



ANTIDIARRHEAL EFFECT OF LIME PEEL EXTRACT ON BISACODYL-INDUCED MICE

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ABSTRACT

Background: Diarrhea is a condition of increased fecal water content of more than 200 grams or 200 ml/24 hours with a liquid or semi-liquid consistency. Complications of diarrhea, dehydration and malnutrition are still world health problems. Lime peel extract contains tannins which have astringent properties. Tannins are indicated as an alternative anti-diarrheal agent by tightening and forming a protective layer on the intestinal mucosa thereby reducing osmolarity. **Aim:** To examine the consistency, fecal water content, and frequency of defecation of bisacodyl-induced diarrhea in mice that are given lime peel extract. **Methods:** This research was an experimental with post test only group design. Thirty-six male mice were included and then divided into 5 groups with bisacodyl induction and 1 normal control group (KM) without bisacodyl induction. The negative control group (KN) was given 0.5 ml Tween 80 1%, the positive control group (KP) was given attapugite 0.4 mg/40gW, treatment group 1 (X1), treatment group 2 (X2), treatment group 3 (X3) were given lime peel extract at graded doses of 125, 250, and 500 mg/KgW. Consistency, fecal water content, and frequency of defecation were observed every 30 minutes for 4 hours by observing the consistency of feces, weighing wet and dry feces to measure the water content, and measuring the frequency of defecation in each group. Data were analyzed statistically using *Kruskal Wallis* test with *Post Hoc Mann Whitney* test. **Results:** There were significant differences between the comparison of defecation frequency in the X3 group and the KM group, for the fecal water content comparison between the X3 group and the KN group, and no significant differences in the following group comparisons. **Conclusion:** Lime peel extract cannot reduce the frequency of defecation, fecal water content, and increase the consistency of feces significantly.

Keywords: Diarrhea, lime peel extract, tannins, astringent, consistency, fecal water content, frequency of defecation

INTRODUCTION

Diarrhea is a condition in which a person defecates with a soft or in the form of watery stool and the frequency is more frequent than usual (three or more times) in one day. The fecal water content which forms a liquid or semi-liquid consistency has more than 200 grams or 200 ml / 24 hours.¹ Based on the duration, diarrhea can be classified into acute diarrhea that past within 2 weeks, persistent diarrhea that last more than 2 weeks or more, and chronic diarrhea that last more than 30 days or years.² Diarrhea can cause mild to severe dehydration as well as malnutrition which causes Severe Acute Malnutrition (SAM) in children under five years.^{3,4} Severity of diarrhea is categorized according to the degree of dehydration.⁵

Diarrhea is still one of the biggest health problems in the world. According to research conducted by The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD), there were around 4.4 billion episodes of diarrhea cases in all age groups in 2016. Furthermore, in children under five, episodes of diarrhea is 1.1 billion cases. According to GBD, cases of diarrhea in individuals

under five have a mortality rate of 70.6 deaths per 100,000 individuals.⁶ Based on Indonesia Health Profile 2017; There were at least 21 cases of diarrhea with a Case Fatality Rate (CFR) of 1.97% or 1.725 deaths.⁷ In 2018, the coverage rate of diarrhea cases in children under five in Central Java is 40.91%. This is higher than the national average of 37.88%.⁸

Diarrhea occurs due to impaired absorption, abnormalities in intestinal motility, and osmotic abnormalities in the intestinal tract. The clinical manifestations of diarrhea are well known, but diarrhea case management is still inadequate.⁹

The management diarrhea and dehydration which is currently used is fluid management by administering Oral Rehydration Solution (ORS) with zinc supplementation parallel to the clinical criteria. Other managements of diarrhea are antibiotics, peristalsis, astringents, absorbents, and selective anti-secretions.¹⁰ Astringent is an antidiarrheal which has a mechanism of action in the process of mucosal shrinkage and controls intestinal irritation resulting in the decrease of fecal water content. One of the example of astringent is tannins. Tannins are



astrigents that are classified as polyphenol compounds. Tannins can bind and precipitate or shrink proteins. Apart from being anti-diarrheal, Tannins are also used as antiseptic in wound healing and anti-bleeding.¹¹

There are many plants that contain tannins, including tea, red wine and lime.¹¹ Lime or *Citrus aurantifolia* contains tannins with different concentrations in different parts of the plant. In 0.5 g of lime peel extract, there are 0.64% tannins.¹² According to the Center for Agricultural Data and Information, the total consumption of oranges of various species in Indonesia was around 1.78 million tons in 2015, and the amount of unprocessed orange peel waste was 500,000 tons per year.¹³ There are still a few innovations for lime peels to reduce the amount of waste in Indonesia.

