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Original Article

Evaluation of Antidiarrheal, Antispasmodic, and Antisecretory Activities of Extract and Fractions of *Castanopsis costata* Leaves in Animal Models

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ABSTRACT

Background: The use of traditional medicinal plants *Castanopsis costata* in the management of diarrhea is an ancient practice in North Sumatra, Indonesia, but its safety and efficacy have not been examined scientifically in animal models. Therefore, this study aims to determine the antidiarrheal effect of *C. costata* leaf extract and fractions *in vivo*. **Methods:** The antidiarrheal activity was evaluated against animal models of diarrhea induced by castor oil, charcoal meal, and entero-pooling test. The extract group received doses of 25, 50, 100, and 200 mg/kg, while the fraction groups was treated with WFCC, EAFCC, and nHFCC, in doses of 50 and 100 mg/kg, respectively. The negative control received 10 mL/kg of the dissolving vehicle, while the positive was treated with loperamide 3 mg/kg. **Results:** The results showed that EECC doses of 200 mg/kg, WFCC, and EAFCC at 50 and 100 mg/kg, respectively significantly delayed the onset of diarrhea, while WFCC and EAFCC at 100 mg/kg significantly reduced the amount of wet feces output and total feces output. In addition, only WFCC and EAFCC (at 50 and 100 mg/kg, respectively) significantly reduced the mean weight of wet feces and total feces. The percentage of maximum diarrhea inhibition was observed at the EAFCC dose of 100 mg/kg. Based on the charcoal meal test results, only EAFCC significantly inhibited the distance traveled by charcoal meal and reduced the peristaltic index at a dose of 100 mg/kg, while the percentage decrease in gastrointestinal motility was 46.87%. Similarly, in the entero-pooling test, WFCC and EAFCC significantly reduced the volume and weight of intestinal contents at a dose of 100 mg/kg, respectively. **Conclusion:** The results confirm that the extract and fractions of *C. costata* have antidiarrheal activity. Therefore, this study provides scientific support for the traditional use of *C. costata* in treating diarrhea.

Key words: *Castanopsis costata,* Diarrhea, Animal models, Traditional medicine, North Sumatra.

INTRODUCTION

In developing countries, such as Indonesia, diarrhea is one of the main causes of morbidity and mortality in both children and adults. This is mainly due to poor hygiene and sanitation. The disease kills more children than malaria, measles, and AIDS.[1] Approximately 1.5 million children die every year due to diarrhea.[2] It is defined as the release of loose or watery stools for at least 3 times in 24 hours and is classified as acute or chronic based on the duration of symptoms. Acute diarrhea is usually caused by bacteria e.g, *Campylobacter*, *Salmonella*, *Shigella*, and *Escherichia coli*, or viruses such as rotavirus. It can also be caused by medications such as antibiotics, anticancer drugs, and antacids that contain magnesium. Meanwhile, chronic diarrhea is usually associated with functional disorders such as irritable bowel syndrome or intestinal diseases such as Crohn's disease. Parasitic infections namely *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium* also cause chronic or persistent diarrhea.[3]

The majority of people in developing countries depend on traditional medicine to treat various diseases.[2] Medicinal plants are usually preferred to treat digestive disorders, such as diarrhea because they contain numerous elements with the potential to increase or neutralize side effects and are considered relatively safe in long-term use.[4] Generally, Indonesians are dependent on the therapeutic benefits of traditional medicine.[5] According to an ethnobotanical survey, several plants have an antidiarrheal role but their therapeutic and safety evaluations have not been reported, an example is *Castanopsis costata*.[6] In the Fagaceae family, *C. costata* leaves are traditionally useful for the treatment of abdominal pain, diarrhea, fever and other health conditions.[7-8] Apart from the therapeutic uses of this plant for diarrhea and abdominal pain, it has been reported to have antioxidant,[9] antihyperlipidemic,[8] and antidiabetic activities.[5]

Several studies found that diarrhea damages the intestinal antioxidant defense system, culminating in further complications and other oxidative stress disorders. Therefore, antioxidants can play an important role in the management of diarrhea.[10-11] There are currently no detailed investigations on the antidiarrheal phytoconstituents of *C. costata*, but it is known to contain alkaloids, flavonoids, tannins, steroids, and others, which majorly have antidiarrheal activities.[8,12] Therefore, this study aims to evaluate the antidiarrheal, antispasmodic, and antisecretory activities of extract and fractions of the *C. costata* leaves using an animal model of castor oil-induced diarrhea.

