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In Vitro and In Vivo Antidiarrheal Activity of Dragon Fruit Peels Methanolic Extract

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ABSTRACT

Diarrhea is a global major health problem reported by 2.5 billion cases annually. Diarrhea accounts for 9% of child death worldwide or the second leading cause of death among toddlers. Many local people have used traditional medicine for diarrhea therapy. Dragon fruit is a herbal plant with high vitamins and various nutritious compounds. Therefore, this study determined antidiarrheal activity from dragon fruit peel methanolic extract (*Hylocereus polyrhizus*). This study was experimental with in vitro and in vivo models. The in vitro model used disc diffusion and microdilution assay against the diarrhea-causing pathogen (*Escherichia coli*). Meanwhile, the in vivo model used male Wistar rats induced by Castro oil. This study showed that the minimum inhibitory concentration (MIC) and minimum killing concentration (MKC) of dragon fruit peel extract against *Escherichia coli* were 50 and 100 mg/ml, respectively. The increased dose of dragon fruit peel methanol extract has increased the antidiarrheal index. The highest and lowest antidiarrheal index was found in the dragon fruit methanol extract-3 (76.56%) and dragon fruit methanol extract-1 (43.28%). The dragon fruit peel methanol extract can be proposed as antidiarrhea and antibacterial therapy against the diarrhea-causing pathogen.
Keywords: Antidiarrheal; Dragon Fruit; Peels; Methanol; Extract

INTRODUCTION

Diarrhea has high morbidity and mortality worldwide, especially in some developing countries (Kardela, Fauziah, and Mayesri, 20,18; Siregar *et al.*, 2019). The Indonesian health minister in an annual report, *Riset Kesehatan Dasar* (Riskesdas) 2018, reported that the prevalence rate of diarrhea was high in children aged 1-4 years old 73,188 cases (11.5%), followed by children aged < 1-year-old (9%), children aged 15-24 years old (6.7%), and the lowest prevalence rate was found in children age of 5-14 years old (6.2%). Moreover, this report also reports that the prevalence rate of diarrhea in North Sumatra in 2018 was 69,517 cases (Kementerian Kesehatan Republik Indonesia, 2019). To date, the development of antidiarrheal still plays a role in reducing the prevalence rate of diarrheal cases instead of disease intervention programs. The development of herbal medicine for antidiarrheal application is the most promising alternative to research extensively.

The local community used traditional medicine to treat diarrhea. One of these biodiversities in Indonesia is dragon fruit, commonly discussed in local community settings. This fruit has several species including the red dragon fruit (Astridwiyanti, Mahendra and Dewi, 2019) that has a high number of vitamins and many other nutrients. Therefore, dragonfruit can improve its lipid profile and neutralize toxins or other harmful substances. In community settings, the utility of dragon fruit is only used as food, and the peels become a wasted product with a limited number of benefits (Niah and Baharsyah, 2018; Winahyu, Candra Purnama and Yevi Setiawati, 2019).

The dragon fruit peel contains various nutrients like vitamin A, vitamin C, vitamin E, alkaloids, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolic, carotene, and phytoalbumin (Pratiwi *et al.*, 2019). Another study by Tang et al. (2021) reported that dragon fruit peels contain more than 37 phenolic compounds, including chlorogenic acid, caffeic acid, ferulic acid, p-coumaric acid, rutin, and isoquercitrin. In addition, Tang et al. reported that the total flavonoid content in red-flesh and white-flesh dragon fruit peel was 11.6 and 10.5 mg GAE/g DW, respectively (Tang *et al.*, 2021).

Various studies have shown the benefits of dragon fruit peel including antioxidant effects, sources of natural pigments, and even antimicrobials. Afandi et al. (2017) demonstrated the formulation's antibacterial activity against six pathogens. The results showed that all extracts inhibit bacterial growth in all bacteria. Except for

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B. cereus, which has very high resistance to the extract, their MBCs were double the MIC concentration. Astridwiyanti et al. (2019) reported that the formulation has antibacterial activity against *S. aureus*. According to the findings of this study, the ethanolic extract of red dragon fruit peel has antibacterial activity against *S. aureus*. According to Hendra et al. (2019), dragon fruit peel extract inhibited the growth of both positive and negative gram bacteria, including *E. coli, K. pneumonia, S. typhimurium, S. aureus, B. subtilis, E. faecalis*. According to the findings of this study, the pigment and peel extracts could be considered a good source of potent natural antibacterial agents. Based on previous reports, dragon fruit peel extract offers antibacterial effects, especially against several gram-negative agents that cause diarrhea, such as *S. aureus, B. subtilis,* and *E.a coli* (Astridwiyanti, Mahendra and Dewi, 2019; Hendra *et al.*, 2019). Therefore, this study aimed to investigate the potential of red dragon fruit peel as an antidiarrhea in vivo and in vitro assay.

