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Antidiarrhoeal screening of Himalayan edible plant *Begonia rubrovenia* and its marker followed by its validation using computational analysis

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Abstract

Background Diarrhoea has become one of the major areas of concern due to its high mortality rate contributing it to be the second largest cause of death in world. To explore the effectiveness of medicinal plant, the present investigation was undertaken to scientifically justify the traditional claim of the ethanolic root extract of the plant *Begonia rubrovenia* (EBV) against diarrhoea.

Results EBV was standardized using HPLC with quercetin as marker and was further subjected to normal fecal excretion study at 100, 200 and 300 mg/kg, p.o. along with quercetin and loperamide. The study confirmed the effectiveness of EBV at 200 and 300 mg/kg followed by quercetin. In castor oil induced diarrhoea rat model, EBV at 200 and 300 mg/kg significantly delayed onset of diarrhoea, reduced the diarrhoeal faecal output which contributed in higher % protection. The effectiveness of EBV at 200 mg/kg was also confirmed through gastrointestinal motility, fluid accumulation and PGE_2 induced enteropooling tests. EBV and its marker quercetin also reduced the elevated level of NO and cytokines and restored the alterations in antioxidant enzymes, ions and enhanced Na^+/K^+–ATPase activity. Molecular docking, dynamics and network pharmacology study confirmed the role of quercetin in modulating the inflammatory mediators IL-1β, TNF-α and EP3 prostanoid receptor, where quercetin formed more stable complex with EP3 prostanoid receptor.

Conclusion The study has scientifically justified the traditional use of the plants *B. rubrovenia* in treating diarrhoea, where quercetin played a critical role in the observed antidiarrhoeal potential of *B. rubrovenia* contributing in maintaining electrolyte balance, antioxidant status and inhibiting inflammatory mediators.

Keywords *Begonia rubrovenia*, Cytokines, Diarrhoea score, Enteropooling test, EP3 prostanoid receptor, Quercetin

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Background

Diarrhoea is an infection mainly associated with gastrointestinal (GI) tract, where defecation rate, volume and consistency of the stools get altered [1]. At present, diarrhoea is assessed to be the most prevalent situation hampering GI tract with 1–5% prevalence rate in adults in developed as well as developing countries like India [2, 3]. According to the WHO and UNICEF reports, about 1 billion diarrhoea cases are estimated worldwide, among which 3 million deaths occurs per year in children's less than five years of age [4].

Different etiological factors attribute to the cause of diarrhoea like infectious agents (viruses, parasites and bacteria's), food allergies, intestinal dysfunction, alcohol consumption, decrease in uptake of bile salts and intake of some drugs, e.g., antineoplastic, antimicrobials, oral hypoglycaemic agents, antiretrovirals, β-blockers, proton pump inhibitors and non-steroidal anti-inflammatory drugs [3]. To handle this diarrhoeal condition, nontherapeutic approach, i.e. oral rehydration therapy is the primary step to maintain water and electrolyte loss along with zinc supplementation [5]. Therapeutic approach used to reduce the most consistent and clinically significant indications of diarrhoea have several side effects like dry mouth, severe constipation, nausea, vomiting, distension of abdomen and cramps [6] along with respiratory tract depression and paralytic ileus (mostly in children's due to loperamide drug) [7]. To minimize the health hazards of these agents, medicinal plants are most suitable approach to treat GI disorders like diarrhoea because they have lots of compounds, which help in efficacy enhancement and side effect neutralization [2]. Thus, to treat GI disorders like diarrhoea, natural entities are the most suitable therapeutic agent and also a good starter for the innovation of new drugs [3].

Begonia rubrovenia C.B. Clarke (Begoniaceae) is an ornamental plant majorly found in North Easton Himalayan regions especially in the states of Arunachal Pradesh, Meghalaya, Tripura and Manipur and is also found in Bangladesh [8]. In Meghalaya, the plant is locally named as Johusia by the Jaintia tribes where, the tender shoots and roots of the plant are commonly used as a vegetable and herbal tea prepared from the leaves, used as a best remedy to treat colic and dysentery like illness [8–10]. Traditionally, *B. rubrovenia* roots are used by the tribal peoples in Meghalaya to treat diarrhoea, liver disorders, stomach problems, peptic ulcers and skin related disorders [8, 10, 11]. Yet, there are very less reports available on the phytochemistry and pharmacological activities on this plant. However, the genus Begonia has been reported to have quercetin, rutin, luteolin, vitexin, isovitexin, orientin, isoorientin, friedelin and beta-sitosterol as some of the major phytoconstituents [12]. The plant under study is traditionally used to treat diarrhoea, however the traditional claim of the plant against diarrhoea is still not scientifically proven. Thus, the current study is an attempt to justify the antidiarrhoeal potential of *B. rubrovenia* using chemical-induced diarrhoea models following *in-vivo* and *in-silico* protocols.

Methods

Extraction and phytochemical evaluation

The plant *B. rubrovenia* was procured from West Khasi hills district of Meghalaya, India and was authenticated by Dr. Dongarwar, a botanist of our institute (Specimen number 10705). Next to authentication, the roots of the respective plant were dried under shade for at least 2–3 weeks. Then the dried roots were ground into coarse powder, further the coarse powder material (500 g) was extracted with ethanol as solvent (1.5 L) by Soxhlet assembly. After extraction, the volume of the collected extract was concentrated by rotary evaporator and the final extract (4.94% w/w) was kept under desiccator for further use. After complete extraction procedure, the obtained extract was screened for primary and secondary phytochemicals following standard test protocols [13]. Further, the screened phytoconstituents (total alkaloids, phenolics, tannins, saponins, and carbohydrates) were quantified as per the procedures described by Prasad et al. [12].

Standardization of extract

The occurrence of quercetin in ethanolic extract of *B. rubrovenia* (EBV) was confirmed by thin layer chromatography (TLC) screening and therefore quercetin ((Sigma-Aldrich, USA) was used as a standard marker for standardization of EBV using High Performance Liquid Chromatography (HPLC). For HPLC analysis, stock solution of EBV (1 mg/mL) and quercetin (0.1 mg/mL) was prepared in methanol. Further, a solution of methanol and water constituting 0.1% formic acid (80:20) was employed as a mobile phase with 10 µL of injection volume and 1.0 mL/min flow rate. The peaks of extract were matched with the standard quercetin peak with respect to retention time and presence of quercetin was confirmed [14].

Animals

Before selection of animals, the experimental protocol for antidiarrhoeal study was approved from the Institutional Animal Ethical Committee (IAEC/UDPS/2022/01 on 21/05/2022). After approval, healthy Wistar rats of either sex (150–200 g) were taken from the registered central animal house of our department (Reg. No.: 92/1999/CCSEA). The animals were placed in their respective cages for at least 1–2 week to acclimatize in surrounding

environment providing temperature, humidity and lightening facility along with feed and water as per standard protocol.

Acute oral toxicity study

Acute oral toxicity (AOT) study was carried out to determine the safety margin of EBV employing Organization for Economic Cooperation and Development (OECD)-425 guidelines. Animals (overnight fasted) under the protocol were administered orally with EBV in increasing manner and different neurological and behavioural parameters were observed like sleep, palpitation, diarrhoea, drowsiness, sedation, tremors, lacrimation, writhing, salivation, gasping, convulsions and lowering respiratory rate for 48 h. Further, for any kind of mortality, these animals were monitored for another 14 days. In addition, weight of organs of the animals treated with EBV and control were measured and difference in their weights was recorded [15, 16].

Normal faeces excretion (NFE) rate

Previously fasted rats (3 h) were randomly categorized into six groups: First group (Normal control) was administered with suspension of 0.5% w/v carboxy methyl cellulose (CMC), second to fourth group was served with three different doses (100, 200 and 300 mg/kg) of EBV (dose selection was confirmed from AOT study), fifth group rats were treated with quercetin 50 mg/kg and the last group was served with standard loperamide drug (Torrent Pharmaceuticals India Ltd., India) at a dose of 2 mg/kg. All the test samples were prepared in 0.5% w/v CMC and were given orally using oral gavage. Weight of faeces in all the groups were noted in wet as well as in dry conditions (dried at 50 °C for 24 h) at 1st, 3rd, 5th and 7th hours after administration of EBV and finally wet to dry ratio was calculated [17].

Castor oil-induced diarrhoea

Castor oil induced (COI) diarrhoea rat model

In this model, castor oil was served as diarrhoea inducing agent, which was given after the gap of one hour of treatment to all the rats under study except the normal control rats. The fasted rats were randomly grouped into six groups, among them group 1 (Normal control) and group 2 (Diarrhoea control) rats received 0.5% CMC suspension. Rats from group 3–5 were administered with EBV at the dose of 100, 200 and 300 mg/kg p.o. respectively, and rats in groups 6 and 7 received quercetin and standard loperamide. All the rats after receiving castor oil (1 ml) were immediately placed in cages previously lined with plastic sheets and different diarrhoeagenic parameters were observed and determined for a period of 4 h after castor oil administration [18, 19].