Lime peels contains many beneficial components for the human body, including polyphenols, essential oils and carotenoids.¹⁴ Lime peels can be used as an antioxidant, antibacterial, antifungal, and antiviral.¹¹ Lime peels also contain tannins which allow it to have antidiarrheal effect.

With sufficient amount and benefits of the tannins contained in lime peels followed by adequate resources, it has not been followed by utilization and innovation from various economic sectors and alternative medicines. To increase the utilization of lime peels, this study examined the effect of the lime peel extract on the consistency, fecal water content, and the frequency of defecation in mice with diarrhea.

METHODS

This research was an experimental with post test only group design. The aim of this research was to figure out the consistency, water content in feces, and the frequency of defecation of mice given lime peel extract. Inclusion criteria for mice are male, weight 25-30 grams, 3 months of age, healthy, appeared active, and no anatomical abnormalities. The criteria for exclusion are mice with behavioral changes and death.

The research samples were obtained using the random allocation sampling method which was then divided into 5 treatment groups with bisacodyl inducement and 1 normal control group (KM) which was not induced by bisacodyl. There were 6 mice per group, therefore the number of samples used were 36 mice. The negative control group (KN) was given 0.5

ml Tween 80 1%, the positive control group (KP) was given attal pugite 0.4 mg /40gBW,¹⁵ treatment group 1 (X1), treatment group 2 (X2), treatment group 3 (X3) were given lime peel extract doses of 125, 250, and 500 mg/KgW.

The independent variable of this study was the dose of lime peel extract. The dependent variables were consistency, water content in feces, and frequency of defecation of mice. For the controlled variable it was the dose of bisacodyl.

In this research, samples were acclimatized for 1 week before the start of treatment, mice were fed standard pellet and water. After that samples were transferred into a plastic cage that has been laid on a parchment paper with an iron mesh holder for 1 hour every day. After randomization and group division, 5 treatment groups except for KM group were induced with bisacodyl 100 mg/KgBW to cause diarrhea.¹⁶ Following the defecation, samples were given drugs or extracts according to the dosage and treatment group.¹⁷ Subsequently the consistency, fecal water content, and frequency of defecation were measured every 30 minutes for 4 hours. Every 30 minutes the filter paper or parchment paper should be replaced with a new one.

Maceration method was used to extract the lime peel. 500 grams of lime peel were removed from the fruit, then washed thoroughly. After that, the lime peel was cut into small pieces and then oven-dried. Lime peel that had been dried, then macerated using 70% ethanol to form a gel. Lime peel extract gel was kept in a glass bottle with room temperature away from the sun. The lime peel extract which was still in the form of gel was weighed and compared with the required amount of emulsion according to the treatment group. After that, the lime peel extract gel was dissolved with 1% to 6 ml tween 80.¹⁷

To analyze the quantity of tannins in lime peel extract, the extract was weighed and dissolved with aquabidest up to 10 ml. Then, with pipette took 1 ml and put it in a 10 ml container that already contained 7.5 ml of aquabidest. After that 0.5 ml of folin denis solvent was added, pour 1 ml of 20% saturated Na₂CO₃ solution. The extract was then incubated for 15 minutes and read through a spectrophotometer with a wavelength of 740 nm. The reading is calculated using standard curve.¹⁸

The data collected were primary data, which were obtained directly from observing the



consistency of feces, weighing wet and dry feces to measure the water content, and calculating the frequency of defecation in each group. Observation were done subjectively and there was no standard provision regarding objective assessment yet. Data was tested with *Saphiro-Wilk*. Then, it was followed by *Kruskal Wallis* test with *Mann-Whitney post hoc* test.

RESULTS

Table 1 shows the average defecation frequency of defecation of each group after the administration of lime peel extract, attalugite, bisacodyl, and Tween 80 1%. X2 had the highest average of defecation compared to X1 or X2. Frequency of defecation observed directly within 4 hours, by comparing the number of defecation before administration of lime peel extract (only bisacodyl) and after administration.