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MATERIALS AND METHODS

Drugs, chemicals, and reagents

Loperamide (Sigma Chemical Company, USA), distilled water, castor oil, activated charcoal, ethanol, ethyl acetate, *n*-hexane and pulvis gummi arabicum (EMSURE® ACS Merck, Darmstadt, Germany) were used for the experiment.

Plant material collection and identification

Fresh leaves of *C. costata* were obtained from the traditional market of Pancur Batu, North Sumatra, Indonesia. The identification of plant specimens was carried out by taxonomists from Herbarium Jatinangor, Department of Biology, Faculty of Mathematics and Science, Universitas Padjadjaran (Code: 219/HB/04/2017) and legalized as *Castanopsis costata*. Afterward, 10 kg of the fresh leaves were transported to the Central Laboratory, Universitas Buana Perjuangan Karawang for extraction and fractionation processes.

Extraction and fractionation

About 2.0 kg of *C. costata* leaf powder was macerated in 70% ethanol for 72 hours to obtain a liquid extract. It was then concentrated using a rotary evaporator at a temperature of 40-50°C. Next, the ethanolic extract was diluted in a 1:3 combination of ethanol and water, while about 100 g was separated by liquid-liquid partitioning using ethyl acetate (EA) (4 x 150 ml) and *n*-hexane (4 x 150 ml), to obtain *n*-hexane fraction (nHFCC), ethyl acetate fraction (EAFCC), and water fraction (WFCC).

Experimental animals

A total of 144 healthy male Swiss albino mice, weighing between 20-32 g and aged 6 to 8 weeks were used for this experiment in which 48 each for the castor oil, charcoal meal, and entero-pooling induced diarrhea model. The animals were obtained from the animal cages of the School of Pharmacy, Institut Teknologi Bandung, Indonesia. The mice were placed in plastic cages with softwood shavings and chips as bedding. They were reared under standard conditions of relative humidity, temperature, 12 h light/12 h dark cycle, and were provided with food as well as water *ad libitum*. All mice were acclimatized to the working environment for 1 week before the start of the evaluation of the pharmacological activity.

Animal grouping and dosing

In all experimental models, mice were randomly divided into 12 groups, 2 control and 10 test consisting of 4 animals each. Group I (negative control) received 10 mL/kg 1% PGA suspension, while Group II (positive control) were treated with the standard drug 3 mg/kg loperamide. The test groups namely III, IV, V, VI, VII, VIII, IX, X, XI, and XII received 25, 50, 100, and 200 mg/kg EECC (EECC 25, EECC 50, EECC 100, and EECC 200), as well as 50 and 100 mg/kg WFCC (WFCC 50 and WFCC 100), EAFCC (EAFCC 50 and EAFCC 100), and nHFCC (nHFCC 50 and nHFCC 100) orally.

Determination of antidiarrheal activity

Castor oil-induced diarrhea: The methods described by previous studies were considered with slight modifications.[13-15] Male Swiss albino mice were fasted for 18 hours with free access to water, then one hour after dosing, each was given 0.5 mL of castor oil orally for the induction of diarrhea and placed one by one in plastic cages with white paper on the floor, the paper was changed every hour for a total duration of 4 hours. During the observation period, diarrhea onset, frequency, as well as wet weight and total feces were recorded. Furthermore, the percentage inhibition of diarrhea and feces output was calculated using the formula described below:[14,16]

$$\% \text{ ID} = \frac{\text{Mean number of (WFNC} - \text{WFT)}}{\text{Mean number of WFNC}} \text{x } 100$$

$$\% \text{ ITFO} = \frac{\text{Mean number of (TFNC} - \text{TFT)}}{\text{Mean number of TFNC}} \text{ x } 100$$

$$\% \text{ RWF} = \frac{\text{Mean number of (WWFNC} - \text{WWFT)}}{\text{Mean number of WWFNC}} \text{ x } 100$$

$$\% \text{ RTFO} = \frac{\text{Mean number of (WTFNC} - \text{WTFT)}}{\text{Mean number of WTFNC}} \text{ x } 100$$

Where, ID = inhibition of diarrhea, WFNC = wet feces in the negative control group, WFT = wet feces in the test group, ITFO = Inhibition of total fecal output, TFNC = total feces of negative control, and TFT = total feces of treated, RWF= reduction in wet feces, WWFNC= weight of wet feces in the negative control group, WWFT= weight of wet feces in the test group, RTFO= reduction in total fecal output, WTFNC= weight of total feces in the negative control group, WTFT= weight of total feces in the in the test group.