COI gastrointestinal transit test model

This model was used to evaluate the antimotility effect of EBV and quercetin by employing charcoal meal. Approximately, 18 h fasted rats were divided into five groups, where 0.5% CMC suspension was prepared and given to Group 1 (Normal control) and Group 2 (Diarrhoea control) rats orally. Group 3 rats were administered with optimized dose of EBV, i.e. 200 mg/kg, which was confirmed from NFE study and COI diarrhoea rat model. Quercetin and atropine (0.1 mg/kg s.c.) (Sigma-Aldrich, USA) were administered to Group 4 and 5 rats, respectively. Further, 1 mL castor oil, was administered to rats, 30 min after the above treatment. This was followed by administration of 1 mL of suspension of 5% deactivated charcoal meal prepared in aqueous tragacanth (10%), 30 min after castor oil administration. Then, all the animals were sacrificed after 30 min of charcoal meal administration and intestinal part was isolated and distance travelled by the charcoal was measured with respect to total length of intestine. Finally, Peristaltic Index (PI) in percentage was calculated and was compared with diarrhoea control rats [18].

COI intestinal fluid accumulation test

Before commencing the protocol, rats were fasted for about 18 h and were categorized into 5 different groups with 6 animals in each group. Group 1 and 2 were served as normal control and diarrhoea control and received 0.5% CMC suspension by oral gavage. Groups 3–5 were treated with EBV (200 mg/kg), quercetin and loperamide, respectively. Diarrhoea was induced by giving castor oil (1 ml) to all rats except normal control rats just half an hour after the treatment. Immediately, 30 min after receiving castor oil, all the rats were sacrificed to remove the small intestine. Intestinal volume and weight of intestinal content of each rat were measured from each group [15].

PGE_2-induced enteropooling

Previously fasted rats were classified into four group (n = 6), where first (Normal control) and second (PGE_2 control) group animals received tragacanth suspension (2% w/v) orally and third and fourth group animals were treated with EBV extract (200 mg/kg) and quercetin (50 mg/kg) orally. PGE_2 (Astra Zeneca, India) was used as an inducing agent and was prepared in saline solution which contained 5% alcohol. PGE_2 was introduced via gavage in rats from groups 2 to 4. About 30 min later, all the rats were killed and small intestine was removed, intestinal volume was measured by using measuring cylinder [15].

Ions concentration and cytokines estimations

The colons isolated during the COI fluid accumulation test were first rinsed, homogenized in deionized water and finally centrifuged. The supernatant received after centrifugation was analysed for the determination of concentration of different ions like Na^{2+}, Ca^{2+}, Cl^{-} and K^{+} with the help of Nulyte Electrolyte Analyzer [20]. Further, cytokines level (IL-1β and TNF- α) were also checked in previously isolated colonic tissues from different groups of rats using marketed Enzyme-linked Immunosorbent Assay (ELISA) kits (R&D Systems).

Na^{+}/K^{+}–ATPase assay

Na^{+}/K^{+}–ATPase assay was carried out to determine the efficacy of EBV and quercetin against Na^{+}/K^{+}–ATPase protein. The colonic tissues collected from the dissected rats from each group were first thoroughly cleaned. Then, they were homogenized according to the method by Gal-Garber et al. [21]. The homogenized solutions obtained were centrifuged and the supernatant collected were utilized for Na^{+}/K^{+}-ATPase assessment as per the method described by Parmar et al. [22].

Biochemical analysis and histopathology

The colonic tissues procured from the sacrificed rats were utilized for biochemical analysis. Initially, the colonic tissues arranged from COI fluid accumulation test were rinsed by tyrode solution, homogenized with 7.4 pH phosphate buffer and supernatants obtained after centrifugation of homogenized solutions were used for the estimations of different biochemical analytes. Nitric acid (NO) was estimated in the colonic segment by adapting the method given by Green et al. [23] and total carbohydrate content was evaluated by Yemm and Willis method [24]. Total DNA and protein concentration were determined by using methods described by Burton [25] and Lowry et al. [26] respectively. Further, the levels of antioxidants (SOD, CAT and LPO) were analysed in rat's colon with the help of standard protocol mentioned by Laloo et al. [27].

Histopathological studies were performed on colons collected from COI fluid accumulation testing. The initially blotted, dried and fixed (10% formalin) colons were dehydrated (with acetone) and finally embedding was carried out using paraffin wax. The colons fixed in paraffin wax were cut into small and thin sections with the help of microtome. Before observing the sections into the microscope (Leica DM-2000, Leica, Germany), they were stained with haematoxylin and eosin staining reagent for clear understanding of the histopathology.

In silico study

Preparation of the ligand

The ligand, quercetin, was prepared using the Marvin sketch tool in the Sanjeevani online program. The resulting 3D structure of the ligand was then imported into Biovia Discovery Studio 2022 (DS 2022) software. To optimize the ligand's energy, an energy minimization process was performed using the CharmM forcefield. Additionally, multiple conformations of the ligand were generated based on an *in-silico* pH of 7.4. Among these conformations, the one with the lowest energy was selected and subsequently docked into the active site of the enzyme protein.

Preparation of the proteins

The PDB structures of the proteins TNF-α (PDB ID: 1TNF), IL1-β (PDB ID: 1I1B), and EP3 prostanoid receptor (PDB ID: 6AK3) were obtained from the RCSB PDB website (https://www.rcsb.org/). The protein structures were prepared using the "Prepare Proteins" protocol incorporated in DS 2022. During the preparation, all water molecules were removed from the proteins and the protein structure was minimized using CharmM forcefield. The entire process was conducted with an *in-silico* pH of 7.4 to ensure consistency.

Molecular docking

The docking process was conducted using the CDOCKER protocol, which is known for its accuracy in predicting ligand–protein interactions. Following the docking simulations, the binding energy of ligand–protein complex was calculated. To refine the ligand conformation and to improve the accuracy of the results, an *in-situ* ligand minimization step was performed. The calculation of the binding energy took into account the non-bonded interactions, with a non-bond list radius of 14.0 Å employed for this purpose. Subsequently, an analysis of the binding poses and ligand orientation within the active site was conducted [28].

Molecular dynamic simulation

Among the three ligand–protein complexes obtained from molecular docking, the complex (Quercetin-6AK3) showing maximum binding energy was further subjected to molecular dynamics simulation using DS 2022. The simulation utilized CHARMm forcefield for both the small molecule and the protein. The system was set up with explicit periodic boundary conditions, maintaining an orthorhombic cell shape. The protein–ligand complex was solvated while ensuring a minimum distance of 7 Å from the boundary. A salt concentration of 0.145 was introduced, with sodium and chloride ions serving as the

cation and anion, respectively. To initiate the simulation, an initial minimization step was performed using the steepest descent method. The minimization was carried out for a maximum of 5000 steps, with an RMS gradient of 0.1. Following the minimization, a standard dynamics cascade was executed, consisting of heating, equilibration, and production phases. The system was gradually heated from an initial temperature of 50–300 K over duration of 20 ps. Equilibration was then performed at 300 K without restraints for 50 ps. During the production phase, the system was simulated in the NPT ensemble at 300 K, using a temperature coupling decay time of 5 ps. The simulation was continued until the Root Mean Square Deviation (RMSD) reached a plateau. Snapshots of the system were saved every 20 ps during the production period. To assess conformational changes in the protein and ligand, several metrics were computed relative to the starting structure. These included the Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and Radius of Gyration (ROG). These metrics provided insights into the dynamics and stability of the protein–ligand complex throughout the simulation [29].

Network pharmacology

Novel bioactive targets based on docking results were selected to perform the network pharmacology. The suitability of the bioactive compound was evaluated using a cheminformatic tool and information regarding the bioactive target proteins was obtained from the UniProt database. The interactions between the bioactive target proteins and other molecules were analysed using STRING software ver 11.5 [30]. All the obtained data were then used to construct a network linking quercetin, target molecules, and protein–protein interaction data by Cytoscape ver 3.9.1 [31].

Statistical analysis

All the experimental data was analysed by one-way and two-way ANOVA using Tukey's multiple comparisons test, where results were represented as mean ± SEM (n = 6). The experimental data was statistically analysed with the help of GraphPad Prism having version 8 and the results were proven to be significant when p values were found to be < 0.05.

Results

Phytochemical standardization

Approximately, 4.94% w/w yield of extract was obtained after extracting the roots from the plant *B. rubrovenia* using ethanol. The results from phytochemical screening revealed that EBV showed the presence of alkaloids, phenols, flavonoids, carbohydrates, proteins, amino acids and steroids. From phytochemical quantification, it was depicted that the extract was found to be rich in total phenolics (362.69 ± 17.44 mg/g GAE), total tannins (250.31 ± 14.47 mg/g TAE) and total flavonoid (197.36 ± 8.45 mg/g RE) contents. During HPLC standardization, well resolved and sharp peaks of quercetin was obtained which was matched with the peak of extract having retention time of 5.2 min and amount of quercetin was found to be 4.72% w/w in EBV (Fig. 1).

AOT study

According to the AOT protocol, the rats under investigation did not depicted any abnormal type of behavioural and neurological symptoms up to the dose level of 2000 mg/kg, which confirmed that EBV up to a dose level of 2000 mg/kg was found to be safe (Details are available as Additional file 1).

