were obtained *via* structure-preparation module implemented in MOE (27), followed by protonation, minimization, and partial charge application. Default parameters were set to optimize the docking experiment. All the compounds were sketched by MOE-Builer module followed by protonation, minimization, and MMFF94 partial charge application. Finally, MOE was utilized to explore the possible interactions.

### Docking protocol

Molecular Docking studies were performed by using MOE-DOCK (27) to study the binding mechanism of the anti-diarrheal compounds (PRA, PRO, LOP, and NIF while PHY used as a standard control among all targets) against four well-known therapeutic target receptors  $\alpha$ -ADRIa,  $\beta$ -ADRI,  $\mu$ -OR, and VGIC, respectively. The MOE-Dock was utilized for docking experiments with all of the above-mentioned receptors. Homology model of  $\alpha$ -ADRIa was constructed and previously reported homology model of  $\beta$ -ADRI [25] while PDB ID's: 4DKL (34) and 6BYO (35) of  $\mu$ -OR and VGIC were retrieved from the Brookhaven Research Collaboratory for Structural Bioinformatics Protein (RCSB) Data Bank (36) to investigate the binding mechanism of anti-diarrheal compounds, respectively.

### Statistical analysis

Data obtained from this study were subjected to one-way analysis of variance (ANOVA), and results are expressed as the mean  $\pm$  standard deviation. Statistical analysis was performed with the aid of Newman-Keuls-*post hoc* test using the software GraphPadPrism® - GraphPad Software, Inc. (version: 6.0); differences were considered significant at  $p \leq 0.05$  with 95% confidence intervals.

## Results and Discussion

### Castor oil-induced diarrhea (In vivo)

Castor oil test has been used for years as to screen and evaluate anti-diarrheal drugs. It has high reproducibility in the number of liquid and formed feces evaluation, since it reduces the absorption and increases secretion of water and electrolytes, besides stimulating peristalsis and accelerating intestinal transit (17)(14). After ingestion, castor oil is hydrolyzed to glycerol and ricinoleic acid by pancreatic lipases. Ricinoleic acid, a major component of the seed oil obtained from mature castor plant seeds or in sclerotium of ergot, is responsible for the diarrheal effect in animals (18). The presence of ricinoleic acid in the small intestine results in the release of prostaglandins and platelet-activating factor (17)(14), thus promoting vasodilation, smooth muscle contraction, and mucus secretion in the small intestine, and resulting in diarrhea (19). In addition, ricinoleic acid promotes the release of nitric oxide (NO) and activation of adenylyl cyclase which causes an increase in cyclic

adenosine monophosphate (cAMP) concentration. This increase in cAMP concentration (a) stimulates peristaltic activity in the intestine, (b) alters the membrane permeability, (c) reduces the activity of the Na<sup>+</sup>K<sup>+</sup>ATPase pump, and (d) decreases the absorption of Na<sup>+</sup> and K<sup>+</sup>; these factors can cause an accumulation of these electrolytes and water in the intestinal lumen (20, 21). Our findings from this study, suggest that PHY and the standards (LOP, PRA, PRO, NIF) significantly ( $p < 0.05$ ) increase latent periods in diarrheal mice when compared to the VEH group. On the other hand, LOP ( $22.6 \pm 3.6$  min) and PRO ( $22.2 \pm 2.8$  min) treatment increased the latency period more than that of PHY, PRA, and NIF groups. However, PHY when co-treated with the standards resulted in the highest increased latency as in the case of PHY + PRO group ( $26.1 \pm 2.6$  min), followed by PHY + PRA, PHY + LOP, and PHY + NIF groups, respectively as shown in Table 2.

Similarly, data in Table 3 indicate that PHY at 50 mg/kg significantly ( $p < 0.05$ ) reduces the number of diarrheal secretions in comparison to the VEH group. The highest reduction of diarrheal secretions by PHY was observed at the 4<sup>th</sup> hour ( $1.6 \pm 0.8$ ). More reduction of diarrheal secretions was observed in LOP and PRO groups. Moreover, PHY's activity was more relevant to the PRA group. Results also revealed that PHY co-treated with the standards effectively reduced diarrheal secretions in all groups. Interestingly, PHY co-treated with PRA and PRO were more effective in reducing diarrheal secretions than the LOP and NIF groups.

