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Biological and computational studies provide insights into *Caesalphinia digyna* Rottler stems

Nazim Uddin Emon [a,b,*], Md Munsur Alam [a], Md Solaman Uddin Sawon [a], Ezazul Hoque Rana [a], Marufa Afroj [a], Mohammad Mehedi Hasan Tanvir [c]

[a] Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong, Chittagong, 4318, Bangladesh
[b] Department of Public Health, School of Science and Technology, Bangladesh Open University, Gazipur, 1705, Dhaka, Bangladesh
[c] Department of Pharmacy, University of Development Alternative, Dhaka, 1209, Bangladesh

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ABSTRACT

Caesalpinia digyna (Rottl.) (Family: Fabaceae) is an essential medicinal plant for it's conventional uses against a kind of human disorders. This research aims to investigate the antidiarrheal, antibacterial and antifungal properties of the methanol extract of the stems extracts of the *C. digyna* (MECD). The *in vivo* antidiarrheal activity of the stem extracts were evaluated by using castor oil-induced diarrhea, castor oil-induced enteropooling and charcoal induced intestinal transit in mice model. Besides, *in vitro* antimicrobial potentiality of MECD was investigated by the disc diffusion method. *In silico* activity of the isolated compounds were performed by Schrödinger-Maestro (Version 11.1) software. In addition, The ADME/T analysis and PASS prediction were implemented by using pass online tools. In the antidiarrheal investigation, the MECD exhibited a notable inhibition rate in all test approaches which were statistically significant ($p < 0.05$, $p < 0.1$, $p < 0.01$). MECD 400 mg/kg showed the maximum antidiarrheal potency in all the test methods. *In vitro* antimicrobial analysis unveiled that, MECD revealed higher potentiality against almost all pathogens and indicates dose-dependent activity against almost all the bacteria and fungi. In the case of *in silico* evaluation of anti-diarrheal, anti-bacterial and anti-fungal activity, all three isolated compounds met the pre-conditions of Lipinski's five rules for drug discovery. Pass predicted study also employed for all compounds. However, The chemical constituents of the *C. digyna* can be a potent source of anti-diarrheal, anti-bacterial and anti-fungal medicine and further modification and simulation studies are required to establish the effectiveness of bioactive compounds.

1. Introduction

Diarrhea is one of the water-borne diseases, endemic in various regions of the world and well-recognized as the significant prosperity threats to the world people, both in tropical and subtropical poor countries [1]. The major causative agents of diarrhea in human beings include are mainly various enteropathogens including *Shigella flexneri, Escherichia coli, Staphylococcus aureus, Salmonella typhi and Candida albican* [2]. Diarrheal illnesses are a potential explanation behind morbidity and mortality especially in children and young people in developing countries. It will be very acute in infants and elderly people because of severe, conceivabely fatality as a result of dehydration [3]. It ranges from the mild and socially inconvenient sickness to a noteworthy reason for death likely because of the extra impacts of unhealthiness among the children in less developed nations. As of now, the treatment for diarrhea is non-specific and is typically planned for diminishing the discomfort and burden of frequent bowel movements [4]. Pharmaceutical drugs are regularly used to treat diarrhea, but adverse effects can be actuated by these drugs [5]. A few antimicrobial and antidiarrheal agents have been utilized against the improvement of microbial infection and diarrhea. The response of conventional medication as an elective type of healthcare and the advancement of microbial resistant from the available antibiotics have driven specialists to investigate the antimicrobial effects of herbal preparations. Various research work has been suggested that medicinal plants can be a promising source of antidiarrheal medication. Plants containing flavonoids, terpenoids, steroids, phenolic compounds, and alkaloids have been accounted for having antimicrobial action as well as antidiarrheal action [6,7].

Medicinal plants, which are recognized as a possible source of therapeutic aids, are playing a crucial role for humans as well as

* Corresponding author. Department of Public Health, School of Science and Technology, Bangladesh Open University, Gazipur, 1705, Dhaka, Bangladesh.
E-mail address: nazim7emon@gmail.com (N.U. Emon).

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unhealthy animals and provides potential health care content in every part of the world in health systems. Herbal medicines are used to prevent and cure infections and diseases of human or animal or to encourage wellbeing and healings [8]. Many on-going research shows that there is an important understanding between ethnological uses by indigenous people in the treatment of explicit symptoms and that the antibacterial, antifungal and antidiarrheal potential are explored in the laboratory [9]. In developing countries, people often use traditional drugs aim to prevent numerical infectious diseases including diarrhea. For that, there is a strong interest in the quest of plants with antidiarrheal and antimicrobial activities that could be used to fight against diarrheal disease. Local healers typically used an amount of medicinal plants with antidiarrheal and antimicrobial properties [10].

Caesalpinia digyna (Family: Fabaceae) is an evergreen shrub which is well known to the ethnic society owing to it's numerical medicinal values. This shrub is also known as Teri Pod, vakerimul and Udakiryaka. This plant is distributed in India, Bruma, Nepal, Bangladesh and China but in Bangladesh the availability of this plant is rare [11]. The herb has been used to prepare a native therapeutic product "Geriforte" which is very effective in the treatment of senile pruritis. Ancient Indian practitioners had found that, this herb have an antipyretic, tonic, astringent and respiratory disease ailment power [12]. The pods of *C. digyna* popularly called "teri pods" have approximately 28% tannin, the tannin in the bark is also 28%, while seed pods have over 54% tannin. The tannin is pure tannin of Gallo and gallic acid. Caesalpinin A, cellallocinnine, ellagic acid, gallic acid, bergenin, bonducellin, intricatinol and tannins were found to be present as a phytoconstituents of *C. digyna* [13]. The structures of intricatinol,E−8-methoxybonducelline, bonducelline, isointricatinol, Z-8 methoxybonducelline, e-eucomine, z-eucomine, caesalpinine A, and caesalpinine C were isolated from *C. digyna* [14–17]. An anti-inflammatory crystalline material, provisionally known as Vikerin was isolated and later confirmed as Bergenin [18,19]. The compound namely 2,3,Dihydro-7-hydroxy-3- [(4methoxyphenyl) Methylene]-4 H-1benzopyran-4-one has been isolated from the twigs and leaves of this plant [20]. Petroleum ether extract of *C. digyna* root was phytochemically examined and four compounds - friedelin, hexacosanoic acid, β-sitosterol and stigmasterol were isolated [21]. The EtOAc fraction of the MeOH extract of *C. digyna* roots were examined through the chromatogram and a new homoisoflavoid, Isointricatinol (1), with eight well-established homoisoflavonoids, three flavonoids, bergenine and 11-O-galloylbergenin were also extracted. In the ancient studies, the root of the *C. digyna* was found to treat diabetes and fever [22]. The powder of this shrub also used by numerous ethnic tribes of the India and Bangladesh in treating diarrhea and other chronic fluxes. *C. digyna* is accounted for the managements of senile pruritis, diarrhea, tuberculosis, tonic disorder, diabetes, etc; [23,24]. Despite extensive plant use, however, biological study has not yet been undertaken on pharmacological and phytochemical aspects to help their traditional use. In this present study, in an attempt to rationalize its indigenous use, the *in vivo* studies were conducted to investigate the phytochemical, antimicrobial and antidiarrheal efficacy of methanol extract of *C. digyna* stems (MECD) in different experimental approaches. Besides, an advanced computational analysis has been propagated to the characterization of its isolated compounds by applying ADME/T, pass prediction, and molecular docking simulation.

2. Materials and methods

Sigma Aldrich Chemicals Co. (St. Louis, MO, USA) has provided methanol, gum acacia, and charcoal, and tween-80 was from BDH Chemicals Ltd. (Poole, UK). Loperamide, amoxicillin, and flucoxacillin have been purshued from the Sanofi Bangladesh Ltd. (Dhaka, Bangladesh). Furthermore, all other chemicals used in this analysis were analytical grade.

2.1. Collection of the plant

Owing to implement the pharmacological and phytochemical analysis of the stems of C. digyna, the test sample (stems) were collected from the shita pahar, prashanti, kaptai, Chittagong, Bangladesh in February 2019. Sajib Rudra, a taxonomist from the Department of Botany, Chittagong University, Bangladesh 4331, performed an effective plant test identification and confirmed the authenticity of the plant (Accession number: CTGUH SR 2793). The stems were washed and dried in the natural shade at 23 ± 2 °C for approximately fourteen days. The seedlings were then dried in the oven for 1 h for better grinding at significantly low temperature (40 °C). The dried stems were then crashed into the powder by using a high capacity granular machine. The powdered materials were placed in a well-closed plastic jar and stored in a cool, and dark place.

2.2. Extraction of the plant material

Approximately 500 g of the dry powdered material was processed and soaked into 2,5 L of methanol in a separate clean glass container. The jar with its materials were wrapped with an aluminum foil and continued intermittent shaking and mixing for 14 days at 23 ± 2 °C. The whole mixture was then filtered through the cotton and followed by no. 1 Whatman twin-ring filter paper (Bibby RE200 Sterilin Ltsd., UK) and subsequently the mixure was condensed on the water bath at 40 °C, so that the solvent can be evaporated. The extract weight from the stalks was 13 g. In the refrigerator, the crude extract was stored under 4 °C.

2.3. Test strains

Investigational bacteria designated to *Staphylococccus aureus, Lactobacillus casei, Bacillus azotoformans, Bacillus azotoformans, Bacillus cereus, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa,* and fungus named to *Candida albicans, Cryptococcus neoformans, Blastomyces dermatitidis, Trichoderma* spp. all the clinical pathogens were obtained from the Department of Pharmacy, International Islamic University Chittagong, Bangladesh.

2.4. Animals

From the BCSIR (Bangladesh Council of Scientific and Industrial Research, Chittagong, Bangladesh) the Swiss Albino mice weighing between 25 to 30 g and matured for 7–8 weeks have been obtained to implement the research. Purified water were facilitated to the mice and the mice were also accommodated with adequate ventilation in the rooms over a normal day/night period. 10 days before the experimentation the animals had been accustomed to lab conditions. Animal handling and management were carried out under universal standards for the use and management of laboratory animals [25].

2.5. Preparatory phytochemical screening

The MECD extract was investigated for the presence or absence of flavonoids, alkaloids, terpenoids, carbohydrate, saponins, tannins, glycosides, phlobotannins, protein, phenolic, and steroid by following the method previously established by Sofowara et al.; [26].

