Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Jan 03; 10(T8):194-199.
https://doi.org/10.3889/oamjms.2022.9458
eISSN: 1857-9655
Category: *T8 -"APHNI: Health Improvement Strategies Post Pandemic Covid-19"*
Section: *Pharmacolgy*

Screening and Antidiarrheal Activity Testing of Sembung Rambat (*Mikania micrantha*) Leaves

Arif Ardianto[1], Dena Munarsih[1], Iin Nur Rahayu[1], Muhammad Muzhil Aslam[1], M. Fahrul Aditya[2], Daru Estiningsih[1]*, Annisa Fatmawati[1], Pujo Hari Saputro[2]

[1]*Department of Pharmacy, Faculty of Health Sciences, Alma Ata University, Kasihan, Indonesia;* [2]*Department of Informatics, Faculty of Computer, Alma Ata University, Kasihan, Indonesia*

Edited by: Sinisa Stojanoski
Citation: Ardianto A, Munarsih D, Rahayu IN, Aslam MM, Aditya MF, Estiningsih D, Fatmawati A, Saputro PH. Screening and Antidiarrheal Activity Testing of Sembung Rambat (*Mikania micrantha*) Leaves. Open Access Maced J Med Sci. 2022 Jan 03; 10(T8):194-199. https://doi.org/10.3889/oamjms.2022.9458
Keywords: Antidiarrheal; Sembung rambat (*Mikania micrantha*); Intestinal transit
***Correspondence:** Daru Estiningsih, Department of Pharmacy, Faculty of Health Sciences, Alma Ata University, Kasihan, Indonesia.
E-mail: daru_estiningsih@almaata.ac.id
Received: 13-Oct-2021
Revised: 21-Nov-2021
Accepted: 02-Dec-2021

Funding: This study was supported by the Ministry of Education, Culture, Research, and Technology of the Directorate General of Higher Education for the 2021 Student Creativity Program grant
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The prevalence of diarrhea in Indonesia is exceedingly high. In consequence, it causes an increment of morbidity and mortality rate in toddlers. Furthermore, every year more than 1.3 billion toddlers suffer from diarrhea, and 3.2 million experience death from this disease. Based on empirical evidence in Pelita Jaya, Pesisir Barat of Lampung, Sembung Rambat (*Mikania micrantha*) leaves are commonly used to treat diarrhea, rheumatism, and bleeding wounds. In addition, alkaloids are one of the compounds contained in sembung rambat leaves that can reduce or inhibit the disposal of waste substances from the body or have antidiarrheal properties.

AIM: The research aims to prove the potential effect of sembung rambat (*M. micrantha*) leaf extract as an antidiarrheal agent by screening and conducting a pharmacological test on male Swiss mice.

METHODS: The phytochemical qualitative analysis was employed to screen the *M. micrantha* leaf extract. Moreover, the antidiarrheal effect was tested using the intestinal transit method of diarrhea in mice induced by Oleum Ricini.

RESULTS: The phytochemical screening results show that there are the secondary metabolites of alkaloids, tannins, flavonoids, and saponins contained in the extract. The measurement of the marker length on the intestine length (ratio) after t = 65 min exhibits that the ethanol extract of sembung rambat (*M. micrantha*) leaf contains an antidiarrheal activity with a dose variation of 100 mg/kg BW, 150 mg/kg BW, and 200 mg/kg BW.

CONCLUSIONS: Ethanol extract of sembung rambat (*M. micrantha*) leaves at various doses of 100 mg/kg BW, 150 mg/kg BW, and 200 mg/kg BW possesses antidiarrheal activities. The most effective dose is 200 mg/kg BW as it performed the closest ratio to the positive control group.

Introduction

Diarrhea is one of the main causes of infant mortality in the world with a fairly high child mortality rate. The thing it also exists in different pathophysiology associated with multiple etiologic agents [1]. Diarrhea is considered one of the major public health problems in Indonesia. This can be seen from the high morbidity and mortality rates, particularly in toddlers. It was estimated that more than 1.3 billion children suffered from diarrhea, and the deaths toll of that in children reaches 3.2 million every year. Children could experience diarrhea attacks about 3.3 times every year and more than 80% of them died at the age of <2 [2].

According to the WHO in 2019, diarrhea is the second leading cause of life expectancy reduction by 1.97 years, just below the lower respiratory tract infections (2.09 years). In addition, in 2016 unhealthy drinking water, poor sanitation, and an unsanitary environment were the main factors responsible for the deaths of 0.9 million people including more than 470,000 infant deaths caused by diarrhea, leading the governments and even world organizations to deal with it [3].

Moreover, the major cause of diarrhea in children under five is malnutrition. 1.7 billion children experience diarrhea every year. Malnourished kids and people living with HIV are at risk for diarrhea and even death. In the past, most people were severely dehydrated which could result in diarrhea. However, another cause of diarrhea nowadays is a septic bacterial infection which can lead to death. Diarrhea is a symptom of infection in the intestinal tract caused by various bacterial, viral, and parasitic infections. The infection can be spread on contaminated food and drink or it can be passed from person to person [4].