The average defecation frequency of defecation of each group were compared using *mann whitney test* (table 2). The result showed there was a significant difference between X3 and KM.

The average fecal water content of each group after the administration of extract and other substances is shown in table 3. X2 group had the highest water content average followed by KN and KM respectively. Other treated group such as X3 and X1 had the lowest average water content.

The average fecal water content of each group were compared using *mann whitney test* (table 4). The result showed there was a significant difference between X3 and KN. Average fecal water content in X3 was lower in average compared to KN.

Table 5 shows the fecal consistency of each group after every 30 minutes for 4 hours after the administration of extract and other substances. X3 had the fastest consistency change from soft to solid in one hour, followed by X1 in two hour. X2 had the longest consistency change compared to other extract treated group.

Table 1. Defecation frequency of each group

Group	Ave. frequency of defecation ±Deviation Std.	Minimum Frequency	Maximum Frequency
KM	2,8750±0,99	1	4
KP	3,6±2,6	1	7
KN	2,75±1,75	1	6
X1	3,25±2,21	1	6
X2	3±2	1	7
X3	4±2,64	1	6

Table 2. Group comparison of defecation frequency

Group	Frequency of Defecation					
	KM	KP	KN	X1	X2	X3
KM	-	0,192	0,71	0,099	0,856	0,036*
KP		-	0,35	0,73	0,261	0,43
KN			-	0,201	0,849	0,085
X1				-	0,142	0,657
X2					-	0,056
X3						-

* $p < 0,05$ Mann Whitney test

Table 3. Fecal water content average of each group

Group	Ave. frequency of defecation ±Deviation Std.	Minimum Content	Maximum Content
KM	65,96±12,93%	50%	90,16%
KP	67,71±18,86%	35,71%	84,5%
KN	73,867±16,95%	33,33%	85%
X1	67,78±24,03%	23,53%	79,1%
X2	68,83±25,64%	25%	95%
X3	48,61±13,3%	33,33%	57,57%

Table 4. Group comparison of fecal water content

Group	Fecal Water Content					
	KM	KP	KN	X1	X2	X3
KM	-	0,503	0,132	0,548	0,433	0,25
KP		-	0,515	0,264	0,987	0,112
KN			-	0,067	0,469	0,024*
X1				-	0,215	0,59
X2					-	0,084
X3						-

* $p < 0,05$ Mann Whitney test



Table 5. Fecal consistency description of each group

Fecal Consistency every 30 minute	Group					
	KM	KP	KN	X1	X2	X3
1	Soft	Soft	Clumpy liquid	Solid	Soft	Soft
2	Soft	Soft	Soft-liquid	Clumpy semi-liquid	Yellowish soft-liquid	Semi-solid
3	Clumpy liquid	Clumpy liquid	Clumpy liquid	Semi-solid	Soft	Solid
4	Semi-solid	Semi-Solid	Liquid	Solid	Clumpy liquid	-
5	Semi-solid	Solid	Clumpy liquid	-	Yellowish clumpy liquid	-
6	Solid	-	Soft	-	Yellowish clumpy liquid	-
7	Solid	-	Soft	-	Solid	-
8	Solid	-	Solid	-	Solid	-

The comparison of the average defecation frequency between groups is shown in the bar chart below (figure 1). Differences between groups were tested by the *Kruskal Wallis* test. Administration of lime peel extract did not significantly affected the frequency of defecation in mice with diarrhea-induced bisacodyl. As can be seen from the bar chart, the administration of bisacodyl without attapulgit in the KN group did not significantly increase the frequency of defecation compared to the KM group.

From the results of the *Mann Whitney* test in table 2, there was no significant difference between the 2 groups, except between the KM and X3 group with $p = 0.036$. The frequency of defecation in the X3 was higher in average compared to KM group.

Comparison of fecal water content between groups is shown in the bar chart in figure 2. Differences between groups were tested by the *Kruskal Wallis* test. Lime peel extract administration did not have a significant effect on the water content of mice faeces.

The results of the *Mann Whitney* test in table 4 show that the water content of mice in the X3 group was significantly lower than KN group which was only induced by diarrhea without the administration lime peel extract. This suggests that lime peel extract, at a dose of 500 mg / kgW may be useful in anti-diarrheal therapy.