Normal faecal excretion rate

Table 1 represents the effect of EBV on normal rate of faecal excretion. The results depicted that the rate of faecal excretion was significantly decreased at EBV 200, EBV 300 mg/kg and quercetin, respectively from 5th hr of treatment. But, in case of standard group, rate of faecal excretion was found to decline after 3rd hr of EBV administration itself. Wet to dry ratio was also found to be low in standard loperamide followed by EBV 200, EBV 300 mg/kg followed by quercetin.

Antidiarrhoeal evaluations

The results of COI diarrhoea model are demonstrated in Table 2, where, we observed that there was a significant delay in onset time in all the treated groups except in group administered with EBV at 100 mg/kg in comparison with the diarrhoeal rats. The other diarrhoeagenic features like total number of faeces, total number of wet faeces, total weight of faeces, total loss in body weight of rats, mean defecation rate, diarrhoea score and % protection also showed significant antidiarrhoeal effect in rats treated with EBV, quercetin and standard drug. From the overall observations, EBV at 200 and 300 mg/kg displayed similar response thus, confirming a ceiling effect of EBV from 200 mg/kg dose therefore, for further antidiarrhoeal studies, EBV 200 mg/kg was considered as optimized dose.

The results of COI gastrointestinal transit test are represented in Fig. 2A. The peristaltic index was found to be maximum in case of diarrhoeal control rats; however, it was significantly decreased in rats treated with EBV 200 followed by quercetin treated group, which confirmed antimotility effect of extract and its marker quercetin.

In COI intestinal fluid accumulation test, the intestinal weight and volume were found to be higher in

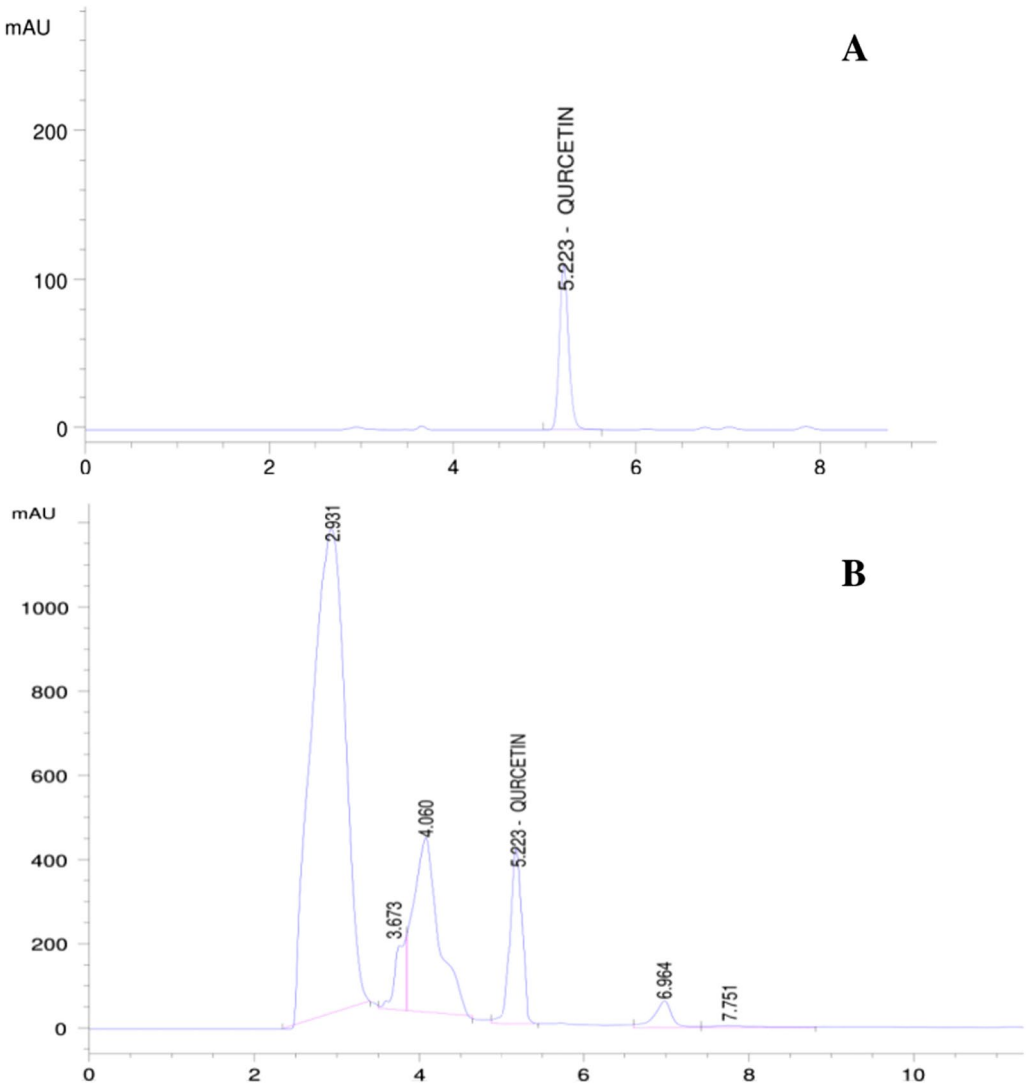

Fig. 1 HPLC chromatogram of EBV representing the presence of quercetin. In figure, **A** HPLC chromatogram of standard quercetin and **B** HPLC chromatogram of EBV showing presence of quercetin

Table 1 Effect of *B. rubrovenia* extract on normal faecal excretion rate

Treatment	Faecal wet weight (g) at various times (h) after treatment				Wet/dry weight of faeces
	1	3	5	7	
Normal Control	0.072 ± 0.003	0.328 ± 0.01	0.543 ± 0.02	0.905 ± 0.03	1.367 ± 0.05
EBV 100	0.379 ± 0.01[a]	0.380 ± 0.01	0.691 ± 0.03[a]	0.672 ± 0.03[a]	1.233 ± 0.07
EBV 200	0.516 ± 0.02[a]	0.542 ± 0.02[a]	0.463 ± 0.02	0.394 ± 0.01[a]	1.070 ± 0.04[a]
EBV 300	0.525 ± 0.02[a]	0.534 ± 0.02[a]	0.442 ± 0.02[a]	0.397 ± 0.01[a]	1.099 ± 0.02[a]
Quercetin	0.490 ± 0.02[a]	0.529 ± 0.02[a]	0.510 ± 0.02	0.470 ± 0.01[a]	1.108 ± 0.03[a]
Loperamide	0.451 ± 0.02[a]	0.299 ± 0.01	0.234 ± 0.01[a]	0.191 ± 0.007[a]	1.058 ± 0.01[a]

Values are mean ± S.E.M. (n = 6)

In table *EBV* Ethanolic extract of *Begonia rubrovenia* at dose level of 100, 200 and 300 mg/kg, p.o.

[a] $p < 0.05$ vs. Normal

[b] $p < 0.05$ vs. Castor oil

Table 2 Effect of *B. rubrovenia* extract on castor oil induced diarrhoea model

Groups	Onset time (min)	Total no of faeces	Total no of wet faeces	Loss in body weight	Total wt of faeces	Mean defecation in 4 h	Diarrhoea score	% Protection
Normal		4.50 ± 0.22		0.16 ± 0.005	0.27 ± 0.01	1.12 ± 0.05	–	100
Castor oil	52.33 ± 2.29	15.33 ± 0.76[a]	10.83 ± 0.74	1.03 ± 0.05[a]	2.07 ± 0.05[a]	3.83 ± 0.19[a]	28.33 ± 2.04	–
EBV 100	68.83 ± 3.04	11.33 ± 0.49[ab]	7.83 ± 0.30[b]	0.90 ± 0.04[a]	1.82 ± 0.08[ab]	2.83 ± 0.12[ab]	15.83 ± 0.79[b]	44.11
EBV 200	119.83 ± 5.54[b]	5.66 ± 0.33[b]	3.50 ± 0.22[b]	0.64 ± 0.03[ab]	0.93 ± 0.04[ab]	1.41 ± 0.08[b]	10.50 ± 0.61[b]	62.93
EBV 300	120.83 ± 6.20[b]	6.50 ± 0.42[b]	3.33 ± 0.21[b]	0.63 ± 0.03[ab]	0.94 ± 0.03[ab]	1.62 ± 0.10[b]	9.83 ± 0.47[b]	65.29
Quercetin	109.50 ± 5.85[b]	7.66 ± 0.33[ab]	3.33 ± 0.21[b]	0.72 ± 0.03[ab]	1.10 ± 0.06[ab]	1.91 ± 0.08[ab]	11.00 ± 0.44[b]	61.17
Loperamide	126.66 ± 6.95[b]	5.83 ± 0.40[b]	2.16 ± 0.47[b]	0.56 ± 0.02[ab]	0.66 ± 0.03[ab]	1.45 ± 0.10[b]	8.00 ± 0.73[b]	71.76

Values are mean ± S.E.M. (n = 6)

In table *EBV* Ethanolic extract of *Begonia rubrovenia* at dose level of 100, 200 and 300 mg/kg, p.o.