2.6. Acute oral toxicity test

The research was carried out under standardized laboratory conditions led by the Environmental Control Development [27]. MECD 1000, 2000, and 3000 mg/kg were given to the group of mice where each group consists of 10 mice. Besides, only vehicle (water) was provided to the control group. The groups were tracked for 48 h. The animals were weighed every day and changes in their normal actions has been observed.

2.7. Antidiarrheal activity

2.7.1. Castor oil induced diarrhea

The related method by Shoba et al. [28]; was followed with slight modification to perform this study. The mice were screened initially by giving 0.5 mL of castor oil and only those indicating diarrhea were chosen for the examination. The test animals affixed overnight with free access to water were randomly allocated to six groups comprising of six mice in each group. The animals of group I (control) received vehicles only (distilled water containing 1% Tween-80). Group-II (positive control/standard drug) received a standard anti-motility drug named loperamide (3 mg/kg body weight) as an oral suspension. Group III, group IV, group V and group VI (test groups) were treated with a suspension of MECD at the oral dose of 50, 100, 200 and 400 (mg/kg body weight) respectively. One hour after administration of test samples, all mice received 0.5 mL of castor oil and afterward, they were independently placed in enclosure's floor of which was fixed with transparent paper. During the observational period, the onset of diarrhea, number and weight of wet stools, the total number and the total weight of fecal yields were recorded. Finally, the percent of diarrheal inhibition (% inhibition of defecation) was determined by using the formula described below.

$$\%\ \text{inhibition of defecation} = \frac{\text{Mean defecation of control}}{\text{Mean defecation of test sample or standard drug}} \times 100$$

2.7.2. Castor oil induced enteropooling

Intraluminal liquid accumulation technique was previously performed by Rudra et al. [29,30]; has been followed. 18 h fasted mice were isolated into six groups of six animals in each group. The animals of group I (control) received vehicles only (distilled water containing 1% Tween-80). Group II (positive control/standard) received standard antimotility drug loperamide (3 mg/kg body weight, p.o) as an oral suspension. The group III, group IV, group V and group VI (test group) were treated with a suspension of MECD at the oral dose of 50, 100, 200 and 400 mg/kg of body weight, respectively. At that point 1 h later, 0.5 mL of castor oil was administered orally to these animals to produce diarrhea. After 2 h, the mice were sacrificed by an overdose of chloroform anesthesia, and the small intestinal tract was ligated both at the pyloric sphincter and at the ileocecal junctions and deserted out. The small intestinal tract was weighed and the intestinal substances were collected by draining into a graduated tube and the volume was estimated. The intestines were reweighed and the fluctuations among full and void intestinal tracts were determined and the findings were contrasted to the average effect of the vehicle. Finally, the percent of intestinal secretion and weight of intestinal substances were determined by using the accompanying equations.

$$\%\ \text{of inhibition by using MVSIC} = \frac{(\text{MVICC} - \text{MVICT})}{\text{MVICC}} \times 100$$

Where, MVSIC is the mean volume of the small intestinal content, MVICC is the mean volume of the intestinal content of the control group and MVICT is the mean volume of the intestinal content of the test groups.

$$\%\ \text{of inhibition by using MWSIC} = \frac{\text{MWICC} - \text{MWICT}}{\text{MWICC}} \times 100$$

Where MWSIC is the mean weight of the small intestinal content, MWICC is the mean weight of the intestinal content of the control group and MWICT is the mean weight of the intestinal content of the test groups.

2.7.3. Gastrointestinal motility test

This assessment was carried out by following the method previously performed by Rudra et al.; [30]. Mice were fasted for 18 h and isolated into six groups comprising of six mice in each. Castor oil was fed orally to these mice to initiate diarrhea. 1-hour later group I received vehicles (distilled water containing 1 % tween 80 orally), group II received standard drug (loperamide 3 mg/kg body weight, p.o) and group III, group IV, group V and group VI (test groups) were treated with a suspension of MECD at the oral dose of 50, 100, 200 and 400 mg/kg body weight, respectively. 1 h after oral administration of MECD, animals received 1 mL of the charcoal meal (10% charcoal suspension in 5% gum acacia) orally. 1 h later, the animals were sacrificed by an overdose of chloroform anesthesia and the distance moved by the charcoal meal from the pylorus to caecum was estimated and the total intestinal transit by charcoal meal was designated. The peristaltic index and percentage of inhibition were estimated by using the executed formula.

$$\text{Peristalsis index} = \frac{\text{Distance travelled by charcoal meal}}{\text{Length of small intestine}} \times 100$$

$$\%\ \text{inhibition} = \frac{(\text{MDc} - \text{MDt})}{\text{MDc}} \times 100$$

Where, MDc = Mean distance traveled by the control, MDt = Mean distance traveled by the test group.

2.8. Antibacterial assay

2.8.1. Disc diffusion antibacterial studies

The antibacterial examination was carried out by disc diffusion protocol which was previously described by Alam et al.; [31]. The disc diffusion process is profoundly effective for the rapid growth of microorganisms. The microorganisms were collected as pure cultures from the International Islamic University Chittagong, Chittagong-4000, Bangladesh). A definite quantity of the test sample was dissolved in a different solvent to obtain a particular concentration (μg/mL). At that point, the sterile filter paper discs having 5 mm in diameter were impregnated with known amounts of the test substances and dried. Such discs and standard antibiotic discs were set on the plate containing an appropriate medium (nutrient agar) seeded with the test organisms. These plates were kept at decreased temperature (4 °C) for 24 h to permit maximum diffusion. The test materials diffuse from the plate to the surrounding medium as per the physical law that controls the diffusion of molecules through agar media. The plates were then kept in an incubator (37 °C) for 18–24 h to allow the growth of organisms. If the test materials have antibacterial action, it will hinder the growth of microorganisms having a clear distinct zone, narrated "Zone of Inhibition". The antibacterial action of the test sample is determined by estimating the diameter of the zone of inhibition in terms of mm. Amoxicillin 30 (mg/disc) was used as a reference standard. Each experiment was repeated three times.

2.8.2. Minimum inhibitory concentration (MIC)

Serial tube dilution technique mentioned by Reiner et al. [32]; was followed using nutrient broth medium to determine the minimum inhibitory concentration (MIC) values of methanol extract of *C. digyna* and antibiotics against the four pathogenic bacteria and three pathogenic fungi. The stock solution was prepared by dissolving 1.024 mg of antibiotics in 2 mL of methanol. Thus solution with a concentration of 512 (μg/mL) was obtained. The standard antibiotic amoxicillin trihydrate solution was made by the same procedure as the concentration was 512 (μg/mL). Thirteen autoclaved test tubes were taken, nine of which were labeled as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and the remaining three were allocated as C_m (medium), C_s (medium + compound) and C_i (Medium + inoculum). Each of 12 test tubes was supplemented with 1 mL of sterile nutrient broth medium or potato dextrose broth medium. In the 1st test tube, 1 mL of the sample solution was added and mixed well. 1 mL content from the 1st test tube was transferred to the 2nd test tube by the sterile pipette and mixed uniformly. Then 1 mL of this mixture was transferred to the 3rd test tube. This process of serial dilution was preceded until the 9th test tube. 1 drop (10 μL) of properly diluted inoculum was added to each of the 9 test tubes and mixed well. For the control test tube, C_s 1 mL of the sample solution was added, mixed well and 1 mL of this mixed content was discarded. This was to verify the medium's clarity in the presence of a diluted solution of the compound. 10 μL of the inoculum was added to the control test tube, C_i to monitor the growth of the organism in the medium used. The control test tube, C_m containing medium only was used to verify the medium's sterility. The control test tubes were incubated for 18–24 h at 37.5 °C.

2.9. Antifungal activity

Antifungal sensitivity test of the MECD was also performed by using the disc diffusion assay mentioned by Espinel-Ingroff A et al. [33]; and the whole testing procedure was carried out as the antibacterial activity test. The only difference was that the period of incubation was 72 h at 25 °C temperature. Potato dextrose agar (PDA) medium was used to perform the antifungal activity and for the subculture of the test organisms. The antifungal activity of the MECD was determined by converting the zone of inhibition (mm) of the 4 pathogens to percent zone of inhibition and subsequent counting total zone of inhibition (TZI) caused by the test groups in comparison to the standard drug Fluconazole.

2.10. In silico studies

2.10.1. Molecular docking analysis: ligand preparation

The structure of three major compounds isolated from *C. digyna* namely Isointricatinol, Isobonducellin, and Z-eucomine were drawn by ChemDraw. The ligands structures were plotted three-dimensionally (3D) by utilizing the LigPrep in Maestro version 11.1 (Schrödinger suite, LLC New York, NY, USA) with the force field OPLS_2005 and pH 7.0 ± 2.0 for ionization state generation that used Epik 2.2 [34].

2.10.2. Molecular docking analysis: protein preparation

3D structures of the protein used for the experiments which including, human kappa opioid receptor (PDB ID: 4DJH) [35], kappa opioid receptor (PDB ID: 6VI4) [36], G protein-coupled receptor (PDB ID: 4XT3) [37], human delta-opioid receptor (PDB ID: 4RWD) [38], (human delta-opioid receptor (PDB ID: 6U1N) [38] for antidiarrheal study and *E.coli* FolC in complex (PDB ID: 1W7K) [39], Aromatic Prenyl Transferase (PDB ID: 1ZB6) [40], Isomerase domain of human glucose (PDB ID: 2ZJ4) [41], RecA mini intein-Zeise's salt complex (PDB ID: 5K08), *E. coli* glucosamine-6-P synthase (PDB ID: 2J6H) [42], Cytochrome P450 14 alpha-sterol demethylase (PDB ID: 1EA1) [43], *Candida Albicans* N-myristoyltransferase (PDB ID: 1IYL) [44], *Candida albicans* dihydrofolate reductase (PDB ID: 4HOE) [45], fungal lanosterol 14α-demethylase (PDB ID: 5HS1) [46], Picolinamide and Benzamide Chemotypes (PDB ID: 6F0E) [47] for both antibacterial and antifungal study were collected from Protein Data Bank in PDB format. Thereupon, the structures were prepared and refined by applying protein preparation wizard (Schrödinger-Maestro version 11.1). Charges and bond orders were assigned, hydrogens were attached to the heavy atoms, selenomethionines were altered to methionine, and waters were eliminated. Using force field OPLS_2005, minimization was performed to set a maximum heavy atom RMSD to 0.30 Å.