Table 5 shows that bisacodyl effects begin to appear in the first 30 minutes or two. Fecal consistency returned to solid at the fifth interval for KP group. In the treatment group that was given lime peel extract, the consistency of the feces returned to solid between the third and the eighth interval. Based on testing the quantity of tannins contained in the lime peel extract, the quantitative content of tannins was 1.305%.

DISCUSSION

The average frequency of defecation in the bisacodyl-induced group without attapulgit 0.4 mg/40 gW administration (KN) was not significantly different when compared to the KP group. This may be caused by suboptimal bisacodyl dose to such an extent that it cannot increase the frequency of defecation. However, looking at the fecal consistency data in the KN group which showed a more watery fecal consistency than the other groups, the researchers concluded that there was an effect of diarrhea induction, and data analysis was continued.

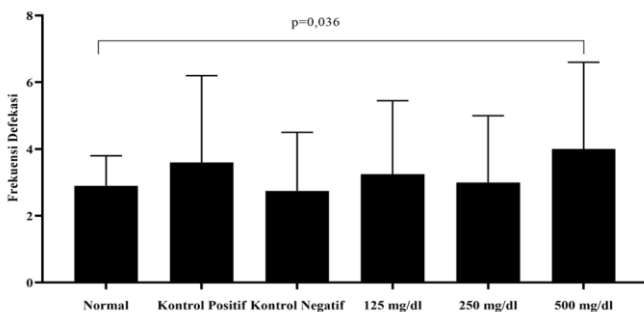


Figure 1. Defecation frequency of each group

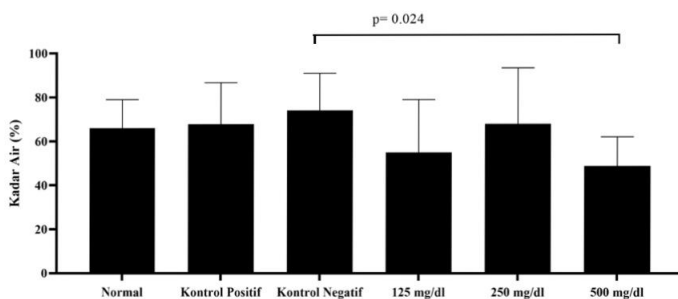


Figure 2. Fecal water content average of each group

Data the frequency of defecation and water content in each group were tested for normality using the Shapiro-Wilk test. The normality test showed an abnormal distribution ($p < 0.05$), therefore hypothesis test was carried out by using a non-parametric test.



The results of data analysis showed that administration lime peel extract did not show a significant reduction in defecation when compared to the KN group which was not given therapy with lime peel extract or attalpugite. Lime peel extract may not have the antidiarrheal effect as expected, or it may act as an antidiarrheal, however it is not through inhibitory action. The results obtained contradict with the research of Juliana (2017) and Adejoh et al. (2018), each research showed a significant reduction in the frequency of defecation of experimental animals which were given lime and sweet orange peel extract therapy when compared to other treatment group and the control group.^{17,19} Both studies indicate the action of tannins in diarrhea with different methods where the first study showed the effect of tannins contained in lime plant stem barks on intestinal motility and the second study indicated the action of tannins contained in sweet orange peels on the inhibition of diarrhea stimulation.

In inter-group difference test on fecal water content, it showed a significant difference between KN group and the treatment group showing a change in fecal consistency. This change also occurred in the KM group which was not given any treatment. The fecal consistency of KM group should not have changed, however, in this study it was seen that diarrhea happened up to the 5th interval and then solidified at the 6th to 8th intervals. There are several things that cause changes in the consistency of feces in the KM group, including: bacterial infection due to an unhygienic environment, or congenital infection acquired from the breeding site. This is also mentioned by Lindsey et al. in their book which states that pathogenic infections in rats or mice can occur in an unhygienic environment as well as possible infections that occur during the breeding and delivery of the subject's animal.²⁰ Another possibility is that the effect of tween 80 which cannot be fully absorbed by the intestinal mucosa and increases the degree of diarrhea.²¹

Observations of changes in fecal consistency after administration of lime peel extract generally showed a faster fecal solidification than the negative control group (KN), the best effect was obtained at the lime peel extract 500 mg/KgW (X3), which more or less comparable to the attalpugite effect. These results are in line with the hypothesis which states that the treatment of lime peel extract 500 mg/KgW (X3) in mice can increase the consistency of feces.