METHODOLOGY

Materials

Dragon fruit skin, methanol (98%), magnesium powder, HCl, aquadest, Mayer's reagent solution, Bouchardat's reagent solution, Dragendorff's reagent solution, 1% Fe (III) Chloride reagent, ethanol (95%), sulfuric acid solution, 0.4 M Pb (II) acetate solution, isopropanol, chloroform, Molisch's reagent, ether solution, acetic acid solution, DMSO, loperamide, NA and NB media, McFarland standard, Na-CMC, and castor oil.

Methods

Study design

This experimental study was performed from May to June 2022 at the Microbiology and Pharmacology Laboratory of Universitas Prima Indonesia. The animal model used was Wistar rats (*Rattus novergicus*) with ethical clearance approved by the Health Research Ethics Committee from Universitas Prima Indonesia with No. 035/ KEPK/UNPRI/X/2021.

Extraction process

This study used fresh dragon fruit peels collected from a traditional market in Medan City. The dragon fruit peel extract was cut, dried, and meshed into the simplicial powder. After that, the dragon fruit simplicial powder was soaked into 98% methanol with a ratio of 1:3. It was regularly stirred and filtered after three days. The residue was re-macerated in the same way two times. Meanwhile, all filtrates were collected in the same container for evaporation. The rotary evaporator underwent evaporation at a temperature of 40-50^{o}C, followed by water bath evaporation until it formed a concentrated extract. After that, the extract yield was determined by dividing the mass of the extract by the mass of the simplicial powder and multiple by 100% (Mutia, 2019; Suhartomi *et al.*, 2020; Chiuman *et al.*, 2021).

Phytochemical analysis

Phytochemical analysis was begun by phytochemical screening to look for some phytochemicals, including phenolic, flavonoid, alkaloid, terpenoid/steroid, tannin, and saponin (Widowati *et al.*, 2017; Depari *et al.*, 2021). After that, the analysis was continued by measurement of polyphenol compounds level by colorimetric assay, described by Mutia et al. and Gulo et al. It included total phenolic, flavonoid, and tannin content, which were expressed as GAE/g DW (Gallic Acid Equivalent per gram Dry Weight), QE/g DW (Quercetin Equivalent per gram Dry Weight), and TAE/g DW (Tannic Acid Equivalent per gram Dry Weight). Folin-Ciocalteu, Aluminium Chloride, and Folin-Denis reagent were the reagent that was used to analyze the total phenolic, flavonoid, and tannin content, respectively (Gulo *et al.*, 2021; Mutia, Ginting, and Yulizal, 2021).

In vitro antidiarrheal assay

This study used two methods to evaluate the antidiarrhea effect of dragon fruit peel extract, including disc diffusion and microdilution methods.

Disc diffusion

Initially, the concentrated dragon fruit peel extract was diluted into five different concentrations, including 250, 200, 150, 100, and 50 mg/ml, with DMSO as the solvent by the volumetric flask. Meanwhile, the standard and control used ciprofloxacin and DMSO at inert concentrations.

After the sterilization, 20 mL of NA media were distributed into some Petri dishes filled with 1 mL *Escherichia coli* suspension (McFarland Standard 0.5). After that, it was homogenized, and every dish was placed in five different disc diffusions that had been diffused by extract, ciprofloxacin, or DMSO. However, the standard and control were only placed two disc diffusions on their dishes. All Petridishes were incubated at 35-37^{o}C for 18-24 hours. At last, the width of the clear zone was measured as the inhibition zone by caliper and expressed as mm (Julianti, Rajah, and Fidrianny, 2017)(Safitri, Nur Adiratna and Drajat S., 2020; Milanda *et al.*, 2021).