Determination of antispasmodic activity

Gastrointestinal motility test by charcoal meal: The mice were fasted for 18 hours with free access to water and treated as previously described, then 0.5 mL of castor oil was administered to each. Approximately 1 mL marker of 5% activated charcoal suspension in distilled water was administered orally 1 hour after. They were then sacrificed with cervical dislocation 1 hour after the administration of activated charcoal suspension. The small intestine was dissected from the pylorus to the caecum and laid lengthwise on white paper. Subsequently, the distance traveled by the charcoal marker and the total length of the small intestine was measured. Peristaltic index and inhibition percentage were calculated using the following formula:[14,16]

$$\text{Perstalstic index (PI)} = \frac{\text{Distance traveled by charcoal meal}}{\text{Total length of small intestine}} \text{x } 100$$

$$\% \text{ Inhibition of motility} = \frac{\text{PI negative control} - \text{PI test}}{\text{PI negative control}} \text{x } 100$$

Determination of antisecretory activity

Castor oil-induced entero-pooling: The activity of *C. costata* leaf extract and fractions on intraluminal fluid accumulation was determined using the method described by Robert *et al*.[17] The mice were fasted for 18 hours, and then treated according to their respective groups. After 1 hour, 0.5 mL of castor oil was administered, and they were sacrificed with cervical dislocation. The stomach of each mice was then opened, the small intestine is ligated at both ends namely at the pyloric sphincter and ileo-cecal valve. The dissected small intestine was weighed and the contents were then collected by squeezing into a measuring tube to measure the volume. Afterward, the intestines were reweighed and the difference was recorded as a measure of the intestinal contents. The percentage reduction in intestinal contents comprising volume and weightwas calculated using the following formula:[14,16]

$$\% \text{ Inhibition volume of intenstinal contents} = \frac{\text{MVICNC} - \text{MVICT}}{\text{MVICNC}} \text{x } 100$$

$$\% \text{ Inhibition of weight of intestinal contents} = \frac{\text{MWICNC} - \text{MWICT}}{\text{MWICNC}} \text{x } 100$$

Where, MVICNC = mean volume of intestinal content of negative control group, MVICT = mean volume of intestinal content of test group, MWICNC = mean weight of intestinal content of negative control group, MWICT = mean weight of intestinal content of test group.

In vivo antidiarrheal index

The *in vivo* antidiarrheal index (ADI) was determined using the following formula:[18]

$$\text{In vivo ADI} = \sqrt[3]{\text{Dfreq} \times \text{Gmeq} \times \text{Pfreq}}$$

$$Dfreq = \frac{\text{Mean of ODT} - \text{Mean of ODNC}}{\text{Mean of ODNC}} \text{ x } 100$$

Where, ODT = onset of diarrhea in treated group, ODNC = onset of diarrhea in the negative control group, *Gmeq* = percent inhibition of motility, *Pfreq* = percent inhibition of diarrhea.

Statistical analysis

All experimental data are expressed as mean±SEM, while data processing and analysis were carried out using SPSS statistical software version 22.0. The statistical significance of the differences between groups was assessed by One-way ANOVA followed by Tukey's post-hoc multiple comparison test. The results were considered significant at a *p*-value less than 0.05.

Ethical approval

All experimental animals were handled and used according to animal care and welfare guidelines.[19] This experimental protocol was approved by the Research Ethics Commission, Universitas Padjadjaran, Bandung, Indonesia (No:278/UN6.KEP/EC/2022).

RESULTS AND DISCUSSION

The yield of extract and fractions produced

The dry ethanol extract of *C. costata* (EECC) was brown powder with a yield of 25.50% obtained from the fixed weight of extract divided by that of the simplicia and multiplied by 100%. The dry fractions of *n*-hexane, ethyl acetate, and water were each brown with yields of 15.00%, 45.00%, and 35.00%, respectively.