Several studies evaluate the effect of lime and sweet orange peel extracts on fecal water content and consistency showed that there was a decrease in water content and solidification of fecal consistency. In a study by Adejoh et al., stem bark extracted from lime plants helped to reduce intestinal motility by inhibiting the movement of fluid in the intestinal mucosa which was caused by inflammation from castor oil.¹⁹ Another research with sweet orange peel extract by Juliana showed at the every level dose of sweet orange peel extract, showed a decrease in water content and solidification of feces consistency.

Another study on the effects of tannins, as a candidate for the active compound in lime peel extract, shows that tannins have the potential to reduce fecal water content.¹⁷ Research conducted by Misra et al. regarding the antidiarrheal effect of Moringa leaf extract by calculating the water content of wet feces and after dried, showing the action of tannins on reducing fecal moisture content.²² Research with a similar method was also carried out by Labu et al. regarding the antidiarrheal effect of the croton plant leaf extract and Sisay et al. regarding the antidiarrheal effect of the myrtle plant leaf extract which also showed the action of one of the active compounds, which are tannins in both leaf extracts in reducing fecal water content.^{23,24} The difference with this research was the type of plant used, the extraction tannin from different part of the plant, and dilution treatment of the extract.

The data analysis indicated that giving lime peel extract can improve water content and fecal consistency due to the fact that there are tannins and other antioxidants in the lime peel extract. Apart from being found in lime peel, tannins are also found in grapes, tea, and immature fruit, and have a function as an astringent that can work in wound healing and antidiarrheal.¹¹ This tannin astringent can form a protein layer in the intestinal mucosa and reduce surface area of the small intestine which ultimately prevents fluid loss. In this study, it is suspected that the astringent properties of tannins contained in lime peel extract can protect the intestinal surface from bisacodil irritants by forming a protein layer around the intestinal lumen. The precipitation of protein by tannins can reduce inflammation and edema in the intestines.²⁵



CONCLUSION

In this study, the consistency and fecal water content of mice in the 500 mg/KgW group were more solid and the frequency of defecation did not change significantly when compared to the KN group which was given 0.5 ml tween 80 1%, then when compared with the KM group, the consistency and fecal water is more solid but the frequency of defecation has not changed significantly.

The comparison of consistency, fecal water content, and frequency of defecation in the subsequent groups did not show a significant decrease nor fecal solidification. The administration of lime peel extract did not significantly reduce the frequency of defecation, fecal water content and fecal consistency.