Table I. Group of Treatment of In Vivo Diarrhea Assay

Group	Treatment
Control	All rats did not receive any treatment.
Standard	All rats received 1.5 mg/ kg BW of loperamide oral suspension
Dragon Fruit Methanol Extract-1	All rats received 400 mg/kg BW extract.
Dragon Fruit Methanol Extract-2	All rats received 200 mg/kg BW extract.
Dragon Fruit Methanol Extract-3	All rats received 1600 mg/kg BW extract.

Microdilution assay

Microdilution assay was performed to determine the Minimum Inhibitory Concentration (MIC) and Minimum Killing Concentration (MKC). This assay used 96-well plates as the container for the media. A 100 μL of NB media was filled into 12 columns on 96-well plates. The 12th column was filled only by NB media and acted as sterile control. Meanwhile, the 11th column added 100 μL of *Escherichia coli* suspension and acted as the growth control. After that, 100 μL dragon fruit peels extract that showed the lowest antibacterial effect in the disc diffusion methods was filled into the first column and homogenized. Then, 1 mL of the mixture in the first column was filled and homogenized in the second column, and it was repeated for the remaining tube (until the tenth column). In the tenth column, 100 μL of the mixture in this column was discarded. Finally, 100 μL of *Escherichia coli* suspension was filled and homogenized into each column from the 1st to the 10th. This 96-well plate was placed at 35-37^{o}C for 18-24 hours in an incubator. The lowest concentration, known as the MIC, showed a clear appearance without bacterial growth. Furthermore, some columns which showed a clear appearance were subcultured into the NB agar by pour and spread plate methods, then stored at 35-37^{o}C for 18-24 hours. The lowest concentration, which showed no bacterial growth in NA media, was the MKC (Julianti, Rajah, and Fidrianny, 2017; Milanda *et al.*, 2021).

Oral suspension formulation

The dragon fruit peel extract was formulated into an oral suspension made of Na-CMC. 400 mg dragon fruit peels methanol extract and 20 mg loperamide were mixed into 10 ml 0.5% Na-CMC to form dragon fruit peels methanol extract and standard, respectively (Kanon, Fatimawati and Bodhi, 2012; Chiuman, 2019; Mutia and Chiuman, 2019).

Acute toxicity assay

The acute toxicity assay was performed by the Fixed Dose Method. This study used five female Wistar rats (2-3 months,150-200 g). The first rat received 2,000 mg/kg BW of dragon fruit peel methanol extract that had been fasted for 4 hours. Then, this rat was observed within one hour after administration of the extract to see physical and behavioral changes in the rat that indicated possible toxicity, such as changes in eating and drinking behavior, locomotor activity, lethargy, and other signs that lead to weakness or distress and death. After 1 hour of observation, the oral acute toxicity assay was continued according to the results of observations in the first mice. Finally, all mice were observed daily to assess for signs of acute toxicity for the next 14 days.

In vivo antidiarrheal assay

Some models, including Castor Oil-Induced Diarrhea, Castro Oil-Induced Gastrointestinal Motility, and Castor Oil-Induced Enteropooling Activity, were used to evaluate the in vivo antidiarrheal effects of dragon fruit peel methanol extract. The in vivo study used 25 male Wistar rats (2-3 months,150-200 g) that acclimatized for one week and rats have been selected accordingly, then received some treatments as described in Table I (Abdela, 2019; Kifle, Atnafie, *et al.*, 2021; Kifle, Kidanu, *et al.*, 2021).

Castor oil-induced diarrhea

Castor oil-induced diarrhea was performed by application of 0.5 ml of Castor oil via the oral route. Before the administration, all rats had fasted for 8 hours. All rats received some treatment according to the treatment group one hour after Castro oil administration. Then, all rats after 4 hours were evaluated for the onset of diarrhea, defecation frequency, and fecal output mass (loose and total stool mass in grams). These data were used to determine both percentages of diarrhea and fecal mass output inhibition by calculation:

Percentage of Diarrhea Inhibition: (a-b)/ a x 100%
The a and b were the average loose feces in the control and sample groups, respectively.

Mass percentage of loose stool output: a/b x 100%
The a and b were the average mass of loose feces in the sample and control groups, respectively.