Evaluation of antidiarrheal activity

Effect of the extract and fractions of *C. costata* leaves on the castor oil-induced diarrhea: The results showed that EECC at 200 mg/kg, WFCC and EAFCC at doses of 50 mg/kg and 100 mg/kg, respectively, significantly delayed the onset of diarrhea with ($p<0.05$) compared to negative control. Meanwhile, only WFCC and EAFCC at a dose of 100 mg/kg significantly reduced the amount of wet feces output and total feces. At doses of 50 mg/kg and 100 mg/kg both fractions also significantly reduced the mean weight of wet stool output and total feces with ($p<0.05$). The maximum percentage of diarrhea inhibition was observed at the EAFCC dose of 100 mg/kg with 65.73%, which is higher than the standard drug LOP 3 with 63.04% as shown in Table 1.

Evaluation of antispasmodic activity

Effect of the extract and fractions of *C. costata* leaves on the castor oil-induced intestinal transit in mice (charcoal meal test): EAFCC significantly inhibited the distance traveled by charcoal powder and reduced the peristaltic index at a dose of 100 mg/kg with ($p<0.05$), while the percentage of decreased gastrointestinal motility was 46.87%. EAFCC at 100 showed a slightly higher antimotility effect than the standard drug LOP 3 with 44.57% as shown in Table 2.

Evaluation of antisecretory activity

Effect of the extract and fractions of *C. costata* leaves on the castor oil-induced entero-pooling (intraluminal fluid accumulation):

Table 1: The antidiarrheal activities of extract and fractions of *C. costata* leaves in castor oil–induced diarrhea in mice model.

Dose (mg/kg)	Onset of Diarrhea (min)	Number of Wet Feces	Number of Total Feces	Average Weight of Wet Feces (g)	Average Weight of Total Feces (g)	% Inhibition of diarrhea	% Reduction in Wet Feces	% Reduction in Total Fecal Output
NC	50.75±8.83	8.55±0.63	12.30±0.35	0.81±0.03	0.92±0.05	–	–	–
LOP 3	224.50±14.61**	3.16±0.23**	5.36±0.28**	0.27±0.04**	0.38±0.07**	63.04	66.67	58.69
EECC 25	56.50±5.89	7.15±0.33	11.04±0.26	0.78±0.04	0.85±0.05	16.37	3.70	7.61
EECC 50	72.75±5.95	6.97±0.18	10.44±0.21	0.61±0.02	0.76±0.04	18.48	24.69	17.39
EECC 100	85.50±6.20	6.47±0.22	10.06±0.13	0.57±0.05	0.64±0.10	24.33	29.63	30.43
EECC 200	115.75±8.02**	6.10±0.36	9.73±0.16	0.52±0.04	0.60±0.05	28.65	35.80	34.78
WFCC 50	106.00±12.46**	5.47±0.22	8.32±0.23	0.24±0.07**	0.36±0.09**	36.02	70.37	60.87
WFCC 100	148.00±13.58**	4.80±0.24**	7.84±0.43**	0.21±0.02**	0.31±0.02**	43.86	74.07	66.30
EAFCC 50	100.50±7.53**	5.03±0.63	8.17±0.12	0.22±0.02**	0.33±0.04**	41.17	72.84	64.13
EAFCC 100	121.25±6.74**	2.93±0.21**	4.62±0.23**	0.17±0.04**	0.27±0.05**	65.73	79.01	70.65
nHFCC 50	61.50±7.82	6.22±0.37	9.92±0.22	0.53±0.03	0.61±0.03	27.25	34.57	33.69
nHFCC 100	78.25±6.02	5.80±0.23	8.44±0.24	0.51±0.04	0.58±0.05	32.16	37.04	36.95

Results are expressed as mean±SEM (n=4); statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test;** shows $p<0.05$ compared to the negative control group; NC: negative control; LOP: Loperamide 3 mg/kg.

Table 2: The antispasmodic effect of extract and fractions of *C. costata* leaves in castor oil–induced gastrointestinal transit (charcoal meal) in mice.

Dose (mg/kg)	Length of Small Intestine (cm)	Distance Traveled by Charcoal Meal (cm)	Peristaltic Index (%)	Inhibition of Motility (%)
NC	52.83±2.02	41.07±2.87	77.74	–
LOP 3	54.21±1.50	23.36±1.77**	43.09**	44.57
EECC 25	53.16±1.11	38.07±1.69	71.61	7.88
EECC 50	53.63±1.20	37.12±1.90	69.21	10.97
EECC 100	53.80±1.14	35.87±1.20	66.67	14.24
EECC 200	53.87±1.19	33.92±1.41	62.97	19.00
WFCC 50	53.95±1.26	31.87±1.74	59.07	24.01
WFCC 100	53.97±1.25	30.15±1.78	55.86	28.15
EAFCC 50	53.65±1.44	32.07±1.94	59.77	23.12
EAFCC 100	54.14±1.31	22.36±1.41**	41.30**	46.87
nHFCC 50	53.70±1.46	34.27±1.77	63.82	17.91
nHFCC 100	53.85±1.45	32.49±1.81	60.33	22.39

Results are expressed as mean±SEM (n=4); statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test; ** shows $p<0.05$ compared to the negative control group; NC: negative control; LOP: Loperamide 3 mg/kg.