[a] $pP < 0.05$ vs. Normal

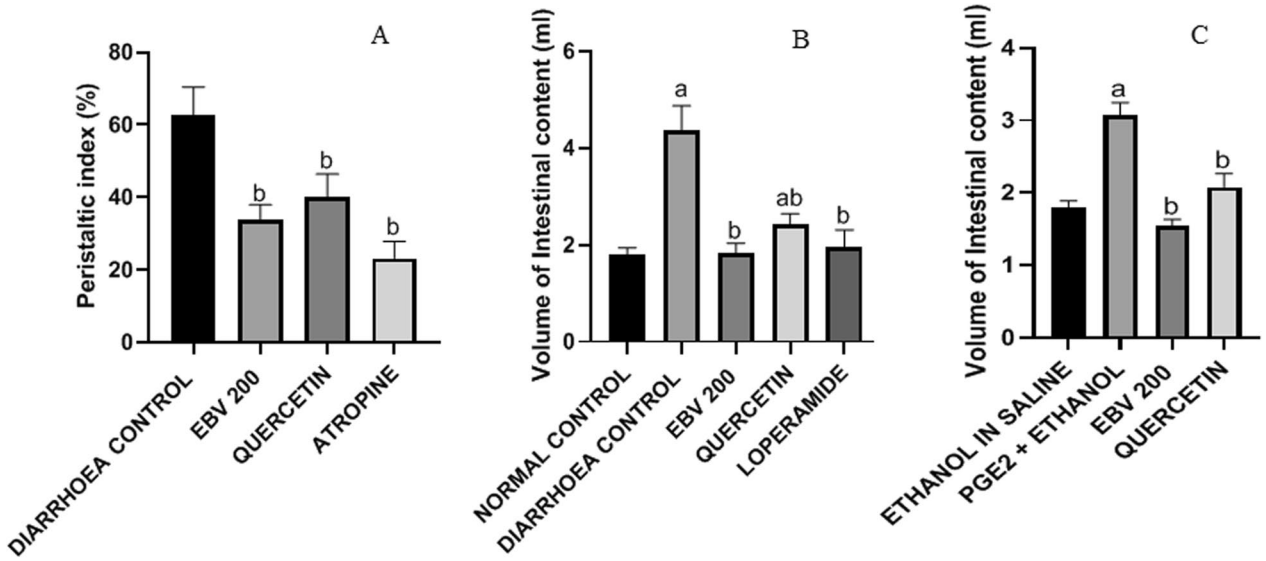

Fig. 2 Effect of EBV and its marker quercetin on COI gastrointestinal transit test (**A**), COI fluid accumulation test (**B**) and PGE_2 induced enteropooling test (**C**). Values are mean ± S.E.M. ($n = 6$), where **a** $p < 0.05$ vs. normal control/ethanol in saline and **b** $p < 0.05$ vs. diarrhoea control/PGE_2 + ethanol. In figure, EBV 200 corresponds to Ethanolic extract of *Begonia rubrovenia* at 200 mg/kg, p.o.

castor oil control rats. Nevertheless, the rats treated with EBV and quercetin showed significant decline in both intestinal weight and volume. The maximum decline was observed in rats who received EBV followed by standard loperamide and quercetin (Fig. 2B). In PGE_2 induced enteropooling test, similar results were obtained where PGE_2 control rats showed maximum intestine volume, however rats receiving EBV and quercetin showed significant decrease in intestinal content (Fig. 2C).

Ions concentration and cytokines estimations

The results of ion concentration determination are given in Table 3, which demonstrated that there was a significant decrease in the ion concentration of colonic tissue of castor oil control rats. However, in case of treatment groups, there was a significant recovery in the altered ions level. Figure 3A and B represents the cytokine profiling, where the results depicted that, groups receiving EBV, quercetin and standard showed significant decrease in the cytokine levels while castor oil control rats showed

Table 3 Effect of *B. rubrovenia* extract on castor oil induced intestinal fluid accumulation

Groups	Weight of intestinal content (g)	Volume of intestinal content (mL)	% Inhibition	Na^+ (mmol/l)	K^+ (mmol/l)	Ca^{2+} (mmol/l)	Cl^- (mmol/l)
Normal	1.47 ± 0.05	1.80 ± 0.05	100	75.44 ± 3.59	28.86 ± 1.35	17.20 ± 0.88	70.06 ± 3.49
Castor oil	4.04 ± 0.28[a]	4.36 ± 0.17[a]	–	57.33 ± 2.62[a]	20.56 ± 1.43[a]	18.90 ± 0.80	52.08 ± 2.13
EBV 200	2.48 ± 0.10[ab]	2.06 ± 0.14[b]	52.59	82.90 ± 4.11[b]	27.40 ± 1.30[b]	18.21 ± 0.77	67.71 ± 3.18[b]
Quercetin	3.09 ± 0.07[ab]	2.41 ± 0.09[ab]	44.57	76.33 ± 3.82[b]	26.88 ± 1.37[b]	18.60 ± 0.92	65.21 ± 2.92[b]
Loperamide	2.02 ± 0.10[b]	1.95 ± 0.07[b]	55.27	84.23 ± 4.16[b]	28.06 ± 1.43[b]	18.70 ± 0.93	70.21 ± 2.89[b]

Values are mean ± S.E.M. (n = 6)

In table *EBV* Ethanolic extract of *Begonia rubrovenia* at 200 mg/kg, p.o.

[a] $p < 0.05$ vs. Normal

[b] $p < 0.05$ vs. Castor oil

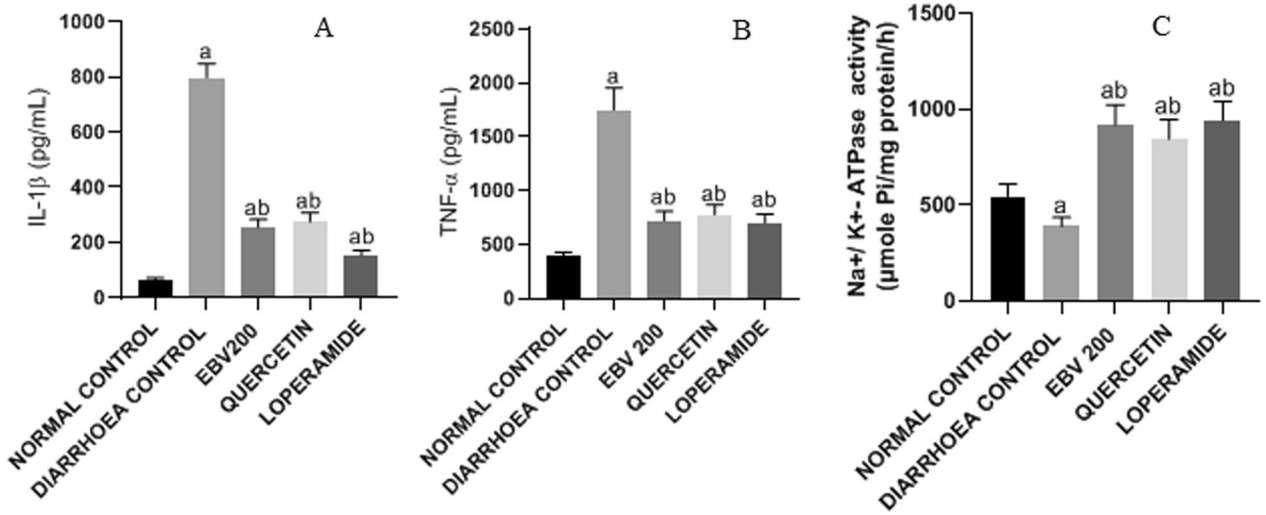

Fig. 3 Effect of EBV and marker quercetin on IL-1β (**A**), TNF-α (**B**) and Na^+/ K^+–ATPase activity (**C**) in colonic tissue of COI diarrhoeal rat.Values are mean ± S.E.M. ($n = 6$). Where a $p < 0.05$ vs. Normal control and b $p < 0.05$ vs. Castor oil induced diarrhoea control. In Figure EBV 200 corresponds to Ethanolic extract of *Begonia rubrovenia* at 200 mg/kg, p.o.

significant hike in the levels of cytokines as compared to normal rats.

Na^+/ K^+–ATPase assay

Figure 3C represents the results of effect of EBV and its marker quercetin on Na^+/ K^+–ATPase. From the overall results, it was observed that the groups treated with EBV and quercetin showed profound increase in Na^+/ K^+–ATPase activity in comparison with the diarrhoeal control rats. The results demonstrated by EBV were quite comparable to that of standard loperamide.

Biochemical estimations and histopathological examination

The results of biochemical evaluations are illustrated in Table 4, where we observed that the NO content was observed to significantly decline, whereas cellular proliferative factors like DNA, carbohydrates and proteins were found to significantly escalate in rats receiving EBV 200, quercetin and standard when compared with rats from diarrhoea control group. The results of antioxidant profiling revealed that, the amount of CAT and SOD went on to significantly decline, while LPO level significantly enhanced in rats from diarrhoea control group. Nevertheless, in treatment group's rats, both the antiperoxidative enzymes showed marked enrichment in their levels followed by decrease in peroxidative enzyme LPO.