2.10.3. Molecular docking analysis: glide standard precession

Using Glide embedded in Schrödinger-Maestro version 11.1, generation of grid and docking test were accomplished [48,49]. In Glide, grids were produced according to the default parameters: van der Waals scaling factor 1.00 and charge cutoff 0.25, designed to the OPLS_2005 force field. A cubic box of particular dimensions centered on the centroid of the active site residues was obtained for the receptor, and the size of the box was set to 14 Å × 14 Å × 14 Å for docking. Molecular docking studies were evaluated by recording the best-docked pose for each ligand with the lowest glide score.

2.11. Pharmacokinetic (ADME) and toxicological properties prediction

Here for determining the pharmacokinetic properties (ADME) of three major compounds, the online tool SwissADME (http://www.swissadme.ch/) was used [50]. Lipinski's rule of five (M.W not more than 500; H-bond donors ≤ 5; H-bond acceptors ≤ 10; Lipophilicity < 5 and molar refractivity ranging from 40 to 130) were considered to evaluate favorable drug-like properties of all compounds [51]. Moreover, the toxicological properties of all the compounds were determined by the web tool admetSAR (http://lmmd.ecust.edu.cn/admetsar2).

3. Statistical analysis

Statistical data interpretation has been presented as mean ± SEM (standard error mean). The data were tested through Graph Pad Prism version 5.0. A one way analysis of variance (ANOVA) has been carried out by Dunnett's test.The P value less than 0.05, 0.01 and 0.001 have been considered statistically significant.

4. Results

4.1. Phytochemical screening

The qualitative phytochemical screening was performed to ensure the presence or absence of secondary plant metabolites. Phytochemical screening results of MECD extract showed in Table 1. From the tabular presentation, it should be mentioned that alkaloids, flavonoids, saponins, and tannins are highly present; carbohydrate, protein, and phenolic compounds are moderately present; glycosides are mildly present and terpenoids are absent in the MECD stems.

Table 1
Phytochemical screening of methanol extract of *Caesalpinia digyna* (Rottl.) stems.

Test group	Name of the Test	Observation
Alkaloids	Meyer's Test	+
	Wagner's Test	+
Carbohydrate	Molisch's Test	+
	Benedict's Test	–
	Fehling Test	+
Flavonoids	Hydrochloric Acid Test	+
Saponins	Foam Test	+
Tannin	Lead acetate Test	+
Glycoside	Acetic Acid test	+
Protein	Nitric Acid Test	+
Phenolic Compounds	Ferric Chloride Test	+
Terpenoids	Salkowski Test	–

(+) = Present; (−) = Absent.

Table 2
Effect of MECD on castor oil induced diarrhea in mice.

Treatment	Dose, route (p.o)	Onset of diarrhea (min)	Average number of wet feces	Average number of total feces	Average weight of wet feces (g)	Average weight of total feces (g)
Group-I	1% tween 80-10 mL/kg	77.16 ± 1.83	11.17 ± 0.79	13.67 ± 0.80	0.37 ± 0.02	0.42 ± 0.02
Group-II	Loperamide-3mg/kg	193.16 ± 8.91***	1.16 ± 0.16***	2.16 ± 0.30***	0.04 ± 0.00***9	0.07 ± 0.005***
Group-III	MECD-50 mg/kg	84.33 ± 2.87*	7.33 ± 0.33***	10.33 ± 0.61	0.28 ± 0.02*	0.38 ± 0.02
Group-IV	MECD-100 mg/kg	105 ± 5.47***	5.5 ± 0.42***	9 ± 0.51***	0.22 ± 0.02**	0.31 ± 0.02**
Group-V	MECD-200 mg/kg	139.33 ± 5.93***	3.16 ± 0.30***	6 ± 0.36***	0.11 ± 0.01***	0.18 ± 0.01***
Group-VI	MECD-400 mg/kg	195.67 ± 8.46***	1.33 ± 0.21***	2.5 ± 0.22***	0.04 ± 0.007***	0.11 ± 0.01***

The given data were presented as mean ± SEM (n = 6); One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ were considered significant compared to the control sample. MECD = Methanol extract of *C. digyna*.

4.2. Acute oral toxicity test

No signs of damage or any physical changed were found during the observation period.

4.3. Antidiarrheal activity

4.3.1. Castor oil induced diarrhea

After administration of MECD (50, 100, 200 and 400 mg/kg), a significant ($p < 0.05, p < 0.01, p < 0.001$) dose-dependent delay in the onset of diarrhea produced upon it was compared with the control. Similarly, there was a reduction in the number of wet feces, total number of feces, weight of wet feces and total weight of all feces. The summary of castor oil-induced diarrhea has been shown in Table 2. However, the data revealed that the percentage of diarrhea inhibitions was 22.31%, 38.79%, 58.15% and 80.64% at doses of 50,100, 200 and 400 mg/kg subsequently.

4.3.2. Castor oil induced enteropooling

With the treatment of MECD, the volume and weight of intestinal substance were significantly ($p < 0.05, p < 0.01, p < 0.001$) decreased in a dose-dependent manner as contrasted individually with control as appeared in Table 3. The most elevated impact on both volume and weight of intestinal substance was accomplished at dose 400 mg/kg of body weight. The percent of inhibition was increased with the elevation of the dose concentration of MECD. The test samples were compared to control (1% Tween-80) and findings were contrasted with standard drug loperamide (3 mg/kg). The mean weight of small intestine content (MWSIC) and mean volume of small intestine content (MVSIC) were calculated for each test sample. The percent inhibition of MECDs were found to be 11.66%, 36.67%, 55.00%, 71.66% in MWSIC 50, 100, 200, 400 mg/kg respectively and the percent inhibition of MVSIC were found to be 8.33%, 37.50%, 54.16%, 64.58% for MECD 50, 100, 200, and 400 mg/kg respectively. On the contrary, the percent of inhibition rate of loperamide (3 mg/kg) were showed 71.66% in MWSIC and 72.91% in MVSIC respectively. Different parameter of castor oil-induced enteropooling has been listed in Table 3.

Table 3
Impact of the methanol extract of *C. digyna* stems on castor oil incited intraluminal fluid accumulation in mice.

Treatment	Dose, route (p.o)	MWSIC (g)	% of inhibition	MVSIC (mL)	% of inhibition
Group-I	1% tween 80-10 mL/kg	0.60 ± 0.02	–	0.48 ± 0.02	–
Group-II	Loperamide-3 mg/kg	0.17 ± 0.09***	71.66	0.13 ± 0.01***	72.91
Group-III	MECD-50 mg/kg	0.53 ± 0.01*	11.66	0.44 ± 0.02	8.33
Group-IV	MECD-100 mg/kg	0.38 ± 0.02***	36.67	0.30 ± 0.02**	37.50
Group-V	MECD-200 mg/kg	0.27 ± 0.01***	55.00	0.22 ± 0.01***	54.16
Group-VI	MECD-400 mg/kg	0.17 ± 0.02***	71.66	0.17 ± 0.01***	64.58

The values are expressed as mean ± SEM (n = 6); One-way analysis of variance (ANOVA) suggested by Dunnett's test. Significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) compared with the control sample. MECD = methanol extract of *C. digyna*. MWSIC = mean weight of the small intestinal content, MVSIC = mean volume of the small intestinal content.

4.3.3. Charcoal induced intestinal transit in mice

In primary treatment, MECD repressed the intestinal travel of charcoal meal at all the treated dosages. The investigation revealed that the rate of gastrointestinal travel of charcoal was decreased with the elevation of test doses respectively. The data revealed that the percentage reduction of gastrointestinal transit of charcoal was 12.21 % ($p < 0.05$), 34.77 % ($p < 0.01$) 49.40 % ($p < 0.001$) and 76.10% ($p < 0.001$)), at doses of 50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively. The maximum dose of this extract showed comparable anti-motility effects to that of the standard drug (78.83%, $p < 0.05$). All the revealed data were calculated with the comparison of control (1% Tween-80). Besides the percent of inhibition of MECD, the total length of the small intestine, distance moved by the charcoal in the small intestine and peristaltic index (%) were also calculated. All the findings have been listed in Table 4.

4.3.4. Antibacterial screening

The methanol extract of *C. digyna* stems yielded very potent activities against both gram-positive and gram-negative bacteria. Evaluation of antibacterial activity was illustrated in Table 5. With the elevation of several concentration, MECD showed sustainable action against almost all the treated bacteria (except *Pseudomonas aeruginosa*). From the

Table 4
Impact of the stem's extracts of *C. digyna* on intestinal transit in mice.

Treatment	Dose, route (p.o)	Total intestinal length (cm)	Distance moved by the charcoal meal (cm)	Peristaltic index (%)	% of inhibition
Group-I	1% tween 80-10 mL/kg	53.42 ± 2.02	38.87 ± 1.80	72.76	–
Group-II	Loperamide-3 mg/kg	49.67 ± 0.67	5.51 ± 0.77***	11.09	84.75
Group-III	MECD-50 mg/kg	50.00 ± 1.83	32.47 ± 1.50*	63.87	12.21
Group-IV	MECD-100 mg\kg	50.00 ± 1.77	23.93 ± 1.03**	47.46	34.77
Group-V	MECD-200 mg/kg	51.67 ± 1.40	13.02 ± 1.27***	36.81	49.40
Group-VI	MECD-400 mg/kg	50.17 ± 1.49	6.73 ± 0.64***	13.14	76.10

The narrated data were presented as Mean ± SEM (n = 6); One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ were considered significant compared to the control sample. MECD = methanol extract of *C. digyna*.

Table 5
The *in vitro* antibacterial activities of MECD and standard drug (amoxicillin) discs.

Test organisms	Diameter of the zone of inhibition (mm)			
	MECD-100 μg/disc	MECD-300 μg/disc	MECD-500 μg/disc	Amoxicillin-30 μg/disc
Staphylococcus aureus	15.67 ± 0.33	18.33 ± 0.34	23.34 ± 0.81	33.60 ± 0.81
Lactobacilllus casei	15.33 ± 0.88	18.67 ± 0.33	22.67 ± 0.67	36.61 ± 0.61
Bacillus azotoformans	13.67 ± 0.89	20.33 ± 0.33	26.33 ± 0.88	34.33 ± 0.87
Corynebacterium species	16.66 ± 67	19.00 ± 1.15	21.61 ± 0.34	36.67 ± 0.81
Bacillus cereus	20.33 ± 0.33	23.33 ± 0.81	20.68 ± 0.30	33.67 ± 0.61
Salmonella typhi	16.67 ± 0.67	21.66 ± 0.88	25.66 ± 1.20	32.66 ± 0.66
Escherichia coli	15.00 ± 0.57	19.67 ± 1.20	22.66 ± 0.34	34.67 ± 1.20
Pseudomonas aeruginosa	–	–	–	35.00 ± 0.57

Table 6
Minimum inhibitory concentration (MIC) of standard amoxicillin tri-hydrate against tested pathogenic microorganisms.