Table 3: The antisecretory effect of extract and fractions of *C. costata* leaves in castor oil–induced gastrointestinal fluid accumulation (entero-pooling) in mice.

Dose (mg/kg)	Volume of Intestinal Contents (mL)	% Inhibition	Weight of Intestinal Contents (g)	% Inhibition
NC	0.92±0.04	–	1.12±0.07	–
LOP 3	0.47±0.05**	48.91	0.61±0.05**	45.53
EECC 25	0.81±0.02	11.95	0.94±0.03	16.07
EECC 50	0.77±0.06	16.30	0.82±0.04	26.78
EECC 100	0.71±0.08	22.83	0.78±0.03	30.36
EECC 200	0.67±0.09	27.17	0.72±0.02	35.71
WFCC 50	0.61±0.06	33.69	0.70±0.05	37.50
WFCC 100	0.51±0.07**	44.56	0.64±0.06**	42.86
EAFCC 50	0.64±0.08	30.43	0.71±0.03	36.61
EAFCC 100	0.42±0.04**	54.35	0.60±0.05**	46.43
nHFCC 50	0.68±0.06	26.09	0.73±0.05	34.82
nHFCC 100	0.61±0.07	33.69	0.70±0.04	37.50

Results are expressed as mean±SEM (n=4); statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test; ** shows $p<0.05$ compared to the negative control group; NC: negative control; LOP: Loperamide 3 mg/kg.

Table 4: The *in vivo* antidiarrheal index of the extract and fractions of *C. costata* leaves.

Dose (mg/kg)	*Dfreq*	*Gmeq*	*Pfreq*	Antidiarrheal Index (ADI)
NC	–	–	–	–
LOP 3	342.36	44.57	63.04	98.71
EECC 25	11.33	7.88	16.37	11.35
EECC 50	43.35	10.97	18.48	20.64
EECC 100	68.47	14.24	24.33	28.73
EECC 200	128.08	19.00	28.65	41.16
WFCC 50	108.87	24.01	36.02	45.49
WFCC 100	191.62	28.15	43.86	61.85
EAFCC 50	98.03	23.12	41.17	45.35
EAFCC 100	138.92	46.87	65.73	75.36
nHFCC 50	21.18	17.91	27.25	21.78
nHFCC 100	54.19	22.39	32.16	33.92

Results are expressed as mean±SEM (n=4); NC: negative control; LOP: Loperamide 3 mg/kg.

The results showed that WFCC and EAFCC significantly reduced the mean volume and weight of intestinal contents at a dose of 100 mg/kg with ($p<0.05$) respectively, compared to negative control. Meanwhile, the percentage inhibition of intestinal volume and weight were 44.56% and 42.86% at 100 mg/kg WFCC as well as 54.35% and 46.43% at 100 mg/kg EAFCC (Table 3).

In vivo antidiarrheal index

The *in vivo* antidiarrheal index (ADI) was measured by considering 3 important parameters such as the delayed onset of diarrhea (*Dfreq*), inhibition of motility (*Gmeq*), and inhibition of diarrhea (*Pfreq*). The highest ADI value was recorded at the EAFCC dose of 100 mg/kg (75.36), which was still below the standard LOP drug at a dose of 3 mg/kg at 98.71 as shown in Table 4.

Diarrhea is caused by an imbalance between absorptive and secretory processes in the gastrointestinal tract. As demonstrated in experimental models, this disease is reportedly caused by castor oil.[16,20] Ricinoleic acid, the active metabolite of castor oil, which is released through lipase action in the small intestine, is known to cause diarrhea.[13,16] It induces the release of prostaglandins through intestinal irritation and inflammation mechanisms, which in turn increase motility as well as the secretion of water and electrolytes. Another mechanism that causes this effect is by activating the G protein-coupled prostanoid receptor (EP3) on intestinal smooth muscle cells.[21] In addition, ricinoleic acid also inhibits sodium-potassium ATPase by forming sodium and potassium salts of ricinoleate in the lumen culminating in increased intestinal permeability.[22]