Figure 4 represents the histopathological changes observed in all the normal, treated and diarrhoea control rat colons. The histopathology of the rat colons in normal groups exhibited well intact and distinct microvilli with normalized glands, whereas the colonic tissues of diarrhoeal rats showed more disruption of epithelia and blunting of the microvilli. Nevertheless, the

Table 4 Effect of *B. rubrovenia* extract on biochemical parameters

Groups	NO (Units in mole/mg of protein)	Total carbohydrates (Conc mg/g of tissue)	Total protein (Units in mg/100 mg of tissue)	Total DNA (Units in mg/100 mg of tissue)	MDA (Units in Mole/mg of protein)	CAT (μMol H_2O_2 consumed/min/mg of protein)	SOD (Units/mg of protein)
Normal	3.59 ± 0.14	1.07 ± 0.05	0.98 ± 0.04	0.15 ± 0.007	7.35 ± 0.38	67.64 ± 2.65	1.75 ± 0.04
Castor oil	10.26 ± 0.51[a]	0.47 ± 0.02[a]	0.37 ± 0.01[a]	0.11 ± 0.005[a]	20.72 ± 1.79[a]	48.77 ± 2.88[a]	1.25 ± 0.02[a]
EBV 200	6.33 ± 0.39[ab]	0.94 ± 0.04[b]	0.73 ± 0.03[ab]	0.14 ± 0.007[b]	13.37 ± 0.55[ab]	108.60 ± 4.36[ab]	2.25 ± 0.09[ab]
Quercetin	8.79 ± 0.39[ab]	0.83 ± 0.04[ab]	0.56 ± 0.03[ab]	0.12 ± 0.005[b]	16.15 ± 0.82[ab]	103.08 ± 5.07[ab]	2.02 ± 0.16[b]
Loperamide	4.68 ± 0.29[b]	1.06 ± 0.05[b]	0.78 ± 0.03[ab]	0.16 ± 0.007[b]	11.16 ± 0.58[b]	120.45 ± 2.97[ab]	2.80 ± 0.14[ab]

Values are mean ± S.E.M. (n = 6)

In table *EBV* Ethanolic extract of *Begonia rubrovenia* at 200 mg/kg, p.o.

[a] $p < 0.05$ vs. Normal

[b] $p < 0.05$ vs. Castor oil

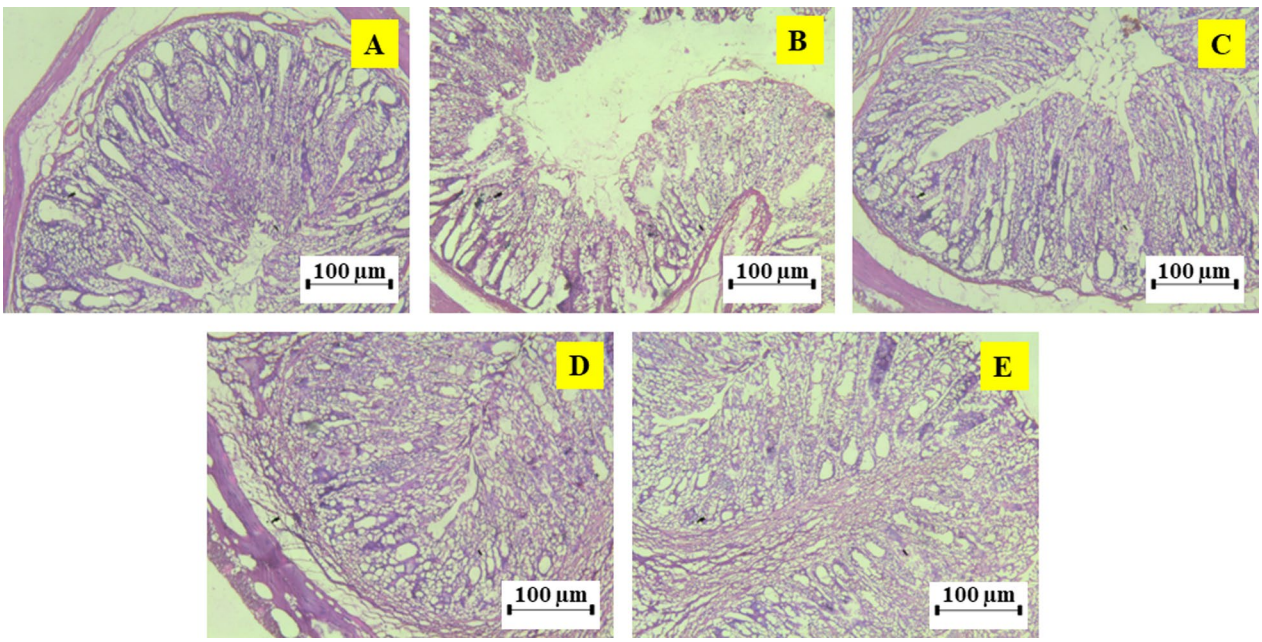

Fig. 4 Histopathological images of colonic sectional part of rats in COI diarrhoea model. [10 × Scale Bar 100 µm]. In figure, **A** Colonic section of Normal control rat, **B** Colonic section of Castor oil control rat, **C** Colonic section of Castor oil induced diarrhoeal rat receiving EBV (200 mg/kg, p.o.), **D** Colonic section of Castor oil induced diarrhoeal rat colon receiving quercetin (50 mg/kg, p.o.), and **E** Colonic section of Castor oil induced diarrhoeal rat colon receiving standard

histopathological view of treated groups demonstrated epithelia free from disruption and well distinct villi, which clearly justified that EBV and quercetin have diarrhoea protecting potential.

In silico study

The calculated binding energies of quercetin with the proteins TNF-α, IL-1β, and EP3 prostanoid receptor are shown in Table 5. The table demonstrated that quercetin exhibited a higher affinity towards the EP3 prostanoid

Table 5 Observations of the molecular docking studies

Molecule	Target protein	PDB ID	Binding energy CDOCKER (Discovery Studio)
Quercetin	TNF-α	1TNF	−106.4030 kcal/mol
	IL1-β	1I1B	−131.2166 kcal/mol
	EP3 prostanoid receptor	6AK3	−150.6988 kcal/mol

receptor compared to TNF-α and IL-1β proteins. This suggests that quercetin may have a stronger binding interaction and potential therapeutic relevance with the EP3 prostanoid receptor. To visualize the binding interactions between quercetin and the proteins, Fig. 5 displays different binding poses of quercetin at the active site of the respective proteins. The figure provides a visual representation of how quercetin interacts with the binding pocket of each protein, highlighting potential key interactions and conformational changes. These binding poses help in understanding the molecular interactions and potential mechanisms of action between quercetin and the target proteins.

The results of the molecular dynamics study are depicted in Fig. 6. The figure provides information on several key parameters that were analysed during the simulation. Firstly, the RMSD of the different conformations was presented in comparison to the initial pose. The RMSD indicates the average deviation or fluctuation of the protein–ligand complex structures throughout the simulation. It helps to assess the stability and conformational changes of the complex over time. The RMSD plot in Fig. 6A demonstrates how the different conformations deviate from the initial structure during the simulation.

Additionally, Fig. 6B shows ROG of the protein over the course of the molecular dynamics study. The ROG is a measure of the compactness or overall size of the protein molecule. Monitoring the ROG provides insights into the protein's structural stability and any potential unfolding or compaction events during the simulation. Furthermore, the RMSF of different amino acid residues of the protein is also depicted in Fig. 6C. The RMSF analysis demonstrates the flexibility or mobility of individual amino acids in the protein. It highlights regions or residues that exhibit significant fluctuations or conformational changes during the simulation. The RMSF plot provides information on the dynamic behaviour of the protein and can identify regions that may play crucial roles in ligand binding or protein function.

Network pharmacology

Quercetin was the bioactive compound obtained from *Begonia rubrovenia* and TNF-α (encoded by the gene TNF), IL1-β (encoded by the gene IL1B) and EP3 (encoded by the gene PTGER3) were the bioactive target proteins as shown by the docking studies. Quercetin was found to be neutrally charged with a molecular weight of 302.23 (> 1250). We found that TNF interacted with 10 different proteins, including IKBKG (encoding NF-kappa-B essential modulator), TRAF2, IL10, BIRC2, FADD, TRADD, and others. Out of 16 molecular processes identified by gene ontology analysis, a process of tumour necrosis factor receptor superfamily binding (GO:0032813) was found with the lowest false discovery rate of 1.77e-07 in which there was modulation of 5 proteins against 48 background proteins (Fig. 7).