Test tube no.	Nutrient broth medium added (mL)	Diluted solution of Standard Amoxicillin tri-hydrate (μg/mL)	Inoculum added (μL)	Growth observation against			
				a	b	c	d
1	1	512	10	–	–	–	–
2	1	256	10	–	–	–	–
3	1	128	10	–	–	–	–
4	1	64	10	–	–	–	–
5	1	32	10	–	–	–	–
6	1	16	10	–	–	–	–
7	1	8	10	–	–	–	–
8	1	4	10	–	+	–	–
9	1	2	10	–	+	+	–
10	1	1	10	+	+	+	+
C_m	1	0	0	–	–	–	–
C_s	1	512	0	–	–	–	–
C_i	1	0	10	+	+	+	+
Results of MIC values (μg/mL)				2	8	4	2

(+) = growth, (−) = no growth, a-*Staphylococcus aureus*, b-*Lactobacillus casei*, c-*Bacillus azotoformans*, d-*Corynebacterium species*.

tabular analysis of MECD against eight pathogenic bacteria, it can be suggested that *Pseudomonas aeruginosa* strain is resistant to MECD extract and *Staphylococcus aureus, Lactobacilllus casei, Bacillus azotoformans, Corynebacterium species, Bacillus cereus, Salmonella typhi, Escherichia coli, strains* are potentially susceptible to MECD. *Bacillus azotoformans, Staphylococcus aureus* and *Salmonella typhi* elevated better sensitivity to MECD extracts than others. Maximum zone of inhibition (26.33 ± 0.88 mm) was attained by the MECD extract at 500 (μg/disc) concentration against *Bacillus azotoformans* among all the gram-positive bacteria and in opposition to *Bacillus azotoformans* and as well as all the gram-negative bacteria, maximum zone of inhibition (25.66 ± 1.20 mm) was attained by *Salmonella typhi* at 500 (μg/disc).

4.3.5. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of amoxicillin trihydrate and MECD has been presented in Table 6 and Table 7.

4.4. Antifungal screening

The MECD extract yielded significant susceptibility against almost all the fungi. From the investigation, it can be narrated that, *Blastomyces dermatitidis* strain has remarkable susceptibility to MECD extract. The maximum zone of inhibition for *Blastomyces dermatitidis* was revealed 21.34 ± 0.89 mm in diameter at MECD 500 μg/disc and the minimum zone of inhibition was revealed 10.67 ± 0.67 mm for *Trichoderma* spp. at MECD 100 μg/disc. The maximum and minimum zone of inhibition for *Candida albicans* have been measured 20.66 ± 0.67 and 12.67 ± 0.61 (mm) at MECD 500 and 100 (μg/disc) respectively where flucloxacillin 20 μg/disc has inhibited 32.66 ± 0.66 mm for the same strain. The Appraisal of antifungal susceptibility was illustrated in Table 8.

Table 8
In vitro antifungal activities of MECD stems and standard drug (flucloxacillin) discs.

Test organisms	Diameter of zone of inhibition (mm)			
	MECD - 100 μg/disc	MECD - 300 μg/disc	MECD - 500 μg/disc	Flucoxacillin - 20 μg/disc
Candida albicans	12.67 ± 0.61	15.33 ± 0.34	20.66 ± 0.67	32.66 ± 0.66
Cryotooccus neoformans	–	–	–	30.33 ± 0.87
Blastomyces dermatitidis	12.34 ± 0.88	15.67 ± 0.67	21.34 ± 0.89	35.61 ± 0.33
Trichoderma spp	10.67 ± 0.67	13.66 ± 1.20	16.67 ± 066	34.67 ± 0.33

Table 7
Minimum inhibitory concentration (MIC) of MECD against tested pathogenic microorganisms.

Test tube no	Nutrient broth medium added (mL)	Diluted solution of MECD extract (μg/mL)	Inoculum added (μL)	Growth observation against						
				a	b	c	d	e	f	g
1	1	512	10	–	–	–	–	–	–	–
2	1	256	10	–	–	–	–	–	–	–
3	1	128	10	–	–	–	–	–	–	–
4	1	64	10	–	–	–	–	–	–	–
5	1	32	10	–	–	+	–	+	–	–
6	1	16	10	–	+	+	–	–	–	+
7	1	8	10	+	+	+	+	+	+	+
8	1	4	10	+	+	+	+	+	+	+
9	1	2	10	+	+	+	+	+	+	+
10	1	1	10	+	+	+	+	+	+	+
C_m	1	0	0	–	–	–	–	–	–	–
C_s	1	512	0	–	–	–	–	–	–	–
C_i	1	0	10	+	+	+	+	+	+	+
Results of MIC values (μg/mL)				16	64	32	16	64	16	32

MECD = methanol extract of *Caesalpinia digyna.* a-*Staphylococcus aureus*, b-*Lactobacillus casei*, c-*Bacillus azotoformans*, d-*Corynebacterium species*, e-*Bacillus cereus*, f-*Salmonella typhi*, g-*Escherichia coli*, Cm (medium), Cs (medium + extract) and Ci (Medium + inoculum).

Table 9
Antidiarrheal docking scores of the selected compounds of *C. digyna*.

Docking Score					
Compounds	4DJH	6VI4	4XT3	4RWD	6U1N
Loperamide	−6.56	−6.23	−5.10	−5.14	−7.55
Isointricatinol	−7.29	−5.20	−5.67	−5.30	−7.83
Isobonducellin	−5.60	−5.50	−5.02	−5.41	−7.59
Z-eucomine	−6.09	−6.33	−5.15	−5.88	−8.70

4.5. In silico analysis

4.5.1. Molecular docking analysis for antidiarrheal study

For antidiarrheal activity, the docking analysis results are presented in Table 9 and Fig. 1. In this investigation, three receptors namely kappa opioid receptors (PDB ID: 4DJH, 6VI4), delta-opioid receptors (PDB ID: 4RWD, 6U1N) and G-protein coupled receptor (PDB ID: 4XT3) were used to analyze the possible antidiarrheal activity of MECD. In case of kappa opioid receptor (PDB ID: 4DJH), Isointricatinol exhibited the highest docking score (−7.29 kcal/mol) better than the reference drug loperamide (−6.56 kcal/mol) and Isobonducellin showed the lowermost docking score (−5.60 kcal/mol). The ranking order of the docking score is: Isointricatinol› Z-eucomine› Isobonducellin. On the contrary as compared to the reference drug, for delta-opioid receptor (PDB ID: 6U1N) Z-eucomine showed the maximum docking score (−8.70 kcal/mol) followed by Isointricatinol (−7.83 kcal/mol) and Isobonducellin (−7.59 kcal/mol).

4.5.2. Molecular docking analysis for antimicrobial study

For antimicrobial activity, the docking analysis results are presented in Table 10 and Fig. 1. Here, three major compounds were docked with ten receptors (PDB ID: 1W7K, 1ZB6, 2ZJ4, 5K08, 2J6H, 1EA1, 1IYL, 4HOE, 5HS1, 6F0E) to assess the possible antimicrobial activity. Among all the receptors, Z-eucomine showed the highest binding affinity (−9.859 kcal/mol) against the receptor 1IYL, on the other hand, Isointricatinol presents the lowest binding affinity (−7.159 kcal/mol). The ranking order of docking score is: Z-eucomine › Isobonducellin › Isointricatinol. Furthermore, Isobonducellin showed the highest binding affinity (−9.424 kcal/mol) against the receptor 1W7K whereas Isointricatinol exhibited lower binding affinity (−6.787 kcal/mol). The ranking order of the docking score is: Isobonducellin › Z-eucomine › Isointricatinol.

4.6. Pharmacokinetic and toxicological (ADME/T) properties prediction

The properties of ADME (absorption, distribution, metabolism, excretion) of the isointricatinol, isobonducellin, and Z-eucomine have been displayed in Table 11. The chosen properties are remarkable to dominance cell permeation, metabolism and bioavailability. Here, in consonance with Lipinski's rule of five, predicted properties of all three compounds met within the range, which is considered to predict as drug-like potential. Moreover, in case of toxicological properties of these selected compounds, the study exhibited that these compounds were non-carcinogenic and non-Ames toxicities.

5. Discussion

The possible local, national, regional, or transnational extinction of an estimated 4000 to 10,000 species of medicinal plants are expected to have significant implications for living standards, economies, and healthcare [52]. The plant produced antidiarrheal drugs available on the market are included Seirogan [53], tormentil root [54], Zangrado [55], Tong-xie-ning [56], and Kampo [57]. In addition, as herbal sources, *Croton lechleri* [6], *Camellia sinensis*, [7]. *Mentha piperita* [8], and *Psidium guajava* [9] are frequently used for the treatment of diarrhea [58]. The intrinsic antidiarrheal and antimicrobial potentials of these medicinal plants were evaluated by a particular choice of medicinal plant (employed ethnopharmacological survey) [59]. A realistic approach with specific animal behavioral methods is necessary to produce a top-class herbal antidiarrheal product. Thoughincredible animal models are available for pharmacological study, each model has its benefits and disadvantages [60]. Syndrome-based methods, novel animal experiments, and well-established research can thus lead to a credible preclinical and clinical conclusion that are either suitable or equivalent to the treatment of pathologic diarrhea and microbial disease [61]. The lack of effective and safe medication and the rising resistance of pathogens to available antibiotics or anti-parasitic agents are more challenging issues for developing countries. Infectious diarrhea, enteric bacterial pathogens, and parasites are a typical form of these diseases [62].