Table II. Antibacterial Activity of Dragon Fruit Peels Methanolic Extract by Disc Diffusion Method in *Escherichia coli*

Concentration	Width of Inhibition Zone (mm)		P-Value
	Mean	SD	
Control[a]	6.00	0.00	
Standard[b]	32.63	1.17	
250 mg/ ml[a]	8.87	1.07	
200 mg/ ml[a]	8.50	1.46	< 0.05
150 mg/ ml[a]	6.94	0.18	
100 mg/ ml[a]	7.43	1.45	
50 mg/ ml[a]	6.00	0.00	

P-value was obtained from the One Way ANOVA; Different superscripts in the same column show significant differences based on Post Hoc Test Tukey HSD

The mass percent of total fecal output: a/b x 100%
The a and b were the average total fecal mass in the sample and control group, respectively.

Castro oil-induced gastrointestinal motility

Castro oil-induced gastrointestinal motility was conducted by grouping all rats and fasting for 8 hours. After that, all rats received some treatment according to the group. After one hour, all rats received 0.5 ml of castor oil via the oral route. After an hour of Castro oil application, all rats received 1 ml of 5% activated charcoal as the marker via the oral route. All rats were sacrificed after an hour of marker administration, and then the small intestine was dissected from pylorus to cecum. After that, the distance from the pylorus to the farthest distance of activated charcoal movement. The formula was used to calculate the peristaltic index:
Peristaltic index: a/b x 100%

The a and b were the displacement distance of the ingested activated charcoal and the length of the entire small intestine, respectively. Meanwhile, the percentage of inhibition of the peristaltic index is formulated by the following formula:
Percent of Peristaltic Index Inhibition: (a-b)/a x 100%

The a and b were the Peristaltic-index of the control and sample groups, respectively.

Castro oil-induced enteropoolong activity

Castro oil-induced enteropoolong activity was conducted by grouping all rats and fasting for 8 hours. After that, all rats received some treatment according to the group. An hour after treatment, all rats received 0.5 ml of castor oil via the oral route. All rats were sacrificed by cervical dislocation, and the small intestine was dissected from pylorus to cecum. Ethical clearance approved by the Health Research Ethics Committee from Universitas Prima Indonesia with No. Letter: 035/ KEPK/UNPRI/X/2021. The contents of the small intestine are then squeezed out and placed in a separate container to measure the volume. Then the small intestine was then re-weighed in a filled and empty condition. These data were used to determine the percent inhibition of volume and mass of the contents of the small intestine with the following formula:
Percent of Mass Intestinal Contents Inhibition: (a-b)/a x 100%
The a and b were the mean mass of the small intestine contents in the control and sample groups, respectively.
Percent of Volume Intestinal Contents Inhibition: (a-b)/a x 100%
The a and b were the average volumes of small intestine contents in the control and sample groups, respectively. Finally, the antidiarrheal effect of dragon fruit methanol extract was expressed as Antidiarrheal Index (ADI), which was determined by the following formula.

$$\text{ADI: } \sqrt[3]{Dfeqx\ Gmeq\ x\ Pfreq}$$

The Dfreq, Gmeq, and Pfreq were Delay defecation or diarrhea onset (% against a control group), Percent of Peristaltic Index inhibition, and Percent of Diarrhea Inhibition, respectively. Meanwhile, the following formulation determined Dfreq as the delayed defecation or diarrhea onset.

$$\text{Dfreq= (a-b)/b x 100\%}$$

The a and b were average onsets of diarrhea in the sample and control groups, respectively.

Data analysis

Initially, all data were analyzed by descriptive statistics of central tendency and data distribution. The analysis continued to an inferential statistic based on data distribution. If

Table III. Determination of MIC Value in Dragon Fruit Peels Methanolic Extract by Microdilution Method in *Escherichia coli*

Concentration	Turbidity	
	I	II
Media Control	-	-
Bacterial Growth Control	+	+
Extract Control	-	-
100 mg/ ml	-	-
50 mg/ ml	-	-
25 mg/ ml	+	+
12.50 mg/ ml	+	+
6.25 mg/ ml	+	+
3.13 mg/ ml	+	+
1.56 mg/ ml	+	+
0.78 mg/ ml	+	+
0.39 mg/ ml	+	+

data distribution was normal, then the data were expressed as mean and SD, and the data were analyzed by one-way ANOVA and followed by Post Hoc Test Tukey HSD. When the data distribution was not normal, then the data were expressed as median with minimum and maximum and analyzed by Kruskal-Wallis.