IL1-β was also found to have 10 different interacting proteins, including CASP1, CXCL1 & 8, CCL3, IL1A, IL10, IL6 and others. Gene ontology data revealed 14 molecular processes, among which cytokine receptor binding (GO:0005126) had the lowest false discovery rate of 6.46e-10, modulating 8 proteins against 264 background proteins. Similarly, EP3 was found to interact with 10 different partners, including GNAS, GNAI1, 2 & 3, GNAQ, PTGDR2, PTGES and others. Gene ontology identified a total of 9 molecular function, among which a molecular function of G-protein beta/gamma-subunit complex binding (GO:0031683), having the lowest false discovery rate of 3.30e-09 was identified, modulating 5 proteins against 20 background proteins (Fig. 7).

Discussion

Diarrhoea is a very common gastrointestinal disorder but ranks second in deaths among all the causes of deaths in developing countries profoundly due to lack of sanitation, unhygienic conditions, contaminated water as well as food, malnutrition, etc. [32]. Diarrhoea is either secretory or osmotic type depending upon its etiology [33]. The present investigation overviewed on the antidiarrhoeal efficacy of EBV using castor oil and PGE_2 induced diarrhoea rat models including molecular docking, dynamics and network pharmacological studies.

Oral acute toxicity study is carried out to determine the safest dose of drug for the evaluation of animal experimental study. Here, for estimation of diarrhoeal study OECD guidelines 425 was implemented. Up to 2000 mg/kg dose of the extract, there were no any abnormal behavioural or neurological signs observed in the treated animals which confirmed the extract to be safe up to above dosing. The normal faecal excretion rate study revealed that, the rate of excretion significantly declined in treated rats, which gives us an idea about the antisecretory potential of *B. rubrovenia* extract [19]. In castor oil induced diarrhoea rat model, diarrhoea was found to be more severe in case of diarrhoea control rats. But, the severity of diarrhoea was reduced in all the treated rats, which was depicted through the results showing delay in onset of diarrhoea, decline in total number of faeces, number of wet faeces and mean defecation rate. Also, the profound antidiarrhoeal activity of the EBV and its marker was confirmed from the low diarrhoea score and high % protection values. Both EBV at 200 and 300 mg/kg dose showed quite similar responses in both the studies, i.e. normal faecal excretion and castor oil induced diarrhoea models. Thus we may presume that, ceiling effect

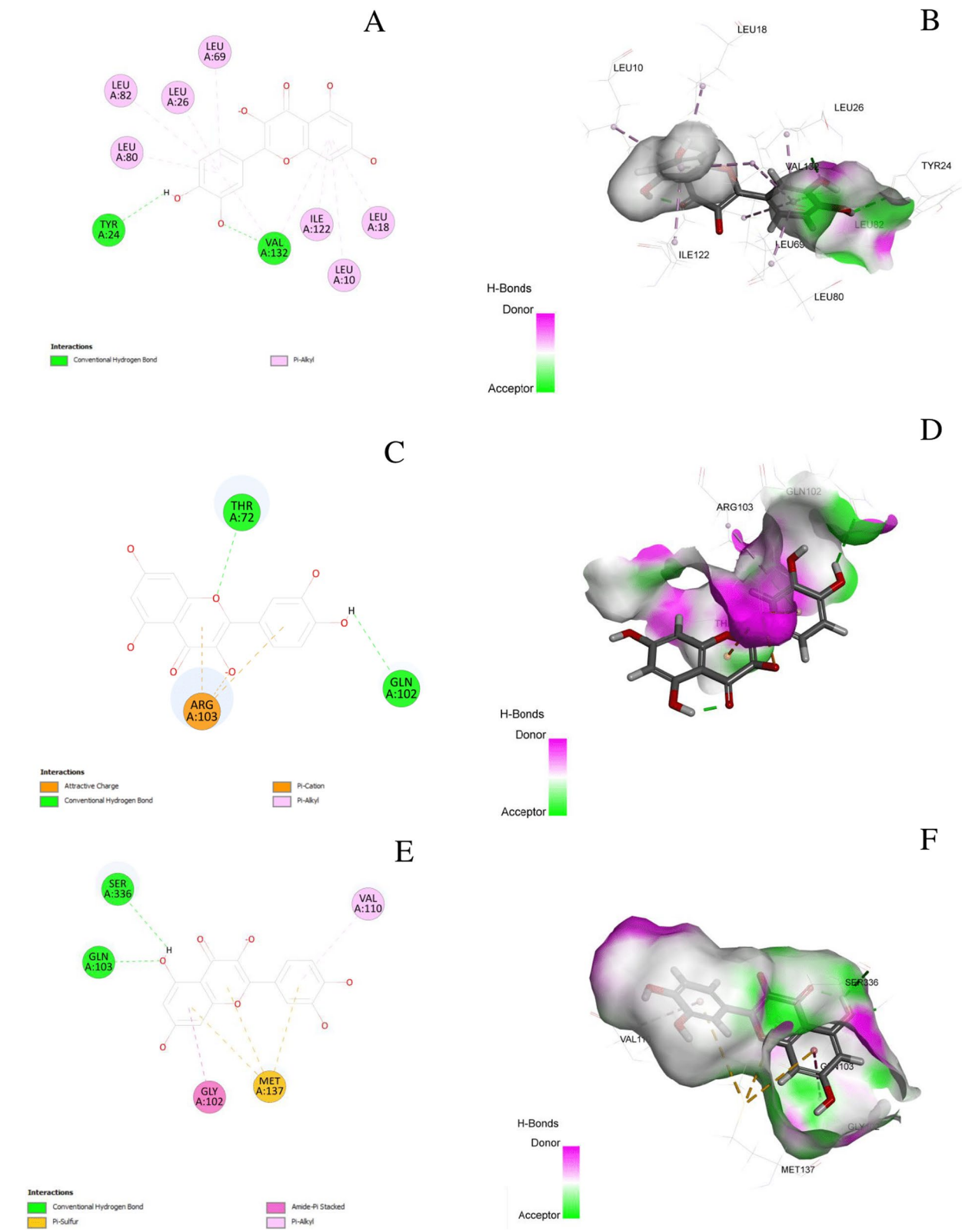

Fig. 5 Molecular docking studies of quercetin against different target proteins. In figure **A** represents 2D interactions of quercetin against IL-1β, **B** represents 3D interactions of quercetin against IL-1β, **C** represents 2D interactions of quercetin against TNF-α, **D** represents 3D interactions of quercetin against TNF-α, **E** represents 2D interactions of quercetin against EP3 prostanoid receptor and **F** represents 3D interactions of quercetin against EP3 prostanoid receptor

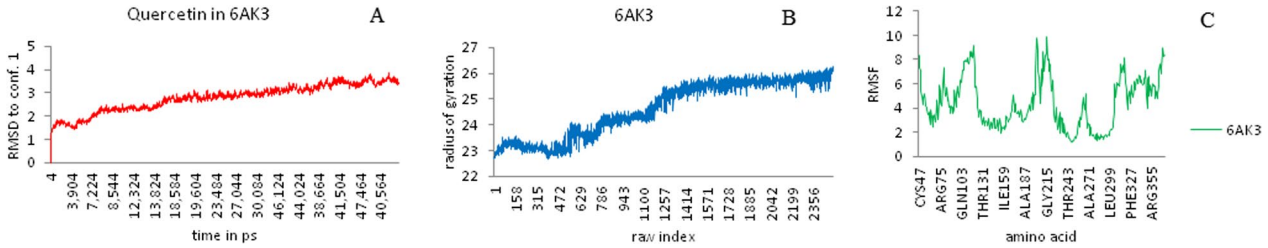

Fig. 6 Molecular dynamics simulations of quercetin against EP3 prostanoid receptor. In figure, **A** Root mean square deviation, **B** Radius of gyration and **C** Root mean square of fluctuation

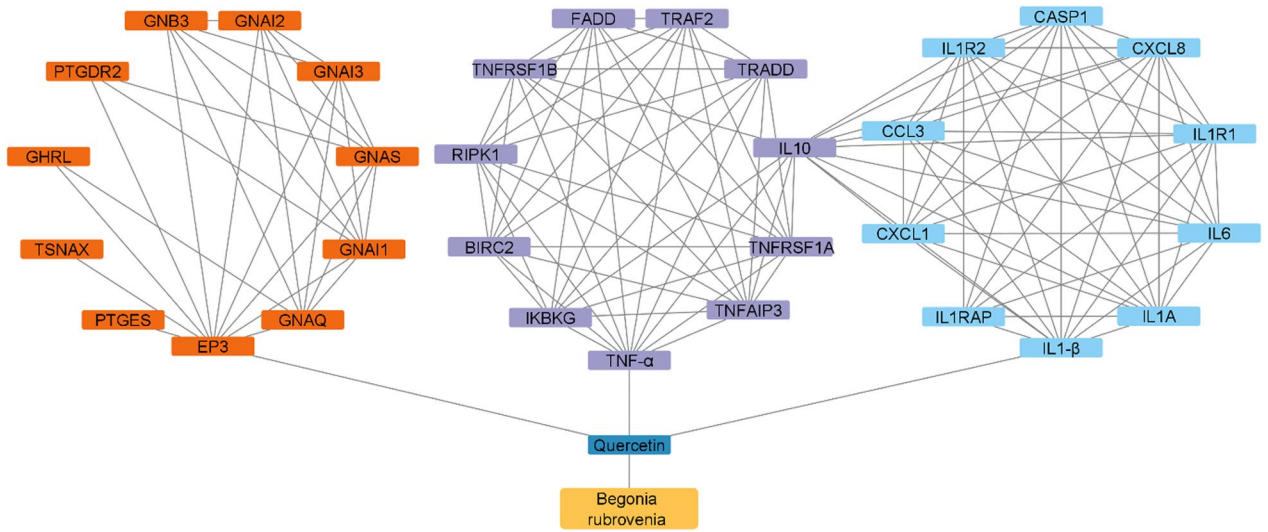

Fig. 7 Interaction network of *Begonia rubrovenia,* quercetin and its molecular targets

was observed from EBV 200 mg/kg and therefore, EBV 200 was considered as effective dose for the next entire antidiarrhoeal evaluations.