Antioxidants are protective in nature; they are generally used to protect body organs from the damaging effects of free radicals. Among the other reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), nitric oxide (NO), at moderate concentrations, has important signaling roles under physiological conditions, however, excessive or sustained NO production may lead to oxidative stress in our body. Additionally, high levels of NO in vascular systems can generate cardiovascular risk factors such as hypercholesterolemia, hypertension, and diabetes mellitus (22). Two excellent reviews which were recently published by Islam and his co-workers suggest that PHY exhibits anti-inflammatory, lipid-lowering, and anti-diabetic ef-

**Table 2.** Latent periods observed in the treated groups.

Treated groups	Latency (min)
VEH	8.4 $\pm$ 2.4
PHY	12.2 $\pm$ 1.8*
LOP	22.6 $\pm$ 3.6*#
PRA	17.1 $\pm$ 1.9*#
PRO	22.2 $\pm$ 2.8*#b
NIF	15.4 $\pm$ 3.9*#
PHY + LOP	24.2 $\pm$ 2.1*#a
PHY + PRA	25.8 $\pm$ 3.2*#b
PHY + PRO	26.1 $\pm$ 2.6*#
PHY + NIF	22.9 $\pm$ 2.6*#d

Values are mean  $\pm$  SD (n = 5); ANOVA oneway followed by Newman-Keuls-*post hoc* test;  $p < 0.05$  when compared to the \*Gr-I, #Gr-II, aGr-III, bGr-IV, cGr-V, dGr-V in respective hour. LOP: Loperamide; PRA: Prazosin; PRO: Propranolol; NIF: Nifedipine.

**Table 3.** Diarrheal secretions of mice in different treatment groups at 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> hours.

Treatment groups	1 <sup>st</sup> hr	2 <sup>nd</sup> hr	3 <sup>rd</sup> hr	4 <sup>th</sup> hr
VEH	14.4 ± 4.3	12.2 ± 3.5	9.1 ± 0.9	8.8 ± 1.5
PHY	9.0 ± 4.9*	3.8 ± 1.3*	2.8 ± 1.3*	1.6 ± 0.8*
LOP	5.2 ± 2.6 <sup>#</sup>	3.8 ± 2.5 <sup>#</sup>	3.4 ± 1.3 <sup>#</sup>	3.2 ± 1.8 <sup>#</sup>
PRA	7.8 ± 2.4 <sup>#</sup>	4.6 ± 1.2 <sup>#</sup>	4.2 ± 2.2 <sup>#</sup>	1.6 ± 0.8 <sup>#a</sup>
PRO	5.4 ± 3.1 <sup>*#b</sup>	4.4 ± 2.2 <sup>#</sup>	2.0 ± 1.0 <sup>*#ab</sup>	1.2 ± 0.8 <sup>*#a</sup>
NIF	8.2 ± 3.7*	4.4 ± 1.7 <sup>#</sup>	3.2 ± 0.8 <sup>*#b</sup>	2.0 ± 1.0 <sup>*a</sup>
PHY + LOP	5.0 ± 1.3 <sup>*#a</sup>	2.0 ± 0.0 <sup>*#a</sup>	1.0 ± 0.2 <sup>*#a</sup>	0.6 ± 0.4 <sup>*#a</sup>
PHY + PRA	3.6 ± 1.1 <sup>*#b</sup>	3.4 ± 1.4 <sup>*b</sup>	1.2 ± 0.8 <sup>*b</sup>	0.4 ± 0.3 <sup>*b</sup>
PHY + PRO	4.4 ± 2.7 <sup>*#c</sup>	3.4 ± 1.8*	2.4 ± 1.7*	1.4 ± 0.9*
PHY + NIF	7.2 ± 1.6 <sup>#</sup>	6.4 ± 2.9*	2.6 ± 1.9*	1.2 ± 0.8*