RESULT AND DISCUSSION

Characteristic of extract

This study used 2,000 g of fresh dragon fruit peels, then dried to form 1896.9 g of dry Simplicia powder. The dry Simplicia powder was soaked in 7,500 mL of methanol solution as a solvent for maceration. After that, the maceration resulted in 79.97 g of concentrated dragon fruit peel methanol extract.

Phytochemical analysis

The result of the phytochemical screening shows that the dragon fruit peel methanolic extract had some phytochemicals, including phenol, flavonoid, alkaloid, and tannin. The total polyphenol compound content, which shows that the total phenol, tannin, and flavonoid of dragon fruit peel methanolic extract were 10.52 ± 0.89 GAE mg/g extract, 3.41 ± 0.27 TAE mg/g extract, and 1.37 ± 0.07 QE mg/g extract, respectively.

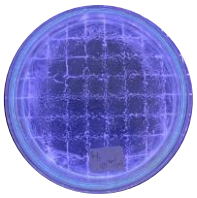
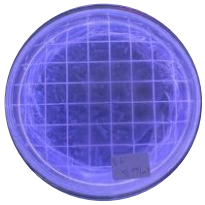
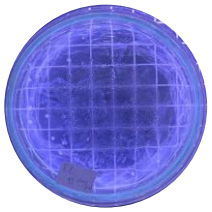
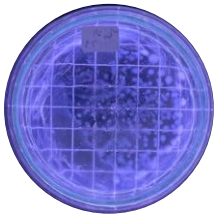
In vitro antidiarrheal assay

The result of the disc diffusion assay showed that there was a significant difference in the inhibition zone in all concentrations of dragon fruit peel methanol extract (p-value <0.05). The widest average diameter of the inhibition zone was found in the standard group (ciprofloxacin), which was 32.63 mm, and the narrowest was found in the control group (DMSO) was 6.00 mm. The width of the inhibition zone in the control group was not shown as the clear zone, and this value indicated the width of disc diffusion. It means that the control group does not show any antibacterial activities. On the other hand, the increasing concentration of dragon fruit peel methanol extract also increased the width of the inhibition zone. The highest inhibition zone in various concentrations at 250 mg/ml (8.87 mm), followed by 200 mg/ml (8.50 mm), 150 mg/ml (6.94 mm), 100 mg/ml (7.43 mm), and the lowest concentration (50 mg/ml) did not show any antibacterial activities, which was shown as the width of disc paper of 6.00 mm.

The antibacterial activity analysis was continued to the microdilution assay to look for the MIC and MKC. The lowest concentration of dragon fruit peels methanol extract, which still showed the antibacterial effect (100 mg/ml), was subcultured into the NB in 96-well plates into several concentrations for obtaining the MIC, and the result of this subculture was described in Table III. The lowest concentration of dragon fruit peel extract, which showed no turbidities, was 50 mg/ml, defined as MIC. The two lowest concentration, 50 and 100 mg/ml, was then subcultured into the NA media to determine the MKC value, and the result of this subculture was described in Table IV.

Based on Table IV, the 50 mg/ml showed some *Escherichia coli* bacteria growth. However, the higher concentration, 100 mg/ml, showed no bacteria growth. Interestingly, the width of the inhibition zone of 100,150, and 200 mg/ml were better than the positive control treated with

Table IV. Determination of MKC Value in Dragon Fruit Peels Methanolic Extract by Microdilution Method in *Escherichia coli*

Concentration	Repetition	Bacterial Growth	Images of inhibition zone
100 mg/ ml	I	-	
	II	-	
50 mg/ ml	I	+	
	II	+	

loperamide. Hence, MIC and MKC of dragon fruit peel methanol extract against *E.a coli* were 50 and 100 mg/ ml, respectively.

Acute toxicity assay

The acute toxicity assay during 14 days revealed that all mice did not show any acute toxicity signs, including changes in eating and drinking patterns, changes in activity locomotor, lethargy, weakness, or distress, until death, up to 2000 mg/kg BW.