In the animal model of castor oil-induced diarrhea, WFCC and EAFCC produced significant antidiarrheal effects on various measured parameters such as diarrhea onset, amount of wet feces, and weight feces at each dose of 100 mg/kg with $p<0.05$. Meanwhile, EECC at a dose of 200 mg/kg produced a significant difference only in the onset of diarrhea, while WFCC and EAFCC at a dose of 50 mg/kg each significantly influenced the onset of diarrhea and weight feces. The high antidiarrheal activity of both fractions in many parameters might be due to the presence of secondary metabolites, including tannins, flavonoids, phenolics, and saponins.[8] Tannins and flavonoids presumably cause the antidiarrheal activity of *C. costata* by increasing colonic water and electrolyte reabsorption. Besides, tannins reduce intestinal irritability, thereby lowering the peristaltic index.[23] They have also been reported to denature proteins in the intestinal mucosa by forming protein tannates that decrease secretion and inhibit intestinal motility through changes in intracellular Ca^{2+} levels.[24-26] Tannins produce a temporary protective layer of coagulation proteins on the intestinal mucous membrane, which desensitizes sensory nerve endings and reduces provocative peristaltic stimulation.[27] This compound also forms a protective pellicle that prevents the absorption of toxic substances. It is an astringent, polyphenolic compound that binds and precipitates or shrinks proteins.[27,28] Similarly, flavonoids are known as intestinal antimotility and inhibitors of water as well as electrolyte secretion. They also have antioxidant activity which might play a role in suppressing the catalytic activity of various enzymes including those for prostaglandin synthesis.[26,29-31] This compound has astringent actions, increases intestinal mucosal resistance, as well as reduces intestinal secretion and transit.[2] Therefore, the higher

antidiarrheal activity observed in WFCC and EAFCC might be due to the availability of these phytochemical compounds in the fractions.

In the assessment of antidiarrheal activity by the gastrointestinal motility model, only the EAFCC dose of 100 mg/kg showed a significant difference ($p<0.05$) in the peristaltic index compared to the negative control. This antimotility activity might be due to the effect of secondary metabolites contained in *C. costata*. Several studies reported that tannins decrease intestinal peristaltic by inhibiting intracellular Ca^{2+} inflow.[16,32] Meanwhile, flavonoid compounds are known to inhibit intestinal motility.[33]

The secretory component of diarrhea was assessed using the enteropooling model, WFCC and EAFCC at a dose of 100 mg/kg each showed a significant reduction in volume and weight of intestinal contents compared to negative control ($p<0.05$). The increase in weight and volume of small intestinal contents might be due to activation of the nitric oxide pathway through the effects of ricinoleic acid.[34] A previous study stated that the presence of phytochemical compounds such as flavonoids, terpenoids, alkaloids, and steroids reduces the synthesis of nitric oxide.[14] Meanwhile, tannins have been reported to affect the activity of cystic fibrosis transmembrane conductance regulator proteins which transport chloride ions from epithelial cells to the lumen thereby reducing the secretion in the small intestine and colon.[35] Tannins also reduce intestinal secretions by inhibiting the inflow of intracellular Ca^{2+}.[32] The antidiarrheal index (ADI) was used to evaluate the antidiarrheal effect based on various parameters from different models. This is the combined number of *Dfreq, Gmeq,* and *Pfreq* meaning percentage delay in diarrhea onset, inhibition of motility, and inhibition of diarrhea. For all extract, the value increased with dose, while EAFCC at 100 mg/kg had a higher ADI value, indicating a superior antidiarrheal effect compared to other extract and fractions. Based on these results, the antidiarrheal mechanism of action for *C. costata* is associated with inhibition of water secretion, reduced intraluminal fluid accumulation, or increased water absorption, and delayed onset of diarrhea. These findings are consistent with the goal of drug therapy in diarrhea, namely to reduce fecal water by increasing fluid absorption or reducing fluid secretion, or both.[27]

CONCLUSION

Based on the results, the extract and leaf fractions of *C. costata* have antidiarrheal, antispasmodic, and antisecretory activities. This provides a scientific basis for the traditional use as antidiarrheal agents in medical practice in North Sumatra. Meanwhile, further studies are needed to isolate the specific phytochemicals responsible for the antidiarrheal activity and determine their mechanism of action at the molecular level.