The present examination is to investigate the presence of antidiarrheal and antimicrobial potentiality of *C. digyna*. Laxity of the guts can be deposited as the consequent defecation of feces at low consistency which might be occurred because of impairment in the vehicle of water and electrolytes in the intestinal tract. Diarrhea results from the quick development of fecal stuff through the large intestine [63]. To introduce diarrhea, castor oil was used in all methods because castor oil induces diarrhea as a result of its active metabolite, ricinoleic acid which is liberated by the action of lipases in the upper part of the small intestine [64]. The activity of castor oil is originated by ricinoleic acid, a hydroxylated fatty acid exempt from castor oil by intestinal lipases. Despite the wide-spread application of castor oil in traditional and folk medicine, the molecular mechanism by which ricinoleic acid acts still remain unknown. Prostaglandin E2 receptor 3 (EP3) receptor is specifically activated by ricinoleic acid and that it mediates the pharmacological effects of castor oil. In mice, due to lack of EP3 receptors, the laxative effect and the uterus contraction-induced upon ricinoleic acid are absent. Although a conditional deletion of the EP3 receptor gene in intestinal epithelial cells did not affect castor oil-induced diarrhea, mice lacking EP3 receptors only in smooth-muscle cells were unresponsive to this drug. Thus, the castor oil metabolite ricinoleic acid activates intestinal and uterine smooth-muscle cells via EP3 prostanoid receptors. These findings identify the cellular and molecular mechanism underlying the pharmacological effects of castor oil and indicate the role of the EP3 receptor as a target to induce laxative effects [65]. Castor oil-induced diarrhea has been possessed to evaluate the onset of diarrhea, the number of wet feces, and the frequency of feces. In our investigation, MECD significantly delayed the fecal time, retarded the weight of the wet feces, and reduced the frequency of wet feces upon castor oil-induced diarrhea was produced in Swiss albino mice. In the castor oil-induced diarrhea the percent of inhibition rate of feces was mostly found in 200 mg/kg and 400 mg/kg and shows dose-dependant potentiality upon contrast with the control. The effect of MECD 400 mg/kg is likely to the standard drug loperamide (3 mg/kg).As a corollary, the retardation of onset of diarrhea, weight of wet feces and frequency of diarrhea by administering MECD indicates the existence of antidiarrheal potentiality in castor oil induced diarrheal model.

Enteropooling model was aimed to assess the secretary components of diarrhea. In this model, the MECD extract showed significant retardation in both MWSIC and MVSIC at all tested doses as compared to control. In the present study, it has been characterized that castor oil is liable to the diarrheal activity because of containing nitric oxide in it. This diarrheal effectiveness includes reduction of general liquid misappropriation by obstruction of intestinal Na^+, K^+ ATPase activity enactment of adenylate cyclase or mucosal cAMP intervened dynamic secretion [66]. It is supposed that the active metabolite, ricinoleic acid might activate the nitric oxide pathway and incited nitric oxide (NO) dependent gut secretion [67]. MECD significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$) lessened the secretory effect which was propagated by nitric oxide as well as ricinoleic acid. Therefore, It can be presumed that the presence of flavonoids implicated in attenuation of NO synthesis [68] and MECD contains these types of substances which presume to acts

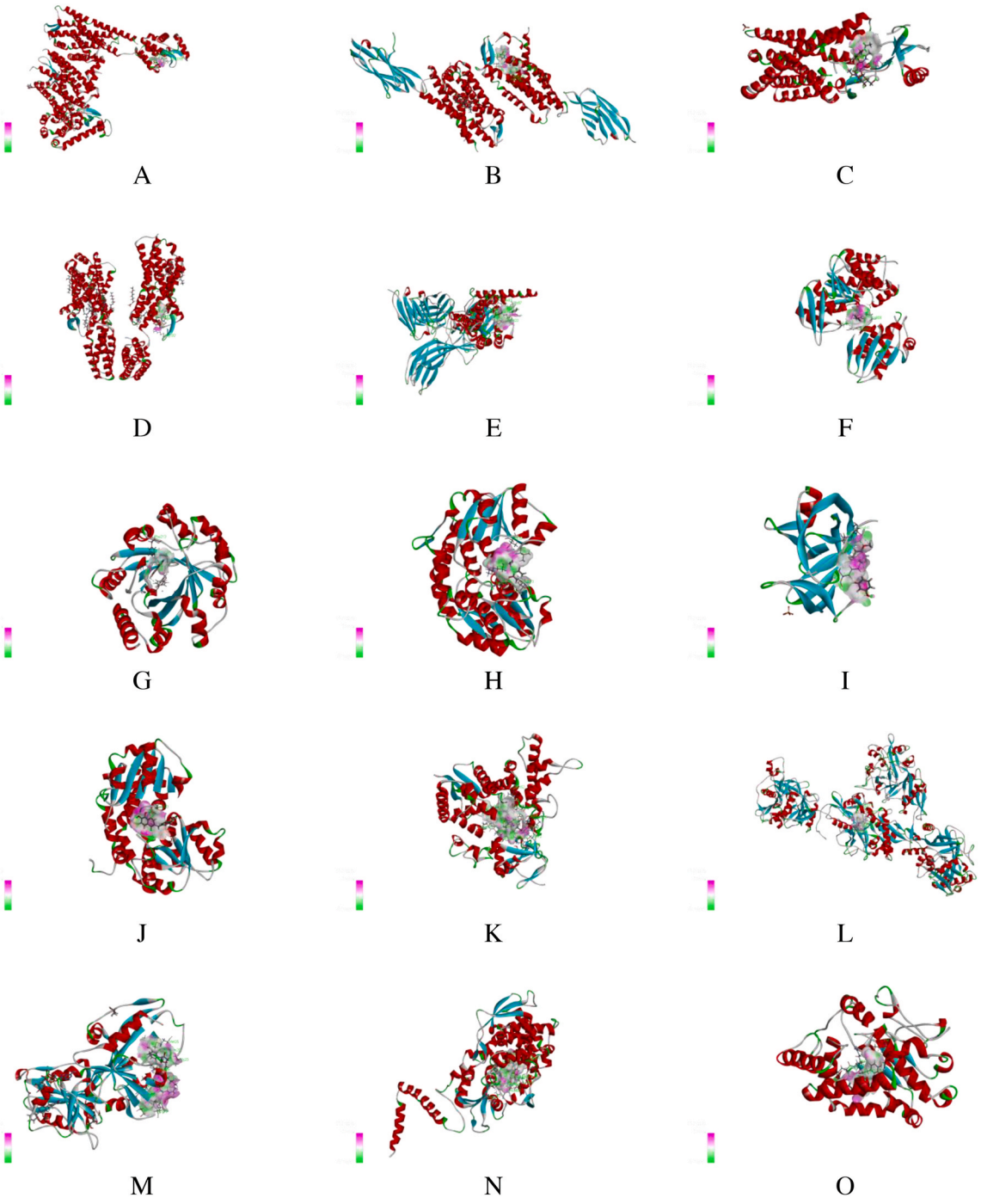

Fig. 1. 3D structure of the best ligand and receptor interaction. A = Isointricatinol with kappa opioid receptor (PDB ID: 4DJH), B = Z-eucomine with kappa opioid receptor (PDB ID: 6VI4), C = Isointricatinol with G-protein coupled receptor (PDB ID: 4XT3), D = Z-eucomine with the delta-opioid receptor (PDB ID: 4RWD), E = Z-eucomine with the delta-opioid receptor (PDB ID: PDB ID: 6U1N), F = Isobonducellin with the E.coli FolC in complex (PDB ID: 1W7K), G = Isobonducellin with the Aromatic Prenyl Transferase (PDB ID: 1ZB6), H = Z-eucomine with the Isomerase domain of human glucose (PDB ID: 2ZJ4), I = Isointricatinol with the RecA mini intein-Zeise's salt complex (PDB ID: 5K08), J = Isointricatinol with E. coli glucosamine-6-P synthase (PDB ID: 2J6H), K = Isobonducellin with the Cytochrome P450 14 alpha-sterol demethylase (PDB ID: 1EA1), L = Z-eucomine with the Candida Albicans N-myristoyltransferase (PDB ID: 1IYL), M = Isointricatinol with the Candida albicans dihydrofolate reductase (PDB ID: 4HOE), N = Isobonducellin with the fungal lanosterol 14α-demethylase (PDB ID: 5HS1), O = Isointricatinol with the Picolinamide and Benzamide Chemotypes (PDB ID: 6F0E).

against nitrous oxide implicated defecation secretion. Regarding the statement of Siqueira, C.F.d.Q., et al.; [69], it can be narrated that the anti-secretory effects of MECD presumably observed because of having tannin and flavonoids.

The charcoal induced intestinal transit in mice implicated that the mean separation gone by the charcoal plug was greatest for the control. During the analysis, the charcoal meal method was chosen to pursue the displacement of the gastrointestinal content due to the fact that the retardation of gastrointestinal motility is one mechanism by which numerous antidiarrheal agents can act [70]. Activated charcoal, employed in the gastrointestinal transit model as a marker, has been followed for more than 60 years as a basic tool for assessing the impact

Table 10
Antibacterial and antifungal docking scores of the selected compounds of *C. digyna*.

Docking Score										
Antibacterial						Antifungal				
Compounds	1W7K	1ZB6	2ZJ4	5K08	2J6H	1EA1	1IYL	4HOE	5HS1	6F0E
Amoxicillin and Fluconazole	−7.77	−8.16	−6.14	−4.80	−7.50	−7.77	−8.27	−7.62	−5.93	−6.12
Isointricatinol	−6.78	−8.18	−5.07	−5.08	−6.36	−7.82	−7.15	−8.07	−5.92	−7.63
Isobonducellin	−9.42	−8.70	−4.11	−3.85	−7.64	−8.57	−8.45	−8.00	−6.40	−6.09
Z-eucomine	−7.25	−8.10	−6.24	−4.48	−7.54	−7.51	−9.85	−7.41	−6.39	−7.30

Table 11
Pharmacokinetic and toxicological properties of the compounds of *C. digyna* for good oral bioavailability.

Compounds	M.W(g/mol)	HBA	HBD	Log P	Ames Toxicity	Carcinogens
Isointricatinol	302.32	5	2	2.58	Non-Ames toxic	Non-carcinogenic
Isobonducellin	284.31	4	2	2.17	Non-Ames toxic	Non-carcinogenic
Z-eucomine	298.33	4	2	1.77	Non-Ames toxic	Non-carcinogenic

M.W = molecular weight, HBA = Hydrogen bond acceptor, HBD = Hydrogen bond donor, Log P = Lipophilicity.

of laxatives [71]. This strategy is a pointer of the maximum distance moved by the marker (activated charcoal) through its administration and the appraisal of its way in the small intestinal tract over a period of time [72]. In the current experiment, MECD suppressed the conduction of charcoal marker in a dose-dependent pathway. The peristaltic index and distance travel by the charcoal meal was least in the MECD 200 mg/kg and 400 mg/kg of body weight respectively upon contrasted with the control. This finding proposed that the extracts act on all parts of the intestinal tract. Retardation in the motility of gut muscles enforces the remain of substances in the intestinal tract [73]. This permits better water absorption from the gut. Medications that restrain intestinal transit in pathophysiological states are compelling in relieving diarrhea [74]. It is however presumed that the decrease in the intestinal propulsive development in the gastrointestinal transit model might be because of antispasmodic properties of the test extract [75]. Secondary metabolites, for example, flavonoids and tannins are accounted for to have antidiarrheal movement because of their capacity to restrain intestinal motility. Subsequently, the significant anti-motility impact of the extract might be identified with the synergistic inhibitory impact of flavonoids and tannins on charcoal induced gastrointestinal transit test [76,77].