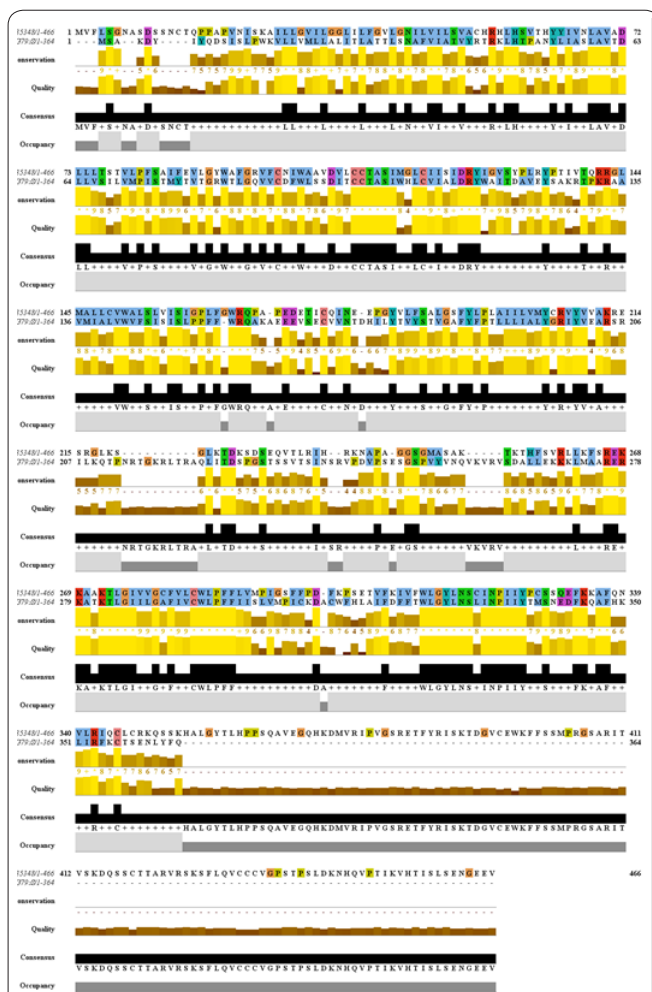
Values are mean ± SD (n = 5); ANOVA oneway followed by Newman-Keuls *post hoc* test; p < 0.05 when compared to the <sup>a</sup>Gr-I, <sup>#</sup>Gr-II, <sup>a</sup>Gr-III, <sup>b</sup>Gr-IV, <sup>c</sup>Gr-V, <sup>d</sup>Gr-V in respective hour; PHY: Phytol; LOP: Loperamide; PRA: Prazosin; PRO: Propranolol; NIF: Nifedipine.

fects in experimental animals (15). In another study, Islam and colleagues reported that PHY cansignificantly scavenge reactive and harmful species such as ROS and RNS, and NO (23). Thus, results from this investigation are in line with the previously accomplished studies.

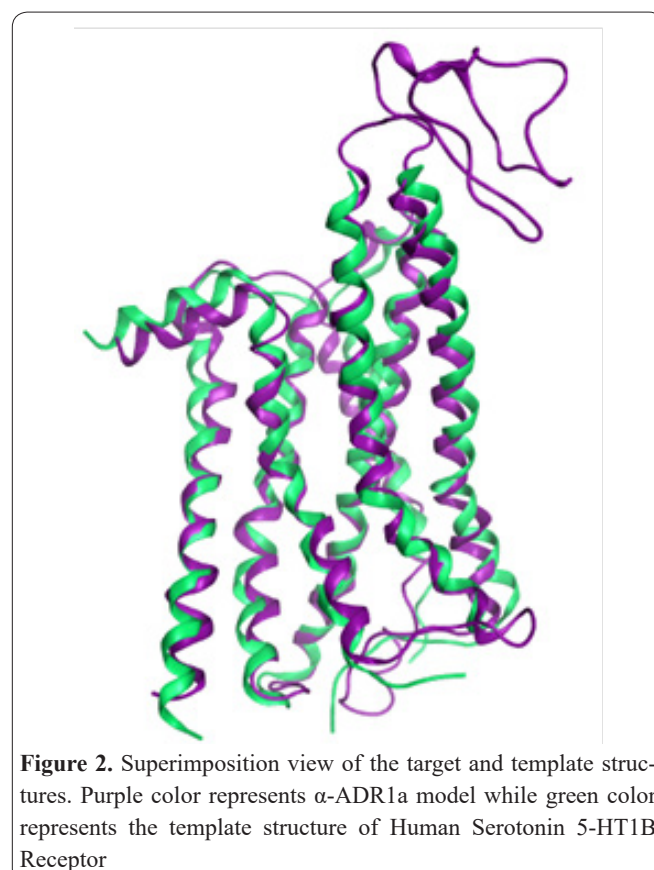
**α-ADR1a homology model**

The amino acid Sequence of α-ADR1a, retrieved from Uniprot (Accession ID:P35348),was subjected to NCBI Blast Program for the selection of best homologous template. Human Serotonin 5-hydroxytryptamine 1B (5-HT1B) Receptor (PDB ID:6G79) (24) showed