ETHICAL APPROVAL

All research procedures received ethical clearance from the Health Research Ethics Commission of the Faculty of Medicine, Diponegoro University before conducting the research. The Ethical Clearance number is 63/EC/H/FK-UNDIP/VI/20120.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Simadibrata K, Marcellus dan Daldiyono. 2007. Diare Akut. In: Sudoyo AW, Setiyohadi, B., Alwi, I., K Simadibrata, M., Setiati, S., eds. Buku Ajar Ilmu Penyakit Dalam. Edisi keempat-Jilid I. Jakarta: Pusat Penerbitan Departemen Ilmu Penyakit Dalam Fakultas Kedokteran Universitas Indonesia. 408-413.
2. Wiryan N, Wibawa I. Pendekatan Diagnostik Dan Terapi Diare Kronis. *J Intern Med.* 2007;8(1):66-78.
3. Juffrie M. Gangguan Keseimbangan Cairan dan Elektrolit pada Penyakit Saluran Cerna. *Sari Pediatr.* 2004;6(1):52-9.
4. Asg A, Das SK, Chisti MJ, Afroze F. Childhood Diarrhea and Severe Malnutrition. 2014;(May 2015).
5. Lamberti LM, Fischer Walker CL, Black RE. Systematic review of diarrhea duration and severity in children and adults in low- and middle-income countries. 2012;12(1):276.
6. Troeger C, Blacker BF, Khalil IA, Rao PC, Cao S, Zimsen SR, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis.* 2018;18(11):1211-28.
7. Kementerian Kesehatan Republik Indonesia. Profil Kesehatan Indonesia 2017. 2018. 496 p.
8. Kementerian Kesehatan Republik Indonesia. Profil Kesehatan Indonesia 2018. 2019. 156 p.
9. Lamberti LM, Fischer Walker CL, Black RE. Systematic review of diarrhea duration and severity in children and adults in low- and middle-income countries. 2012;12(1):276.
10. Faure C. Role of Antidiarrhoeal Drugs as Adjunctive Therapies for Acute Diarrhoea in Children. *Int J Pediatr.* 2013;2013:1-14.
11. Ashok PK, Upadhyaya K. Tannins are Astringent. *J Pharmacogn Phytochem.* 2012;1(3):45-50.
12. Ezeabara C. Determination of Tannin Content in Various Parts of Six Citrus Species. *J Sci Res Reports.* 2014;3(10):1384-92.
13. Kementerian Pertanian Republik Indonesia. Outlook Komoditas Pertanian Subsektor Hortikultura Jeruk 2015. Jakarta: Pusat Data dan Sistem Informasi Pertanian Kementerian Pertanian; 2015.
14. Rafiq S, Kaul R, Sofi SA, Bashir N, Nazir F, Ahmad Nayik G. Citrus peel as a source of functional ingredient: A review. *J Saudi Soc Agric Sci.* 2018 ;17(4):351-8.
15. Oktaviani L, Busman H, Nurcahyani N. Uji Anti Diare Ekstrak Rimpang Rumput Teki (*Cyperus rotundus* L.) Dibandingkan dengan Obat Attapulgit Pada Mencit (*Mus musculus* L.) Jantan yang Diinduksi Oleum ricini. 2016;4(2):15-21.
16. Anggraeni L. Studi Perbandingan Efektivitas Penggunaan PEG 350 dan Bisakodil pada



Abraham Talent Bawadi Sibarani, Hesti Triwahyu Hutami,
Nani Maharani, Yora Nindita

- Konstipasi yang Diinduksi Morfin [Skripsi]. Surabaya (Indonesia): Universitas Airlangga; 2016.
17. Juliana R. Uji Efek Antidiare Ekstrak Kulit Jeruk Manis (*Citrus sinensis* L.) pada Tikus Putih Jantan (*Rattus novergicus*) yang Diinduksi Magnesium Sulfat [Skripsi]. Palembang (Indonesia): Politeknik Kesehatan Palembang; 2017.
 18. Mukhriani, Nonci FY, Mumang. Penetapan Kadar Tanin Total Ekstrak Biji Jintan Hitam (*Nigella sativa*) Secara Spektrofotometri UV-VIS. Jf Fik Uinam. 2014;2(4):154–8.
 19. Adejoh IP, Nnedimkpa AJ, Chizoba AJ, Benjamin AE, Chukwuemeka N-AP. Effects of Aqueous Stem Bark Extract of *Citrus aurantifolia* on the Gastrointestinal Tract of Wistar Rats. Asian J Res Med Pharm Sci. 2018;5(3):1–7.
 20. Committee on Infectious Diseases of Mice and Rats, National Research Council. Infectious Diseases of Mice and Rats. Infectious Diseases of Mice and Rats. 1991. Washington, D.C: Institute of Laboratory Animal Resources, Commission on Life Sciences.
 21. U.S. Departement of Health and Human Services. Toxicology and Carcinogenesis Studies Of Polysorbate 80 (CAS No. 9005-65-6) In f344/N Rats and B6C3F₁ Mice.1992. North Carolina: National Toxicology Program.
 22. Misra A, Srivastava S, Srivastava M, Sharad Srivastava C. Evaluation of anti diarrheal potential of *Moringa oleifera* (Lam.) leaves.4 J Pharmacogn Phytochem. 2014;2(5):43–6.
 23. Labu ZK, Laboni FR, Mamun MMA AI, Howlader MSI. Antidiarrhoeal activity and total tannin content of ethanolic leaf extract of *Codiaeum variegatum*. Dhaka Univ J Pharm Sci. 2015;14(1):87–90.
 24. 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.
 25. Mondal S. Pharmaceutical Inorganic Chemistry (BP104T) UNIT– IV : Astringents. 2017; (Gitam University).