The disclosure of effective antibiotics, vaccines, and different items or methods have diminished the overwhelming effect of infectious illnesses and improved personal satisfaction. However, the adequacy of numerous antibiotics is being threatened by the rise of microbial resistance from existing chemotherapeutic agents due to their aimless and inappropriate use [78]. Even though pharmaceutical companies have introduced several new antibiotics in the last years, resistance to these drugs has elevated at a high rate and has now become a global concern [24]. The infliction of some antibiotics is affiliated with adverse effects, including immune suppression, allergy, and hypersensitivity [79]. Acceptance of medicines from such plant origins as an elective type of healthcare is expanding due to their promising source of novel antibiotic models. Moreover, these ingredients may have various mechanisms of action than conventional medications and could be of clinical significance to improve healthcare [80]. The plant-determined natural product speaks to an appealing source of antimicrobial agents since they are regular and affordable, particularly for bucolic societies [81]. Our study was to assess the antimicrobial effect of MECD and from the investigation, it was noticed that MECD is highly susceptible to *Bacillus azotoformans, Staphylococcus aureus* and *Salmonella typhi* strains, less susceptible to *Bacillus cereus* and rest of the gram-positive and negative bacteria are moderately susceptible strains against MECD. As well as, MECD is susceptible to some pathogenic fungi (*Candida albicans, Cryptococcus neoformans, Blastomyces dermatitidis, Trichoderma* spp). The Diameter of zone of inhibition (mm) of *Cryptococcus neoformans* is most likely to the standard drug which was applied to conduct the antifungal test. Some of the phytochemical compounds e.g., glycoside, saponin, tannin, flavonoids, terpenoid, and alkaloids, have been reported to have antimicrobial activity [82]. From our phytochemical evaluation we have noticed the presence of alkaloid, carbohydrate, flavonoids, saponin, tannin, glycosides, potein, and phenolic compounds. Therefore, several gram positive and gram negative bacteria as well as some fungi showed susceptibility against MECD. So it can be inferred that MECD can be the source of antimicrobial agents.

Studies on molecular docking have been widely used to forecast ligand-target interactions and to gain a deeper understanding of natural products' biological activity. It also provides additional insights on potential mechanisms of action and binding mechanisms within the binding pockets of different proteins [83]. To consider the fact, a molecular docking study has been conducted to explain and validate the experimental results found in biological study. The experimental findings are confirmed in this process. Three representative molecules within the *C. digyna* were chosen for docking study in order to gain a deeper insight regarding the witness of biological activity (antidiarrheal, antibacterial, and antifungal). These compounds were then docked against fifteen targets for antidiarrheal, antibacterial, and antifungal study respectively. In antidiarrheal molecular docking study, several amino acid residues reacted by the hydrogen bonds with selected compounds resulting in docking values between - 5.02 and - 8.70 kcal/mol. From those findings, it can be inferred that, by interactions with these target proteins, the phytoconstituents are significantly responsible for the antidiarrheal activity. Besides, in the antibacterial docking study, molecular docking with the selected receptors has been accomplished and after interactions between the bioactive compounds and enzymes, the docking score has been assumed ranging from - 3.85 to - 9.42 kcal/mol. These findings indicate that the selected compounds have very high antibacterial potential. Among all the compounds isobonducellin have shown the highest docking score against Cytochrome P450 14 alpha-sterol demethylase (PDB ID: 1EA1) in the antifungal docking study. The results has been compared to the respective standard drugs and finding yeilded more potent binding affinity (receptor – ligand) than the standard drugs.

We have further established the pharmacokinetic/drug likeliness and toxicological properties of the selected compounds because these interpretations were significantly intended as essential considerations for the development of a new drug, clinical trial, and biological review of the isolated compounds [84]. Throughout this case, the pharmacokinetic properties of the major compounds of *C. digyna* were determined by using an online method (SwissADME), which was based on the Lipinski five rules. Lipinski's rule of 5 reports that the molecular weight of an orally administered drug/compounds should be < 500 amu, the value of lipophilicity is ≤ 5, hydrogen bond acceptors <5, and the hydrogen

bonds donor is ≤ 10. When any drugs or compounds break this rule, the bioavailability of the compound can be questionable [85]. The findings suggest all compounds met the requirements of Lipinski's five rules, suggesting that these compounds are effective drug candidates and potentially preferable for oral use. Besides, we have used the admetSAR online tool to evaluate the toxicological properties of the selected phytochemicals, as the safety of the compounds is an important parameter for becoming a good drug [86]. In this research, no ames toxicity and carcinogens were identified as a threat.

Therefore, it is also claimed that numerous plant shows antidiarrheal activity supposed to having antibacterial potentiality [87]. MECD demonstrated high susceptibility against some pathogenic bacteria and fungi, It additionally contains flavonoid and phenolic compound which might be the major gradual factors for the antidiarrheal action, notwithstanding its antisecretory, antimotility and gastrointestinal transit hindrance impacts were observed in this investigation, its overwhelming antimicrobial properties also reinforcing a presumption that MECD can be a strong candidate for diarrhea of diverse etiologies including those with the infectious segment.

6. Conclusion

The study is to validate the application of methanol extract of *caesapinia digyna* (Rottl.) stems as an antidiarrheal substance used in conventional folk medicine in Bangladesh. In our investigation, it is almost clear that MECD can be another fountain of antibacterial and antifungal agents against several pathogenic strains. It is additionally assumed that, the antimicrobial effect of MECD may be associated with flavonoid and the phenolic compound which also incites antidiarrheal activity. Furthermore, we can state that, this present theoretical work of three isolated compounds from *Caesalphinia digyna* (Rottl.) stems can be useful in the studies of the next experimental work. Therefore, further investigations are suggested to isolate and identify other dynamic constituents in charge of antimicrobial and antidiarrheal potential.

Declaration of competing interest

The authors declare no conflict of interest for this research.

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Ethical Consideration

All biological activities were performed following the ethical statements of the Declaration of Helsinki 2013. Principles of the Swiss Academy of Medical Sciences and Swiss Academy of Sciences were strictly followed in case of treating and handling of the model animals and mice were euthanized employing the Guidelines for the Euthanasia of Animals: 2013 edition [88].

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Author contributions

NUE: conceptualization, desiging, methodology, investigations, data analysis, software, validation, original draft preparation, review and editing; MMA: data curation, investigations; MSUS: data analysis, investigations; review and editing EHR: data curation, investigations; MA: data curation, investigations; MMHT: data curation, investigations.