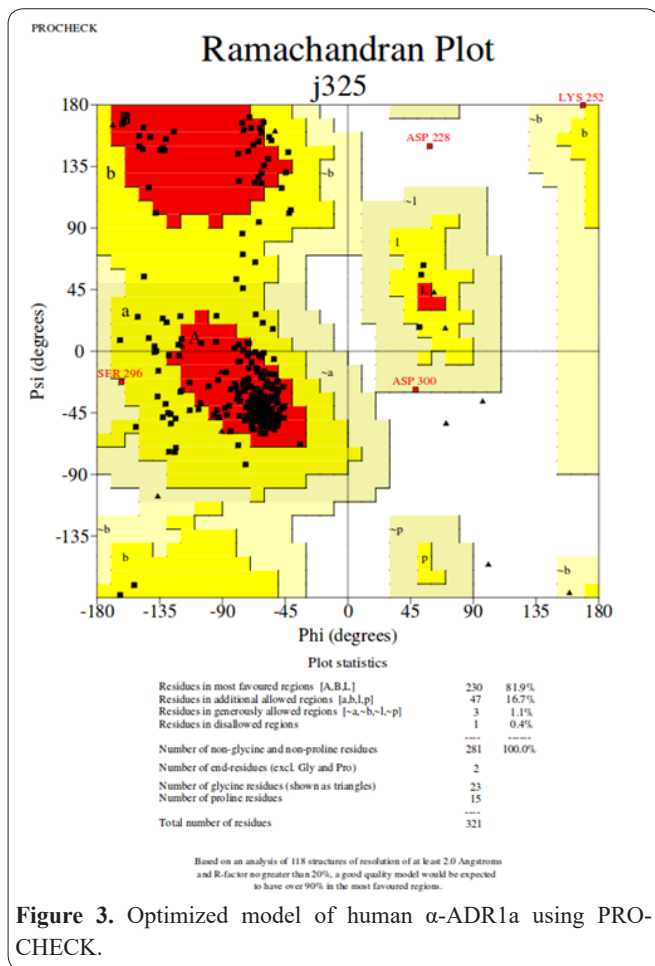
38% identity with the target sequence. On the other hand, multiple sequence alignment was performed to observe the conservation between the target and the template sequence illustrated in Figure 1. Homology model of α-ADR1a was generated by Swiss model. The 3D model of α-ADR1a revealed an excellent agreement with the experimentally determined 3D structure of human serotonin 5-HT1B Receptor. The Figure 2 showed superimposed view of α-ADR1a model and serotonin 5-HT1B Receptor. The calculated polypeptide backbone (Cα, C, and N atoms) root mean square deviation (RMSD) of α-ADR1a model against Serotonin 5-HT1B Receptor was observed to be 0.28Å. Optimization of α-ADR1a model was achieved by using GROMACS 5.1. (25), whereas validation of α-ADR1a homology model was acquired through the use of Ramachandran plot performed by PROCHECK (26) and illustrated in Figure 3. Furthermore, binding site exploration of α-ADR1a was performed by MOE-Site finder module



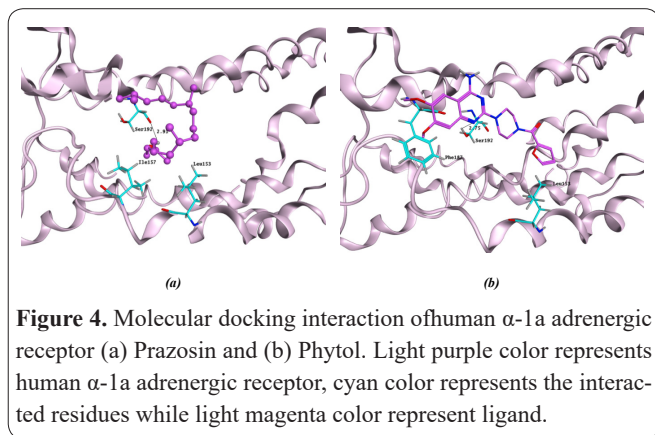
**Figure 1.** Multiple sequence alignment of the α-ADR1a sequence with Human Serotonin 5-HT1B Receptor sequence using Clustal Omega.