Castor oil obtained from the plant *Ricinus communis*, when ingested orally gets metabolized into ricinoleic acid by the intestinal lipase enzymes. This acid forms lesions on the intestinal mucosal membrane, which leads to changes in the permeability of mucosal fluid and disturbance in the electrolyte and water transport that results in hypersecretion and finally diarrhoea [34]. In the present study also, the intestinal volume significantly increased in diarrhoea control rats, whereas it was significantly recovered in rats treated with EBV and its marker quercetin as demonstrated in the results of COI fluid accumulation diarrhoea model. Ricinoleic acid metabolized from castor oil, after oral administration, increases the peristaltic activity of the intestinal smooth muscle [35]. The reduction in the peristaltic movement was observed in EBV and quercetin treatment group as demonstrated through the COI transit study showing very low peristaltic index. The antidiarrhoeal efficacy of EBV against inflammatory diarrhoea was also evaluated by using PGE_2 induced enteropooling study. PGE_2 enhances the gastrointestinal motility by interacting with EP3 receptors of the intestine thus, increase the intestinal volume which leads to diarrhoea [32]. The results of our analysis showed that the extract and quercetin were found to be effective in reducing the fluid volume, which might be due to inactivation of EP3 receptors expressed in the small intestine.

Ricinoleic acid present in the intestine promotes the release of NO by activating the inducible nitric oxide synthase (i-NOS). Over expression of NO results in increased level of c-AMP mediated through activation of adenyl cyclase. This overall condition decreases the Na^+-K^+- ATPase activity [32]. The results demonstrated that, there was an over expression of NO in diarrhoeal rats, which tend to normalize after treating the animals with extract and quercetin, that ultimately enhanced the Na^+-K^+- ATPase activity. The upsurge in NO results in elevated oxidative stress, which is one of the important parameter responsible for provoking various types of gastrointestinal malfunctioning [34]. This oxidative stress decreases, the CAT and SOD level and increases the LPO level, which was depicted from the diarrhoea control

group rats. However, in case of EBV and quercetin treated rats, there was restoration of altered change in the levels of oxidative enzymes, which helps in the maintenance of oxidative stress. Na^+-K^+- ATPase, a basolateral protein enzyme, plays a major role in transport and maintenance of nutrients and electrolytes [14]. Studies have suggested that diarrhoeal condition hampers the cellular proliferative factors (DNA, proteins and carbohydrates) causing decreases in their levels [36]. The cellular proliferative enhancing effect of the extract, quercetin and standard drug was confirmed through the increase in these parameters. The inducing agent castor oil, causes irritation to the intestinal mucosal membrane through ricinoleic acid resulting into inflamed mucosa and consequently release of inflammatory mediators like TNF-α and IL-Iβ, which was observed in diarrhoea control group [35]. Our results also showed that, there was significant decline in the above mediators after treatment, which suggests the role of EBV in treating inflammatory type of diarrhoea. The histopathological examination of colonic tissues confirmed the protective nature of the extract and its marker quercetin showing recovery from the destruction of epithelia and blunting of villi as observed in treated groups.

Flavonoids, alkaloids, tannins, terpenoids, saponins and steroids from the medicinally active plants plays critical role in treating diarrhoea [37]. Our extract was found to be rich in phenolics, tannins, flavonoids which was confirmed from the quantification results. Quercetin belongs to flavonoid category and its presence in the extract was confirmed through the HPLC analysis data. Quercetin was reported to have antidiarrhoeal potential by relaxing the smooth muscles of intestine, which ultimately inhibits the bowel movement by reducing the release of Ca ions (intracellularly) through the sarcoplasmic reticulum [38]. From the overall antidiarrhoeal study we may presume that, quercetin might be one of the major responsible phytoconstituent in the extract for the observed antidiarrhoeal activity in combination with other phytochemicals.

The molecular docking results indicated that, quercetin exhibited a strong affinity towards all the target proteins, particularly the human EP3 prostanoid receptor, with a binding energy of -150.69 kcal/mol. Quercetin was found to have two hydrogen bonds with active site amino acids viz. GLN:103 and SER:336 together with other non-bonded interactions. This suggests that quercetin forms stable interactions with the active site of the EP3 prostanoid receptor, indicating its potential as a ligand for this protein. Subsequently, a molecular dynamics simulation revealed that the RMSD value reaches a plateau after 5 ns, indicating that the complex has achieved stability. This suggests that quercetin remained compatible and firmly bound within the active site of the protein throughout the simulation. Regarding the receptor protein itself, the ROG analysis showed minimal and insignificant fluctuations. This implies that, the protein maintained its overall compactness and stability during the molecular dynamics simulation study. The limited fluctuation in ROG indicates that the receptor protein structure remained relatively intact and does not undergo significant conformational changes or unfolding. Additionally, the RMSF analysis of the amino acid residues further supports the stability of the receptor protein molecule [29]. The RMSF plot indicated that the individual amino acid residues exhibited minimal fluctuation and remained relatively stable throughout the simulation. This suggested that the receptor protein structure is robust and maintained its stability, supporting its functionality and ability to interact with ligands like quercetin [28]. Findings from this in silico studies conducted in this research suggest that quercetin has the potential to interact with the EP3 prostanoid receptor, which may contribute to its antidiarrheal activity.

Quercetin was found to exhibit wide-ranging effect on several molecules in the network analysis. TNF-α, besides interacting with several receptor molecules was also predicted to modulate apoptotic adaptor molecule FADD, caspase regulator BIRC2, and NF-kappa-B modulator IKBKG [30]. IL1-β was found to interact with significant molecules such as Caspase1, CXCL1 which has chemotactic activity for neutrophils, CCL3 with inflammatory and chemokinetic properties and several interleukins. IL10 molecule was found in the network of both TNF-α and IL1-β indicating extended molecular level effect. Further EP3 molecules has several important predicted functional partners like Appetite-regulating hormone GHRL, Translin-associated protein X TSNAX, Microsomal prostaglandin-e synthase 1 PTGES, and several Guanine nucleotide-binding proteins. The network analysis showed that quercetin can have diverse range of effects by interacting with these proteins and modulate the molecular pathway [31].

Conclusion

The present study concludes that, the antidiarrhoeal potential of EBV may be due its secretion inhibitory and antipropulsive effect. In addition, EBV also showed reduction in NO level, restoration of ions and antioxidants, decreased proinflammatory cytokines expression and enhancement of Na^+-K^+- ATPase activity which may promotes its diarrhoea protecting efficiency. Thus, we have successfully justified the traditional use of *Begonia rubrovenia* in treating diarrhoea, where quercetin played a major role in combination with other phytoconstituents.

Abbreviations

AOT	Acute oral toxicity
COI	Castor oil induced
DE	Diosgenin equivalent
DFE	D-fructose equivalent
EBV	Ethanolic extract of *B. rubrovenia*
GAE	Gallic acid equivalent
IL-1β	Interleukin 1β
NFE	Normal faeces excretion
PDB	Protein data bank
PGE_2	Prostaglandin E_2
RE	Rutin equivalent
RMSD	Root mean square deviation
ROG	Radius of gyration
RSMF	Root mean square fluctuation
TAE	Tannic acid equivalent
TNF- α	Tumour necrosis factor α

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-024-00592-7.

Additional file 1. Acute oral toxicity study.

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Author contributions
PRI, DL and SKP: Conceptualization, supervision, investigation, RSP, DL and NYY: Collected the plant material, authenticated and initial phytochemical standardization, RSP, NYY, SRD, and DL: Performed the initial pharmacological protocol, RSP, PS, MD, and SKS: Performed the biochemical analysis and cytokine profiling, RSP, SRD and JK: Performed the histopathological and ion analysis, JK, NR and SKS: Performed the docking dynamics and network pharmacology, RSP, SKP, PS and JK: Performed statistical analysis and interpretation of results, RSP, DL, NR, NYY and SRD: Prepared the initial draft of the manuscript, SKP and PRI: Finalized the manuscript and responsible for communication of manuscript to the Journal.

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Availability of data and materials
The data could be made available as on request of the journal.