References

[1] D. Laloo, S. Hemalatha, Ethnomedicinal plants used for diarrhea by tribals of Meghalaya, Northeast India, Phcog. Rev. 5 (2011) 147–154, https://doi.org/10.4103/0973-7847.91108.
[2] K. Konaté, K. Yomalan, O. Sytar, M. Brestic, Antidiarrheal and antimicrobial profiles extracts of the leaves from Trichilia emetica Vahl.(Meliaceae), Asian Pacific Journal of Tropical Biomedicine 5 (2015) 242–248.
[3] C. Bern, J. Martines, I. de Zoysa, R.I. Glass, The magnitude of the global problem of diarrhoeal disease: a ten-year update, Bull. World Health Organ. 70 (1992) 705–714.
[4] X. Shang, X. Miao, F. Yang, B. Li, X. Guo, H. Pan, Y. Zhang, J. Zhang, The antidiarrheal activity of the non-toxic dihuang powder in mice, Front. Pharmacol. 9 (2018), https://doi.org/10.3389/fphar.2018.01037.
[5] S. Semenya, A. Maroyi, Medicinal plants used by the Bapedi traditional healers to treat diarrhoea in the Limpopo Province, South Africa, J. Ethnopharmacol. 144 (2012) 395–401.
[6] K. Chukwuka, J. Ikheloa, I. Okonko, J. Moody, T. Mankinde, The antimicrobial activities of some medicinal plants on Escherichia coli as an agent of diarrhea in livestock, Adv. Appl. Sci. Res. 2 (2011) 37–48.
[7] K.S. Komal, A. Rana, Herbal approaches for diarrhoea: a review, Int. Res. J. Pharm. 4 (2013) 31–38.
[8] K. Pathak, R.J. Das, Herbal medicine-a rational approach in health care system, International Journal of Herbal Medicine 1 (2013) 86–89.
[9] D.S. Fabricant, N.R. Farnsworth, The value of plants used in traditional medicine for drug discovery, Environ. Health Perspect. 109 (2001) 69–75.
[10] K. Konaté, K. Yomalan, O. Sytar, M. Brestic, Antidiarrheal and antimicrobial profiles extracts of the leaves from Trichilia emetica Vahl. (Meliaceae), Asian Pacific Journal of Tropical Biomedicine 5 (2015) 242–248, https://doi.org/10.1016/S2221-1691(15)30012-5.
[11] N.U. Emon, I. Jahan, M.A. Sayeed, Investigation of antinociceptive, anti-inflammatory and thrombolytic activity of Caesalpinia digyna (Rottl.) leaves by experimental and computational approaches, Advances in Traditional Medicine (2020) 1–9.
[12] R. Srinivasan, M. Chandrasekar, M. Nanjan, B. Suresh, Antioxidant activity of Caesalpinia digyna root, J. Ethnopharmacol. 113 (2007) 284–291.
[13] C.B. Yogesh, A. Kumar, N.K. Goswami, A report on pharmacognostical and quality control parameters of root of Caesalpinia digyna (Rottler), J. Pharmacogn. Phytochem. 9 (2020) 1943–1946.
[14] S.K. Roy, A. Srivastava, S.M. Jachak, Analysis of homoisoflavonoids in Caesalpinia digyna by HPLC-ESI-MS, HPLC and method validation, Natural product communications 7 (2012), 1934578X1200700922.
[15] S.B. Mahato, N.P. Sahu, E. Müller, P. Luger, Stereochemistry of a macrocyclic spermidine alkaloid from Caesalpinia digyna Rottl. X-Ray determination of the structure of caesalpinine C (celallocinnine), Journal of the Chemical Society, Perkin Transactions 2 (1985) 193–196.
[16] S. Mahato, N. Sahu, P. Luger, Structure of caesalpinine A: a novel spermidine alkaloid from Caesalpinia digyna Rottl, J. Am. Chem. Soc. 105 (1983) 4441–4445.
[17] R.P. Rastogi, B. Mehrotra, Compendium of Indian Medicinal Plants, Central Drug Research Institute, 1990.
[18] T.O. Soine, Naturally occurring coumarins and related physiological activities, J. Pharmaceut. Sci. 53 (1964) 231–264.
[19] J. Harborne, H. Baxter, J. Moss, Phytochemical Dictionary, Taylor & Francis, Padstow, UK, 1999.
[20] S. Mitra, R. Kannan, R. Sundaram, Pharmacognostical and physicochemical characteristics of roots of lesser known medicinal plant Caesalpinia digyna rottl, Ancient Sci. Life 26 (2007) 35.
[21] R. Srinivasan, M. Chandrasekar, M. Nanjan, Phytochemical investigations of Caesalpinia digyna root, E-Journal of Chemistry 8 (2011) 1843–1847.
[22] R. Kumar, D.K. Patel, S.K. Prasad, D. Laloo, S. Krishnamurthy, S. Hemalatha, Type 2 antidiabetic activity of bergenin from the roots of Caesalpinia digyna Rottler, Fitoterapia 83 (2012) 395–401.
[23] S.K. Roy, U.C. Agrahari, R. Gautam, A. Srivastava, S.M. Jachak, Isointricatinol, a new antioxidant homoisoflavonoid from the roots of Caesalpinia digyna Rottler, Nat. Prod. Res. 26 (2012) 690–695, https://doi.org/10.1080/14786419.2010.548813.
[24] Herbpathy. https://herbpathy.com/Uses-and-Benefits-of-Caesalpinia-Digyna-Cid6634.
[25] I.o.L. Care, U.o.L. Animals, N.I.o. Resources, Guide for the Care and Use of Laboratory Animals, National Academies, 1985.
[26] H.O. Edeoga, D. Okwu, B. Mbaebie, Phytochemical constituents of some Nigerian medicinal plants, Afr. J. Biotechnol. 4 (2005) 685–688.
[27] R. Viran, F.Ü. Erkoç, H. Polat, O. Koçak, Investigation of acute toxicity of deltamethrin on guppies (Poecilia reticulata), Ecotoxicol. Environ. Saf. 55 (2003) 82–85.
[28] F.G. Shoba, M. Thomas, Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea, J. Ethnopharmacol. 76 (2001) 73–76.
[29] A. Robert, J.E. Nezamis, C. Lancaster, A.J. Hanchar, M.S. Klepper, Enteropooling assay: a test for diarrhea produced by prostaglandins, Prostaglandins 11 (1976) 809–828.
[30] S. Rudra, A. Tahamina, N.U. Emon, M. Adnan, M. Shakil, M. Chowdhury, H. Uddin, J.W. Barlow, M.S. Alwahibi, M. Soliman Elshikh, Evaluation of various solvent extracts of tetrastigma leucostaphylum (dennst.) alston leaves, a Bangladeshi