**Figure 2.** Superimposition view of the target and template structures. Purple color represents α-ADR1a model while green color represents the template structure of Human Serotonin 5-HT1B Receptor



**Figure 3.** Optimized model of human  $\alpha$ -ADR1a using PROCHECK.



**Figure 4.** Molecular docking interaction of human  $\alpha$ -1a adrenergic receptor (a) Prazosin and (b) Phytol. Light purple color represents human  $\alpha$ -1a adrenergic receptor, cyan color represents the interacted residues while light magenta color represent ligand.

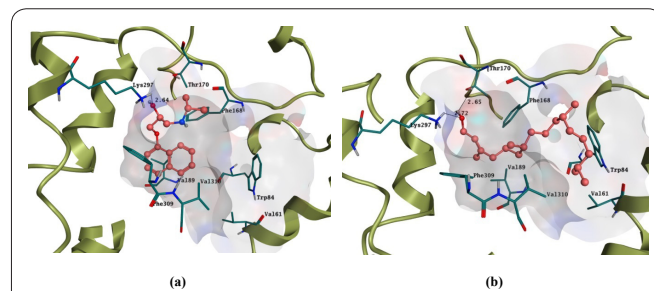
implemented in the MOE Suite (27).

**$\alpha$ -1a Adrenergic Receptor ( $\alpha$ -ADR1a)**

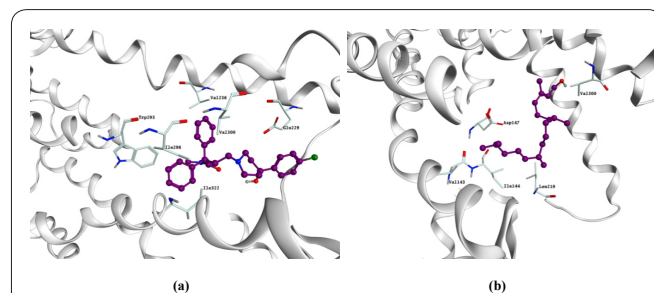
Molecular docking of PRA and PHY compounds was accomplished to investigate the binding mode of  $\alpha$ -ADR1a as shown in Figure 4. The amino pyrimidine moiety of PRA mediates a hydrogen bond at a distance of 2.75Å with the hydroxyl group of Ser192, whereas Phe187 exhibits  $\pi$ - $\pi$  interaction with the benzene ring and  $\pi$ -CH<sub>3</sub> interaction with PRA. On the other hand, PHY showed good interaction with the hydroxyl group of Ser192 at a distance of 2.91Å, while the methyl groups of Leu153 and Ile157 exhibiting hydrophobic interaction with the aliphatic chain of PHY. Docking score of PRA and PHY are -5.2932 and -5.3114, respectively.

**$\beta$ -1 Adrenergic Receptor ( $\beta$ -ADR1)**

$\beta$ -ADR1 provides remedial solutions of cardiovascular diseases and effective treatment of asthma.



**Figure 5.** Molecular docking interaction of  $\beta$ -1 adrenergic receptor (a) Propranolol and (b) Phytol. Lime green color represents  $\beta$ -1 adrenergic receptor, dark cyan color represents the interacted residues while soft pink color represent ligand.



**Figure 6.** Molecular docking interaction of  $\mu$ -opioid receptor (a) Loperamide and (b) Phytol. White color represents  $\mu$ -opioid receptor, grey color represents the interacted residues while magenta color represent ligand.