Declarations

Ethics approval and consent to participate
All the pharmacological experimental protocols were performed after approval from Institutional Animal Ethical Committee of Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra, India (Ref. No. IAEC/UDPS/2022/01 dated 21/05/2022) and were conducted in accordance with accepted standard guidelines of National Institutes of Health Guide for Care and Use of Laboratory Animals. Studies involving plants must include a statement specifying the local, national or international guidelines and legislation and the required or appropriate permissions and/or licences for the study: The plant under investigation was collected from the West Khasi hills district of Meghalaya, India and all the necessary approval right from authentication of plant material approval of the research were been taken from the state government University authorities.

Consent for publication
The consent from all the co-authors have been taken regarding submission of the manuscript to Future Journal of Pharmaceutical Sciences for possible publication.

Competing interests
The authors have disclosed that there are no conflicts of interest. Further, consent from all the co-authors has been taken and the authors are entirely responsible for the composition and content of the article.

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References

1. da Costa DS, dos Santos Negreiros P, da Silva VG, Nunes DB, Acha BT, Quintans-Junior LJ, de Souza Araújo AA, Araújo TDSL, de Melo Sousa FB, Medeiros JVR, Lima FDCA (2020) Antidiarrheal activity of farnesol in rodents: pharmacological actions and molecular docking. Eur J Pharmacol 874:172986
2. Birru EM, Asrie AB, Adinew GM, Tsegaw A (2016) Antidiarrheal activity of crude methanolic root extract of Idigofera spicata Forssk (Fabaceae). BMC Complement Altern Med 16(272):1–7
3. Pessoa MMB, Neto HD, de Oliveira LE, Batista LM (2020) Antifungal activity and antidiarrheal activity via antimotility mechanisms of (-)-fenchone in experimental models. World J Gastroenterol 26(43):6795–6809
4. UNICEF (2015) Geneva: prevention and control of cholera outbreaks: Who policy and recommendations. pp. 90–115
5. Greenland K, Chipungu J, Chilengi R, Curtis V (2016) Theory-based formative research on oral rehydration salts and zinc use in Lusaka, Zambia. BMC Public Health 16:312. https://doi.org/10.1186/s12889-016-2984-2
6. Wu PE, Juurlink DN (2017) Clinical review: loperamide toxicity. Ann Emerg Med 70:245–252. https://doi.org/10.1016/j.annemergmed.2017.04.008. (**PMID: 28506439**)
7. Zarghami M, Rezapour M (2017) Loperamide dependency: a case report. Addict Health 9:59–63 (**PMID: 29026504**)
8. Kumari A, Baskaran P, Staden JV (2017) In vitro regeneration of *Begonia homonyma*—a threatened plant. S Afr J Bot 109:174–177
9. Jaiswal (2010) Culture and ethnobotany on Jaintia tribal community of Meghalaya Northeast India—a mini review. Indian J Tradit Knowl 9:38–44
10. Kayang H (2007) Tribal knowledge on wild edible plants of Meghalaya, Northeast India. Indian J Tradit Knowl 6:177–181
11. Laloo D, Hemalatha S (2011) Ethnomedicinal plants used for diarrhea by tribals of Meghalaya, Northeast India. Pharmacogn Rev 5:147–154
12. Prasad RS, Dhaswadikar SR, Laloo D, Dhobi M, Itankar PR, Prasad SK (2022) Quality control profiling, nutritional analysis and phytochemical standardization of a vegetable root *Begonia roxburghii*. Vegetos. https://doi.org/10.1007/s42535-022-00446-8
13. Trease GE, Evans WC (2002) Pharmacognosy. W.B. Saunders Company Ltd., Elsevier Publication, London
14. Hirudkar JR, Parmar KM, Prasad RS, Sinha SK, Lomte AD, Itankar PR, Prasad SK (2020) The antidiarrhoeal evaluation of *Psidium guajava* L. against enteropathogenic *Escherichia coli* induced infectious diarrhoea. J Ethnopharmacol 251:112561

15. Prasad SK, Laloo D, Kumar M, Hemalatha S (2013) Antidiarrhoeal evaluation of root extract, its bioactive fraction and lupinifolin isolated from *Eriosema chinense*. Planta Med 79:1620–1627
16. Christudas S, Duraipandiyan V, Agastian P, Ignacimuthu S (2013) Antidiabetic effect of plumbagin isolated from *Plumbago zeylanica* L. root and its effect on GLUT4 translocation in streptozotocin-induced diabetic rats. Food Chem Toxicol 50:4356–4363
17. Tangpu V, Yadav AK (2004) Antidiarrhoeal activity of *Rhus javanica* ripen fruit extract in Albino mice. Fitoterapia 75:39–44
18. Adeyemi OO, Akindele AJ, Ogunleye EA (2009) Evaluation of the antidiarrhoeal effect of *Sanseviera liberica* gerome & Labroy (Agavaceae) root extract. J Ethnopharmacol 123:459–463
19. Prasad RS, Yenorkar NY, Dhaswadikar SR, Sinha SK, Rai N, Sharma P, Kulkarni O, Kumar N, Dhobi M, Laloo D, Gurav SS, Itankar PR, Prasad SK (2023) A systematic antidiarrhoeal evaluation of a vegetable root *Begonia roxburghii* and its marker flavonoids against nonpathogenic and pathogenic diarrhoea. Food Biosci 53:102672
20. Rao CV, Vijayakumar M, Sairam K, Kumar V (2008) Antidiarrhoeal activity of the standardized extract of *Cinnamomum tamala* in experimental rats. J Nat Med 62:396–402
21. Gal-Garber O, Mabjeesh SJ, Sklan D, Uni Z (2003) Nutrient transport in the small intestine: Na+, K+-ATPase expression and activity in the small intestine of the chicken as influenced by dietary sodium. Poult Sci 82:1127–1133
22. Parmar KM, Bhagwat DS, Sinha SK, Katare NT, Prasad SK (2019) The potency of eriosematin E from *Eriosema chinense* Vogel. against enteropathogenic *Escherichia coli* induced diarrhoea using preclinical and molecular docking studies. Acta Trop 193:84–91
23. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982) Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem 126:131–138
24. Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by Anthrone. Biochem J 57:508–514
25. Burton K (1956) A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 62:315–323
26. Lowry OH, Rosebrough NJ, Farr AL, Randall RT (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–276
27. Laloo D, Prasad SK, Sairam K, Hemalatha S (2013) Gastroprotective activity of ethanolic root extract of *Potentilla fulgens* wall. Ex Hook J Ethnopharmacol 148:505–514
28. Johari S, Sharma A, Sinha S, Das A (2019) Integrating pharmacophore mapping, virtual screening, density functional theory, molecular simulation towards the discovery of novel apolipoprotein (apoE ε4) inhibitors. Comput Biol Chem 79:83–90
29. Lin CH, Chang TT, Sun MF, Chen HY, Tsai FJ, Chang KL, Fisher M, Chen CYC (2011) Potent inhibitor design against H1N1 swine influenza: structure-based and molecular dynamics analysis for M2 inhibitors from traditional Chinese medicine database. J Biomol Struct Dyn 28(4):471–482
30. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, Jensen LJ, von Mering C (2017) The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible. Nucleic Acids Res 45:D362–D368
31. Shannon P (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504
32. dos Santos Negreiros P, da Costa DS, da Silva VG, de Carvalho Lima IB, Nunes DB, de Melo Sousa FB, Araújo TDSL, Medeiros JVR, Dos Santos RF, Oliveira RDCM (2019) Antidiarrheal activity of α-terpineol in mice. Biomed Pharmacother 110:631–640
33. Tagne MAF, Akaou H, Noubissi PA, Fondjo AF, Rékabi Y, Wambe H, Kamgang R, Oyono JE (2019) Effect of the hydroethanolic extract of *Bixa orellana* Linn (Bixaceae) leaves on castor oil-induced diarrhoea in Swiss albino mice. Gastroenterol Res Pract 2019:6963548
34. Ma YL, Wu ZM, Liu X, Lan JE, Zai WJ, Jin X, Xie H, Mu Q, Hong-Rui Liu HR (2022) Antidiarrheal activity of the extracts of *Valeriana jatamansi* Jones on castor oil-induced diarrhoea mouse by regulating multiple signal pathways. J Ethnopharmacol 298:115560
35. Vanjari H, Mohan MM, Saudagar P, Guthal G, Dalvi M (2020) Protective effect of SKB Gutbiotic against castor oil and *E. coli* induced diarrohea in laboratory animals. Microb Pathog 143:104078
36. Prasad SK, Parmar KM, Danta CC, Laloo D, Hemalatha S (2017) Antidiarrhoeal activity of eriosematin E isolated from the roots of *Eriosema chinense* Vogel. Phytomedicine 24:127–133
37. Mesaik AM, Poh HW, Bin OY, Elawad I, Alsayed B (2018) In vivo anti-inflammatory, anti-bacterial and anti-diarrhoeal activity of *Ziziphus Jujuba* fruit extract. Open Access Maced J Med Sci 6:757–766
38. Ahmad I, Alotaibi BS, Malak N, Asad F, Ullah B, Nasreen N, Khan A, Chen CC (2023) Antidiarrheal potential of *Viola canescens*: in vivo and in silico approaches. Pharmaceuticals 16:489. https://doi.org/10.3390/ph16040489

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