REFERENCES

1. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, *et al*. Child health epidemiology reference group of WHO and UNICEF. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2012;379(9832):2151-61.
2. Asrie AB, Abdelwuhab M, Shewamene Z, Gelayee DA, Adinew GM, Birru EM. Antidiarrheal activity of methanolic extract of the root bark of *Cordia africana*. J Exp Pharmacol. 2016;8(1):53-9.
3. Thapar N, Sanderson IR. Diarrhoea in children: an interface between developing and developed countries. Lancet. 2004;363(9409):641-53.
4. Gilani AH, Rahman AU. Trends in ethnopharmocology. J Ethnopharmacol. 2005;100(1-2):43-9.
5. Alkandahri MY, Sujana D, Hasyim DM,Shafirany MZ, Sulastri L, Arfania M, *et al*. Antidiabetic activity of extract and fractions of *Castanopsis costata* leaves on alloxan-induced diabetic mice. Pharmacogn J. 2021;13(6Suppl):1589-93.
6. Elfahmi, Woerdenbag HJ, Kayser O. Jamu: Indonesian traditional herbal medicine towards rational phytopharmacological use. J Herbal Med. 2014;4(2):51-73.
7. Salim E, Fatimah C, Fanny DY. Analgetic activity of Cep-cepan (*Saurauia cauliflora* Dc.) leaves extract. J Nat. 2017;17(1):31-8.
8. Alkandahri MY, Kusumiyati K, Renggana H, Arfania M, Frianto D, Wahyuningsih ES, *et al*. Antihyperlipidemic activity of extract and fractions of *Castanopsis costata* leaves on rats fed with high cholesterol diet. Rasayan J Chem. 2022;15(4):2350-58.
9. Alkandahri MY, Arfania M, Abriyani E, Ridwanuloh D, Farhamzah F, Fikayuniar L, *et al*. Evaluation of antioxidant and antipyretic effects of ethanolic extract of Cep-cepan leaves (*Castanopsis costata* (Blume) A.DC). J Adv Pharm Educ Res. 2022;12(3):107-12.
10. Nieto N, López-Pedrosa JM, Mesa MD, Torres MI, Fernández MI, Ríos A, *et al*. Chronic diarrhea impairs intestinal antioxidant defense system in rats at weaning. Dig Dis Sci. 2000;45(10):2044-50.
11. Han X, Pang Y, Liu S, Tan Z, Tang S, Zhou C, *et al*. Antidiarrheal and antioxidant activities of Honokiol extract from *Magnolia officinalis* cortex in mice. Trop J Pharm Res. 2014;13(10):1643-51.
12. Ayalew M, Bekele A, Mengistie MG, Atnafie SA. Evaluation of the antidiarrheal activity of 80% methanol extract and solvent fractions of the leaf of *Bersama abyssinica* fresen (Melianthaceae) in mice. BMC Complement Med Ther. 2022;22(1):1-9.
13. Tadesse E, Engidawork E, Nedi T, Mengistu G. Evaluation of the anti-diarrheal activity of the aqueous stem extract of *Lantana camara* Linn (Verbenaceae) in mice. BMC Complement Altern Med. 2017;17(1):1-8.
14. Sisay M, Engidawork E, Shibeshi W. Evaluation of the antidiarrheal activity of the leaf extracts of *Myrtus communis* Linn (Myrtaceae) in mice model. BMC Complement Altern Med. 2017;17(1):1-11.
15. Zewdie KA, Bhoumik D, Wondafrash DZ, Tuem KB. Evaluation of in-vivo antidiarrhoeal and in-vitro antibacterial activities of the root extract of *Brucea antidysenterica* J. F. Mill (Simaroubaceae). BMC Complement Med Ther. 2020;20(1):1-11.
16. Alemu MA, Andargie Y, Sisay W, Mengie T, Desta GT, Tessema TA, *et al*. Antidiarrheal effect of 80% methanol extract and fractions of the leaves of *Ocimum lamiifolium* in swiss albino mice. Evid Based Complement Alternat Med. 2022;2022:1-9.
17. Robert A, Nezamis JE, Lancaster C, Hanchar AJ, Klepper MS. Enteropooling assay: a test for diarrhea produced by prostaglandins. Prostaglandins. 1976;11(5):809-28.
18. Than A, Kulkarni HJ, Hmone W, Tha S. Anti-diarrhoeal efficacy of some Burmese indigenous drug formulations in experimental diarrhoeal test models. Int J Crude Drug Res. 1989;27(1):195-200.
19. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8thedition. Washington (DC): National Academies Press (US); 2011. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54050/https://doi.org/10.17226/12910.
20. Teferi MY, Abdulwuhab M, Yesuf JS. Evaluation of in vivo antidiarrheal activity of 80% methanolic leaf extract of *Osyris quadripartita* Decne (Santalaceae) in swiss albino mice. J Evid Based Integr Med. 2019;24:1-9.
21. Degu A, Engidawork E, Shibeshi W. Evaluation of the anti-diarrheal activity of the leaf extract of *Croton macrostachyus* Hocsht. ex Del. (Euphorbiaceae) in mice model. BMC Complement Altern Med. 2016;16(1):1-11.
22. Komal KS, Rana AC. Herbal approaches for diarrhoea: a review. Int Res J Pharm.2013;4(1):31-8.
23. Tadesse WT, Hailu AE, Gurmu AE, Mechesso AF. Experimental assessment of antidiarrheal and antisecretory activity of 80% methanolic leaf extract of *Zehneria scabra* in mice. BMC Complement Altern Med. 2014;14(1):1-8.