traditional medicine used for the treatment of diarrhea, Molecules (Basel, Switzerland) 25 (2020) 4994.
[31] S. Alam, N.U. Emon, S. Shahriar, F.T. Richi, M.R. Haque, M.N. Islam, S.A. Sakib, A. Ganguly, Pharmacological and computer-aided studies provide new insights into Millettia peguensis Ali (Fabaceae), Saudi Pharmaceut. J. (2020).
[32] R. Reiner, Antibiotics: an Introduction, F. HOFFMAN-LA ROCHE & CO., LTD., BASLE(SWITZERLAND), 1982, 1982.
[33] A. Espinel-Ingroff, B. Arthington-Skaggs, N. Iqbal, D. Ellis, M. Pfaller, S. Messer, M. Rinaldi, A. Fothergill, D. Gibbs, A. Wang, Multicenter evaluation of a new disk agar diffusion method for susceptibility testing of filamentous fungi with voriconazole, posaconazole, itraconazole, amphotericin B, and caspofungin, J. Clin. Microbiol. 45 (2007) 1811–1820.
[34] N.U. Emon, S. Alam, S. Rudra, S.R. Riya, A. Paul, S.M. Hossen, U. Kulsum, A. Ganguly, Antidepressant, anxiolytic, antipyretic, and thrombolytic profiling of methanol extract of the aerial part of Piper nigrum: in vivo, in vitro, and in silico approaches, Food Sci. Nutr..
[35] H. Wu, D. Wacker, M. Mileni, V. Katritch, G.W. Han, E. Vardy, W. Liu, A. A. Thompson, X.P. Huang, F.I. Carroll, S.W. Mascarella, R.B. Westkaemper, P. D. Mosier, B.L. Roth, V. Cherezov, R.C. Stevens, Structure of the human kappa-opioid receptor in complex with JDTic, Nature 485 (2012) 327–332, https://doi.org/10.1038/nature10939.
[36] T. Che, J. English, B.E. Krumm, K. Kim, E. Pardon, R.H.J. Olsen, S. Wang, S. Zhang, J.F. Diberto, N. Sciaky, F.I. Carroll, J. Steyaert, D. Wacker, B.L. Roth, Nanobody-enabled monitoring of kappa opioid receptor states, Nat. Commun. 11 (2020) 1145, https://doi.org/10.1038/s41467-020-14889-7.
[37] J.S. Burg, J.R. Ingram, A.J. Venkatakrishnan, K.M. Jude, A. Dukkipati, E. N. Feinberg, A. Angelini, D. Waghray, R.O. Dror, H.L. Ploegh, K.C. Garcia, Structural biology. Structural basis for chemokine recognition and activation of a viral G protein-coupled receptor, Science (New York, N.Y.) 347 (2015) 1113–1117, https://doi.org/10.1126/science.aaa5026.
[38] G. Fenalti, N.A. Zatsepin, C. Betti, P. Giguere, G.W. Han, A. Ishchenko, W. Liu, K. Guillemyn, H. Zhang, D. James, D. Wang, U. Weierstall, J.C. Spence, S. Boutet, M. Messerschmidt, G.J. Williams, C. Gati, O.M. Yefanov, T.A. White, D. Oberthuer, M. Metz, C.H. Yoon, A. Barty, H.N. Chapman, S. Basu, J. Coe, C.E. Conrad, R. Fromme, P. Fromme, D. Tourwe, P.W. Schiller, B.L. Roth, S. Ballet, V. Katritch, R.C. Stevens, V. Cherezov, Structural basis for bifunctional peptide recognition at human delta-opioid receptor, Nat. Struct. Mol. Biol. 22 (2015) 265–268, https://doi.org/10.1038/nsmb.2965.
[39] M. Mathieu, G. Debousker, S. Vincent, F. Viviani, N. Bamas-Jacques, V. Mikol, Escherichia coli FolC structure reveals an unexpected dihydrofolate binding site providing an attractive target for anti-microbial therapy, J. Biol. Chem. 280 (2005) 18916–18922, https://doi.org/10.1074/jbc.M413799200.
[40] T. Kuzuyama, J.P. Noel, S.B. Richard, Structural basis for the promiscuous biosynthetic prenylation of aromatic natural products, Nature 435 (2005) 983–987, https://doi.org/10.1038/nature03668.
[41] Y. Nakaishi, M. Bando, H. Shimizu, K. Watanabe, F. Goto, H. Tsuge, K. Kondo, M. Komatsu, Structural analysis of human glutamine:fructose-6-phosphate amidotransferase, a key regulator in type 2 diabetes, FEBS Lett. 583 (2009) 163–167, https://doi.org/10.1016/j.febslet.2008.11.041.
[42] S. Mouilleron, M.A. Badet-Denisot, B. Golinelli-Pimpaneau, Glutamine binding opens the ammonia channel and activates glucosamine-6P synthase, J. Biol. Chem. 281 (2006) 4404–4412, https://doi.org/10.1074/jbc.M511689200.
[43] L.M. Podust, T.L. Poulos, M.R. Waterman, Crystal structure of cytochrome P450 14alpha -sterol demethylase (CYP51) from Mycobacterium tuberculosis in complex with azole inhibitors, Proc. Natl. Acad. Sci. U. S. A 98 (2001) 3068–3073, https://doi.org/10.1073/pnas.061562898.
[44] S. Sogabe, M. Masubuchi, K. Sakata, T.A. Fukami, K. Morikami, Y. Shiratori, H. Ebiike, K. Kawasaki, Y. Aoki, N. Shimma, A. D'Arcy, F.K. Winkler, D.W. Banner, T. Ohtsuka, Crystal structures of Candida albicans N-myristoyltransferase with two distinct inhibitors, Chem. Biol. 9 (2002) 1119–1128, https://doi.org/10.1016/s1074-5521(02)00240-5.
[45] G.D. N, J.L. Paulsen, K. Viswanathan, S. Keshipeddy, M.N. Lombardo, W. Zhou, K. M. Lamb, A.E. Sochia, J.B. Alverson, N.D. Priestley, D.L. Wright, A.C. Anderson, Propargyl-linked antifolates are dual inhibitors of Candida albicans and Candida glabrata, J. Med. Chem. 57 (2014) 2643–2656, https://doi.org/10.1021/jm401916j.
[46] A.A. Sagatova, M.V. Keniya, R.K. Wilson, M. Sabherwal, J.D. Tyndall, B.C. Monk, Triazole resistance mediated by mutations of a conserved active site tyrosine in fungal lanosterol 14alpha-demethylase, Sci. Rep. 6 (2016) 26213, https://doi.org/10.1038/srep26213.
[47] V. Pries, C. Nocker, D. Khan, P. Johnen, Z. Hong, A. Tripathi, A.L. Keller, M. Fitz, F. Perruccio, I. Filipuzzi, S. Thavam, T. Aust, R. Riedl, S. Ziegler, F. Bono, G. Schaaf, V.A. Bankaitis, H. Waldmann, D. Hoepfner, Target identification and mechanism of action of Picolinamide and Benzamide Chemotypes with antifungal properties, Cell chemical biology 25 (2018) 279–290.e277, https://doi.org/10.1016/j.chembiol.2017.12.007.
[48] R.A. Friesner, R.B. Murphy, M.P. Repasky, L.L. Frye, J.R. Greenwood, T.A. Halgren, P.C. Sanschagrin, D.T. Mainz, Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein– ligand complexes, J. Med. Chem. 49 (2006) 6177–6196.
[49] R.A. Friesner, J.L. Banks, R.B. Murphy, T.A. Halgren, J.J. Klicic, D.T. Mainz, M. P. Repasky, E.H. Knoll, M. Shelley, J.K. Perry, Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy, J. Med. Chem. 47 (2004) 1739–1749.
[50] N.U. Emon, M. Kaiser, M. Islam, M.F.I. Kabir, M. Jamir, M.A.J. Uddin, M. Islam, Anxiolytic and thrombolytic investigation of methanol extract of Piper nigrum L. fruits and Sesamum indicum L. seeds, J. Adv. Biotechnol. Exp. Ther 3 (2020) 158–164.
[51] N.U. Emon, S. Alam, S. Rudra, S. Chowdhury, J.C. Rajbangshi, A. Ganguly, Evaluation of pharmacological potentials of the aerial part of Achyranthes aspera L.: in vivo, in vitro and in silico approaches, Advances in Traditional Medicine (2020) 1–14.
[52] C.P. Kala, P.P. Dhyani, B.S. Sajwan, Developing the medicinal plants sector in northern India: challenges and opportunities, J. Ethnobiol. Ethnomed. 2 (2006) 32.
[53] T. Kuge, T. Shibata, M.S. Willett, Multiple-dose escalation, safety, and tolerability study of wood creosote, the principal active ingredient of seirogan, an herbal antidiarrheal medication, in healthy subjects, J. Clin. Pharmacol. 43 (2003) 284–290.
[54] M.D. Subbotina, V.N. Timchenko, M.M. Vorobyov, Y.S. Konunova, Y. S. Aleksandrovih, S. Shushunov, Effect of oral administration of tormentil root extract (Potentilla tormentilla) on rotavirus diarrhea in children: a randomized, double blind, controlled trial, Pediatr. Infect. Dis. J. 22 (2003) 706–711.
[55] M.J. Miller, W.K. MacNaughton, X.-J. Zhang, J.H. Thompson, R.M. Charbonnet, P. Bobrowski, J. Lao, A.M. Trentacosti, M. Sandoval, Treatment of gastric ulcers and diarrhea with the Amazonian herbal medicine sangre de grado, Am. J. Physiol. Gastrointest. Liver Physiol. 279 (2000) G192–G200.
[56] W. Gang, T.-q. Li, W. Lei, X. Qing, J. Chang, Y. Zhang, M.-h. Wan, G. Jia, Y. Cheng, X. Huang, Tong-xie-ning, a Chinese herbal formula, in treatment of diarrhea-predominant irritable bowel syndrome: a prospective, randomized, double-blind, placebo-controlled trial, Chin. Med. J. 119 (2006) 2114–2119.
[57] H. Oi, D. Matsuura, M. Miyake, M. Ueno, I. Takai, T. Yamamoto, M. Kubo, J. Moss, M. Noda, Identification in traditional herbal medications and confirmation by synthesis of factors that inhibit cholera toxin-induced fluid accumulation, Proc. Natl. Acad. Sci. Unit. States Am. 99 (2002) 3042–3046.
[58] P. Rawat, P.K. Singh, V. Kumar, Evidence based traditional anti-diarrheal medicinal plants and their phytocompounds, Biomed. Pharmacother. 96 (2017) 1453–1464.
[59] E.M. Williamson, D.T. Okpako, F.J. Evans, Pharmacological Methods in Phytotherapy Research: Volume 1: Selection, Preparation and Pharmacological Evaluation of Plant Material, John Wiley & Sons Ltd, 1996.
[60] J.F. Cryan, F.F. Sweeney, The age of anxiety: role of animal models of anxiolytic action in drug discovery, Br. J. Pharmacol. 164 (2011) 1129–1161.
[61] Y. Miyamoto, L. Eckmann, Drug development against the major diarrhea-causing parasites of the small intestine, Cryptosporidium and Giardia, Front. Microbiol. 6 (2015) 1208.
[62] K. Hodges, R. Gill, Infectious diarrhea: cellular and molecular mechanisms, Gut Microb. 1 (2010) 4–21.
[63] P.C.B. Silva, J. Clementino Neto, A.D.S.d. Silva, T.M.S. Silva, M.d.F. Agra, F.d. A. Cavalcante, Antidiarrheal activity of Solanum asterophorum in mice, Revista Brasileira de Farmacognosia 22 (2012) 131–136.
[64] S.R. Kulkarni, A.B. Pandit, Enzymatic hydrolysis of castor oil: an approach for rate enhancement and enzyme economy, 2005.
[65] S. Tunaru, T.F. Althoff, R.M. Nüsing, M. Diener, S. Offermanns, Castor oil induces laxation and uterus contraction via ricinoleic acid activating prostaglandin EP3 receptors, Proc. Natl. Acad. Sci. Unit. States Am. 109 (2012) 9179–9184.
[66] T.S. Gaginella, P. Bass, Laxatives: an update on mechanism of action, Life Sci. 23 (1978) 1001–1009.
[67] N. Mascolo, A.A. Izzo, G. Autore, F. Barbato, F. Capasso, Nitric oxide and castor oil-induced diarrhea, J. Pharmacol. Exp. Therapeut. 268 (1994) 291–295.
[68] M. Sisay, E. Engidawork, W. Shibeshi, Evaluation of the antidiarrheal activity of the leaf extracts of Myrtus communis Linn (Myrtaceae) in mice model, BMC Compl. Alternative Med. 17 (2017) 103.
[69] C.F.d.Q. Siqueira, D.L.V. Cabral, T.J.d.S. Peixoto Sobrinho, E.L.C. de Amorim, J. G. de Melo, T.A.d.S. Araújo, U.P. de Albuquerque, Levels of tannins and flavonoids in medicinal plants: evaluating bioprospecting strategies, J Evid Based Integr Med 2012 (2012).
[70] P.A. Akah, V.N. Offiah, Gastrointestinal effects of Allamanda cathartica leaf extracts, Int. J. Pharmacogn. 30 (1992) 213–217.
[71] T.S. Gaginella, N. Mascolo, A.A. Izzo, G. Autore, F. Capasso, Nitric oxide as a mediator of bisacodyl and phenolphthalein laxative action: induction of nitric oxide synthase, J. Pharmacol. Exp. Therapeut. 270 (1994) 1239–1245.
[72] L.A. Gurgel, Avaliação experimental da atividade antidiarréica do látex do Croton urucurana Baill, Fortaleza, 2000, Dissertação, Pós-Graduação em, 2000.
[73] V. Tangpu, A.K. Yadav, Antidiarrhoeal activity of Rhus javanica ripen fruit extract in albino mice, Fitoterapia 75 (2004) 39–44, https://doi.org/10.1016/j.fitote.2003.08.015.
[74] F. Borrelli, F. Capasso, R. Capasso, V. Ascione, G. Aviello, R. Longo, A.A. Izzo, Effect of Boswellia serrata on intestinal motility in rodents: inhibition of diarrhoea without constipation, Br. J. Pharmacol. 148 (2006) 553–560.
[75] G. di Carlo, G. Autore, A.A. Izzo, P. Maiolino, N. Mascolo, P. Viola, M.V. Diurno, F. Capasso, Inhibition of intestinal motility and secretion by flavonoids in mice and rats: structure-activity relationships, J. Pharm. Pharmacol. 45 (1993) 1054–1059, https://doi.org/10.1111/j.2042-7158.1993.tb07180.x.
[76] G. Di Carlo, G. Autore, A. Izzo, P. Maiolino, N. Mascolo, P. Viola, M. Diurno, F. Capasso, Inhibition of intestinal motility and secretion by flavonoids in mice and rats: structure-activity relationships, J. Pharm. Pharmacol. 45 (1993) 1054–1059.
[77] E. Tadesse, E. Engidawork, T. Nedi, G. Mengistu, Evaluation of the anti-diarrheal activity of the aqueous stem extract of Lantana camara Linn (Verbenaceae) in mice, BMC Compl. Alternative Med. 17 (2017) 190.
[78] M.M. Cowan, Plant products as antimicrobial agents, Clin. Microbiol. Rev. 12 (1999) 564–582.

[79] https://www.worldgastroenterology.org/guidelines/global-guidelines, 2018. https://www.worldgastroenterology.org/guidelines/global-guidelines.
[80] M.I. Okeke, C.U. Iroegbu, E.N. Eze, A.S. Okoli, C.O. Esimone, Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity, J. Ethnopharmacol. 78 (2001) 119–127.
[81] A. Ghosh, B.K. Das, A. Roy, B. Mandal, G. Chandra, Antibacterial activity of some medicinal plant extracts, J. Nat. Med. 62 (2008) 259–262.
[82] R.R. Mendonça-Filho, Bioactive phytocompounds: new approaches in the phytosciences, Modern phytomedicine: turning medicinal plants into drugs, 2006.
[83] S. Khan, M. Nazir, N. Raiz, M. Saleem, G. Zengin, G. Fazal, H. Saleem, M. Mukhtar, M.I. Tousif, R.B. Tareen, Phytochemical profiling, in vitro biological properties and in silico studies on Caragana ambigua stocks (Fabaceae): a comprehensive approach, Ind. Crop. Prod. 131 (2019) 117–124.
[84] C.A. Lipinski, Lead-and drug-like compounds: the rule-of-five revolution, Drug Discov. Today Technol. 1 (2004) 337–341.
[85] M.-Q. Zhang, B. Wilkinson, Drug discovery beyond the 'rule-of-five', Curr. Opin. Biotechnol. 18 (2007) 478–488.
[86] F. Cheng, W. Li, Y. Zhou, J. Shen, Z. Wu, G. Liu, P.W. Lee, Y. Tang, admetSAR: a Comprehensive Source and Free Tool for Assessment of Chemical ADMET Properties, ACS Publications, 2012.
[87] M. Ilyas, A. Haruna, N. Ilyas, Plant constituents with antidiarrhoeal properties, Bull Sci Assoc Nigeria 10 (1995) 5–12.
[88] E.J. Emanuel, Reconsidering the declaration of Helsinki, Lancet 381 (2013) 1532–1533.