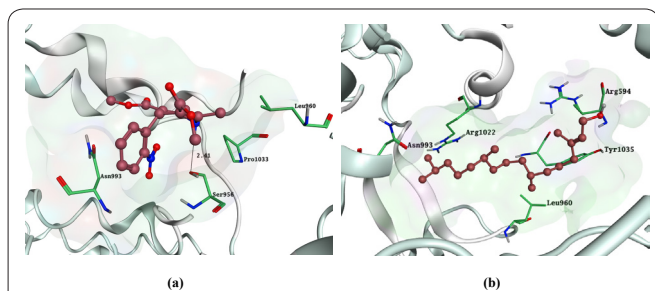
Homology model of  $\beta$ -ADR1, previously reported by Ul-Haq *et al.* (28), was utilized in this study. PRO and PHY were docked into the  $\beta$ -ADR1. Results show that the hydroxyl moiety of PRO mediating two hydrogen bonds with Lys297 at a distance of 2.64 and 2.47Å. In addition, multiple hydrophobic interactions were observed with Val89, Val92, Phe168, Thr170, Phe309, and Val310. Similarly, the hydroxyl group of PHY also mediates two hydrogen bond interactions with Thr170 and Lys297 with the distance of 2.65 and 2.72Å, respectively. Additionally, multiple hydrophobic interactions were observed with Val61, Trp84, Val89, Phe168, Phe309, and Val310 as illustrated in Figure 5. The docking scores of PRO and PHY are -6.8701 and -6.8701, respectively.

**$\mu$ -opioid Receptor ( $\mu$ -OR)**

$\mu$ -OR represents an important opioid target for the occurrence of pain, diarrhea, chronic pulmonary edema, cough, and shivering. Shown in Figure 6. LOP are molecular docking of  $\mu$ -OR with PHY and LOP. For LOP, docking results show multiple hydrophobic interactions with the  $\mu$ -opioid receptor. In addition, the methyl group of Val236, Ile296, Val300, and Ile322 display hydrophobic interactions with the aromatic rings of LOP while Trp293, and Tyr326 show  $\pi$ - $\pi$  stacking. For PHY, the aliphatic chain exhibits hydrophobic interactions with the methyl group of Val143, Val300, Ile144, and Leu219. Docking Scores of LOP and PHY are -7.9117 and -6.8200, respectively.

**Voltage-gated ion channel (VGIC)**

VGIC are a class of transmembrane proteins that form ion channels that are activated by changes in the electrical membrane potential near the channel. These channels are associated with multiple heritable



**Figure 7.** Molecular Docking Interaction of Voltage-Gated Ion Channel (a) Nifedipine and (b) Phytol. Light mint color represents voltage-gated ion channel, green color represents the interacted residues while plum color represent ligand.

human diseases, including episodic ataxia type 2, familial hemiplegic migraine type 1, congenital stationary night blindness type 2, and other autosomal dominant diseases. NIF forms a hydrogen bond with the hydroxyl group of Ser956 with a distance of 2.41Å. In addition, Leu960 and Ile991 exhibit hydrophobic interaction with the aromatic ring of NIF, while Pro1033 shows  $\pi$ -CH<sub>3</sub> interaction with the NIF. On the other hand, PHY exhibits the hydrophobic interaction with Arg594, Leu960, Asn993, Arg1022, and Tyr1035, whereasthehydroxyl group of Ser956 mediates hydrogen bond with a distance of 2.60Å as depicted in Figure 7. Docking scores of NIF and PHY are -5.7210 and -6.0747, respectively.

In summary, findings from this investigation suggest that PHY, a natural compound, displays remarkable anti-diarrheal effect in castor oil-induced diarrheal mice. In this study, we have for the first-time investigated its anti-diarrheal effect in mice; results showed that PHY at 50 mg/kg (p.o.) significantly augmented the latency period, with reduction in diarrheic sections during the observation period (4 h). It exhibited better anti-diarrheal effect when co-treated with the standard drugs, PRA, PRO, LOP, and NIF, where the effect was more prominent when it was co-treated with  $\alpha$  and  $\beta$  receptor blocking agents, PRA and PRO, respectively. Although clinical trials on PHY are yet to be performed, it has many important biological effects on microorganisms, and other test animals. In addition, molecular docking study indicated that the responsible receptor moieties are  $\alpha$ -ADR1 and  $\beta$ -ADR1 that may be blocked by PHY through interaction with Ser192, and Thr170 and Lys297, respectively. Taken all together, PHY may exert its anti-diarrheal effect through  $\alpha$  and  $\beta$  receptors, especially  $\alpha$ -ADR1 and  $\beta$ -ADR1 blocking pathway. Docking scores also suggested that PHY blocked by  $\alpha$ -ADR1 and  $\beta$ -ADR1 as compare to  $\mu$ -OR and VGIC. However, more detailed studies are required to establish the safety and efficacy of this compound before it can be used as an anti-diarrheal drug.

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This research received no external funding.

## Conflicts of Interest

The authors declare no conflict of interest.

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