In vivo antidiarrheal assay

Based on data from the in vitro antibacterial and acute toxicity assay results, in vivo antibacterial assay was used in 100, 200, and 400 mg/kg. Initially, the result of castor oil-induced diarrhea of dragon fruit peel methanol extract has affected the onset of diarrhea, frequency of diarrhea, and mass of loose feces (p-value <0.05). Administration of dragon fruit peel methanol extract at the highest dose can prolong the onset of diarrhea. Whereas the frequency of diarrhea and mass of loose or total feces significantly decreased with increasing extract doses. The best antidiarrheal effect was found in the highest concentration. It showed that the average onset of diarrhea, frequency of diarrhea, mass of loose feces, and mass of total feces were 69.20 minutes, 5.20 times, 5.20 g, and 1.13 g, respectively. These results also showed that Castro oil successfully induced diarrhea.

Based on experimental data, the antidiarrheal activity was expressed as the percentage of defecation inhibition and the percent of loose and total feces output, even though not as good as loperamide. The highest percent inhibition of defecation among the group was the Dragon Fruit Peel Methanol Extract-3 (183.61%), followed by the Dragon Fruit Methanol Extract-2 (132.79%), and the lowest was Dragon Fruit Methanol Extract-1 (95.08%). Meanwhile, the highest percent mass of loose feces was found in the Dragon Fruit Methanol Extract-1 (73.53%), followed by the

Table V. Analysis of Antidiarrheal Activity from Dragon Fruit Peels Methanol Extract with In Vivo Antidiarrheal Assay

Groups	Delay in defecation, *min* (Dfeq)	Gut Meal Travel Distance (Gmeq)	Purging Frequency in Number of Loose Feces (Pfreq)	In Vivo ADI
Control	-	-	-	-
Standard	218.03	52.14	54.04	85.01
Dragon Fruit Peels Methanol Extract-1	95.08	32.22	26.47	43.28
Dragon Fruit Peels Methanol Extract-2	132.79	40.16	37.50	58.48
Dragon Fruit Peels Methanol Extract-3	183.61	47.83	51.10	76.56

Dragon Fruit Methanol Extract-2 (62.50%), and the lowest was found in the Dragon Fruit Methanol Extract-3, which was 48.90%. Finally, the percent mass of total feces had a similar result to that of loose feces. The lowest percent mass of total feces was found in the Dragon Fruit Methanol Extract-2 (90.04%), followed by Dragon Fruit Methanol Extract-1 (87.86%), and the lowest was Dragon Fruit Methanol Extract-3, which was 86.75%.

The analysis of antidiarrheal was continued with the Castro Oil-Induced Gastrointestinal Motility method to evaluate the gastrointestinal motility or transition. The dragon fruit peel methanol extract significantly reduced the gastrointestinal motility or transition and the peristaltic index (P-value <0.05), even though not as good as loperamide. In addition, the small intestine length of all rats ranged from 115.80 cm to 126.20 cm (P-value > 0.05). The ability of dragon fruit peel methanol extract to reduce intestinal peristalsis in this study was described as the percent inhibition of the peristaltic index. The highest percent inhibition of peristaltic inhibition in groups that received dragon fruit peel extract was showed by the Dragon Fruit Peels Methanol Extract-3 (47.83%), followed by the Dragon Fruit Peels Methanol Extract-2 (40.16%), and the lowest was found in the Dragon Fruit Peels Methanol Extract-1, which was 47.83%.

Furthermore, the analysis of the antidiarrheal activity was continued to the enteropoolong activity method. The dragon fruit peel methanol extract has reduced the volume and mass of the small intestine content (P value <0.05). These results showed better performance compared to standard treatment with loperamide. The increased dragon fruit peel extract dose decreased the small intestine volume. The lowest volume of small intestine among the dragon fruit peels extract was found in Dragon Fruit Peels Methanol Extract-3 (0.09 ml [48.28%]), followed by Dragon Fruit Peels Methanol Extract-2 (0.10 ml [42.53%]), and the highest was found in the Dragon Fruit Peels Methanol Extract-1 (0.14 [20.69%]). Meanwhile, the mass of the small intestine content showed a similar result to the volume. The increased dose of extract has decreased the mass of the small intestine content. The lowest mass was found in Dragon Fruit Peels Methanol Extract-3 (5.71 g [15.59%]), followed by the Dragon Fruit Peels Methanol Extract-2 (6.06 g [13.17%]), and the highest was found in the Dragon Fruit Peels Methanol Extract-1 (6.19 g [7.10%]).