24. Jia Q, Su W, Peng W, Li P, Wang Y. Anti-diarrhoea and analgesic activities of the methanol extract and its fractions of *Jasminum amplexicaule* Buch.-Ham. (Oleaceae). J Ethnopharmacol. 2008;119(2):299-304.
25. Belemtougri RG, Constantin B, Cognard C, Raymond G, Sawadogo L. Effects of two medicinal plants *Psidium guajava* L. (Myrtaceae) and *Diospyros mespiliformis* L. (Ebenaceae) leaf extracts on rat skeletal muscle cells in primary culture. J Zhejiang Univ Sci B. 2006;7(1):56-63.
26. Birru EM, Asrie AB, Adinew GM, Tsegaw A. Antidiarrheal activity of crude methanolic root extract of *Idigofera spicata* Forssk. (Fabaceae). BMC Complement Altern Med. 2016;16(272):1-7.
27. Wibowo DA, Nailufar F, Tjandrawinata RR. Antidiarrheal effect of DLBS1Y62, a bioactive fraction of *Uncaria gambir* Roxb. dried sap extract, in wistar rats. J Exp Pharmacol. 2021;13:669-75.
28. de Jesus NZ, de Souza Falcão H, Gomes IF, de Almeida Leite TJ, de Morais Lima GR, Barbosa-Filho JM, *et al*. Tannins, peptic ulcers and related mechanisms. Int J Mol Sci. 2012;13(3):3203-28.
29. Venkatesan N, Thiyagarajan V, Narayanan S, Arul A, Raja S, Kumar SGV, *et al*. Anti-diarrhoeal potential of *Asparagus racemosus* wild root extracts in laboratory animals. J Pharm Pharm Sci. 2005;8(1):39-45.
30. Kusumawati AH, Farhamzah F, Alkandahri MY, Sadino A, Agustina LS, Apriana SD. Antioxidant activity and sun protection factor of Black Glutinous Rice (*Oryza sativa* var. glutinosa). Trop J Nat Prod Res. 2021;5(11):1958-61.
31. Farhamzah, Kusumawati AH, Alkandahri MY, Hidayah H, Sujana D, Gunarti NS, *et al*. Sun protection factor activity of Black Glutinous Rice emulgel extract (*Oryza sativa* var. glutinosa). Indian J Pharm Educ Res. 2022;56(1):302-10.
32. Yacob T, Shibeshi W, Nedi T. Antidiarrheal activity of 80 % methanol extract of the aerial part of *Ajuga remota* Benth (Lamiaceae) in mice. BMC Complement Altern Med. 2016;16(1):303-8.
33. Vezza T, Rodríguez-Nogales A, Algieri F, Utrilla MP, Rodriguez-Cabezas ME, Galvez J. Flavonoids in inflammatory bowel disease: A review. Nutrients. 2016;8(4):1-22.
34. Mascolo N, Izzo AA, Autore G, Barbato F, Capasso F. Nitric oxide and castor oil-induced diarrhea. J Pharmacol Exp Ther. 1994;268(1):291-95.
35. Soares S, Brandão E, Guerreiro C, Soares S, Mateus N, de Freitas V. Tannins in food: Insights into the molecular perception of astringency and bitter taste. Molecules. 2020;25(11):1-26.

GRAPHICAL ABSTRACT

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