Furthermore, our research shows that more doses of the extract resulted in a higher antidiarrheal index. However, this value is not better than the standard group, which received the commonly used antidiarrheal drug, loperamide. The highest antidiarrheal index value was found in the standard group (85.01%), followed by the Dragon Fruit Peels Methanol Extract-3 (76.56%), the Dragon Fruit Peels Methanol Extract-2 (58.48%), and the lowest was found in the Dragon Fruit Peels Methanol Extract-1 (43.28%).

The antidiarrheal effect of dragon fruit peel is due to pectin and various phytochemicals that act as absorbent and antibacterial agents. Some previous studies have been performed to evaluate the antibacterial effects of dragon fruit peels. Manihuruk et al. (2017) reported that dragon fruit peel aqueous extract had an antibacterial effect against various bacteria, including *S. aureus* ATCC 25923 (12.38 ± 2.36 mm), *P. aeruginosa* ATCC 27853 (10.09 ± 0.96 mm), *S. enterica* ser. *T.* ATCC 14028 (8.25 ± 1.37 mm), *B. cereus* (8.11 ± 2.85 mm), and *E. coli* ATCC 25922 (7.70 ± 2.39 mm). Furthermore, this antibacterial effect could not be separated from the presence of phytochemicals, such as phenols, flavonoids, triterpenoids, steroids,

saponins, and tannins. Flavonoids are reported to have antibacterial effects by inhibiting bacterial nucleic acid synthesis, cell membrane function, and metabolism pathway. Flavonoids prevented the interconnection and hydrogen bonding of A and B rings on the nucleotide base, which inhibits the formation of DNA and RNA. The location of the hydroxyl group at position 2', 4' or 2', 6' is hydroxylated on ring B, and 5,7 hydroxylated on ring A plays a vital role in the antibacterial activity of flavonoids. Flavonoids also cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes due to interactions between flavonoids and bacterial DNA (Manihuruk, Suryati, and Arief, 2017). Khotimah et al. also reported that the MIC values of the ethanol, n-hexane, and ethyl acetate fractions of dragon fruit peel extract for *E. coli* were 80, 20, and 80 mg/ml, respectively. The MIC values for *S. aureus* are the same MIC value of 10 mg/ml (Khotimah *et al.*, 2021).

The antidiarrheal effect of dragon fruit peels may also cause by the presence of pectin. Norulfairuz et al. (2017) reported that the extraction time would affect the pectin in dragon fruit peel extract (16-20%). Pectin is a complex carbohydrate molecule used in various food applications as a gelling, thickening, stabilizing, and emulsifying agent.

Furthermore, Subagio et al. (2020) reported that pectin could increase stool consistency because pectin will absorb and binding with water and fat, thereby increasing the consistency of the stool. Pectin is related to excess mucus from the digestive tract and neutralizes toxins from bacteria and irritant substances. The current study used was castor oil-induced diarrhea, where the pectin in dragon fruit peel extract can also bind to the structure of ricinoleic acid in Castrooil, thereby preventing castro oil from binding to the intestinal surface, preventing gastrointestinal irritation, and preventing the onset of secretory diarrhea (Zaidel *et al.*, 2017; Sari, 2020; Subagio *et al.*, 2020).

CONCLUSION

Overall, it can be concluded that the dragon fruit peel methanol extract has an antibacterial and antidiarrheal effect. The MIC and MKC of dragon fruit peel extract against *Escherichia coli* were 50 and 100 mg/ml, respectively. Furthermore, the highest dose of dragon fruit methanol extract (400 mg/ kg BW) has the highest antidiarrheal index, w6.56%. Thus, it can prolong the onset of diarrhea (69.20 ± 4.02 minutes), decrease the frequency of defecation (5.20 ± 0.84 times), liquid stool mass (0.27 ± 0.04 gr), and total stool mass (1.13 ± 0.04).

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