According to the Basic Health Research in 2018, the prevalence of diarrhea in Indonesia was recorded at 18,225 (9%) at the age of <1 year, 73,188 (11.5%) at the age of 1–4 years, 182,338 (6.2%) at the age of 5–14 years, and 165,644 (6.7%) in children aged 15–24 years [5].

Based on the morbidity survey conducted by the Diarrhea Sub-Directorate of the Ministry of Health from 2000 to 2010, there was a tendency for diarrhea incidence to increase. In 2000, the incidence rate of diarrheal disease was 301/1,000 population, in

2003 it rose to 374/1,000 population, in 2006 it rose to 423/1,000 population, and in 2010 it reached 411/1000 population. Extraordinary events of diarrhea are also still commonly found, along with the high Case Fatality Rate (CFR). In 2008, there were outbreaks in 69 sub-districts with a total of 8,133 cases and 239 deaths (CFR 2.94%). In 2009, there were extraordinary events in 24 sub-districts with a total of 5,756 cases with 100 deaths (CFR 1.74%), while in 2010, there was a diarrhea outbreak occurred in 33 sub-districts with a total of 4,204 patients and 73 deaths (CFR 1,74%) and diarrhea is the second leading cause of death in children under 5 years. Therefore, there must be an innovative action to develop antidiarrheal drugs [6].

Based on the data, efforts should be made to address the morbidity and mortality caused by diarrhea in Indonesia. One of the efforts that can be done is the development or the use of herbal medicines which are generally known to have minimal side effects. Thus, it is expected that the development of these drugs can later be consumed directly by the community ranging from toddlers to adults. Observation facts in Pelita Jaya village, Kec. Pesisir Selatan Kab. Pesisir Barat Lampung, where the vines (*Mikania micrantha*) are used by the community as traditional antidiarrheal medicine.

The observation found some facts that in Pelita Jaya village, Pesisir Selatan Subdistrict, Pesisir Barat, Lampung, *M. micrantha* is used by the people as traditional antidiarrheal medicine. Sembung rambat leaf (*M. micrantha*) is a species of plant possessing great potential as an alternative antibacterial that can be developed. However, this plant is rarely utilized, because it grows around the vicinity of the rubber and oil palm plantations which are considered by many as a plant with no efficacy. Be that as it may, based on the results of phytochemical analysis, sembung rambat leaf extract contains active substances in the form of secondary metabolites such as alkaloids, saponins, flavonoids, steroids, tannins, and terpenoids [7].

Furthermore, based on this empirical evidence, this study was conducted to prove the hypothesis that sembung rambat (*M. micrantha*) leaf extract can be utilized as antidiarrheal by carrying out phytochemical screening to the secondary metabolites with antidiarrheal effects and pharmacological tests on male Swiss mice. The animal testing was carried out to determine the antidiarrheal activity of *M. micrantha*. A quantitative analysis was also conducted to figure out the content of alkaloid compounds found in *M. micrantha* which is regarded to be efficacious as antidiarrheals as well as to determine the effective dose which can later be used directly by the public.

The phytochemical results show that sembung rambat leaves contain alkaloids, triterpenoids, and steroids [7], one of which is an alkaloid compound with antidiarrheal, anti-diabetic, anti-microbial, and anti-malarial properties. Alkaloids are the most abundant secondary metabolites containing nitrogen atoms, which are often found in plant and animal tissues. Almost all alkaloid compounds come from plants, especially angiosperms. More than 20% of angiosperm species contain alkaloids [8].

Methods

Tools and materials

Tools

Maceration tools, glassware, tissue, masks, medical gloves, rotary evaporator, mouse cages, weighing scales for test animals, basins, feeding tube, 50 ml glass beakers, ruler, mice surgical instruments, dropping pipettes, Erlenmeyer flask, and volumetric flask were used. The tools used were obtained from the pharmacology laboratory and phytochemical laboratory of Alma Ata University, Yogyakarta.

Ingredients

Ethanol 96%, Aquadest, HCl, 10% NaOH, $FeCl_3$, Wagner reagent, 5% norit as marker, control (loperamide), test preparation (*mikania leaf extract*), CMC Na 0.5%, and Oleum Ricini. The materials used were obtained from CV. Chem-Mix Primary.

Research location

This study was conducted at the Pharmacology Laboratory and the Phytochemical Laboratory of the Pharmacy Undergraduate Study Program, Alma Ata University, Yogyakarta.

Sample

This study used male Swiss mice weighing approximately 20–35 g with an average age of 8 weeks. The mice were divided into five groups and each group consisted of four mice obtained from the experimental animal development unit, UGM.

Determining the plant

The samples of sembung rambat leaves were obtained from Pangandaran area, West Java. Determination of plants aims to ensure the accuracy of the plant species to avoid errors in the usage of the material that will be studied.

Making ethanol extract of sembung rambat (M. micrantha) leaves

Simplicia was extracted by employing the maceration method [9] using 96% ethanol. The extract

was obtained from the evaporated solvent using a rotary evaporator, then was concentrated on a water bath. The extraction was carried out by 2× remuneration.

Phytochemical test

The phytochemical screening was carried out to determine what groups of compounds were contained in the ethanol extract of sembung rambat leaves. The phytochemical examination was carried out by conventional examination of alkaloids, tannins, flavonoids, and saponins.

Antidiarrheal test using the intestinal transit method

The antidiarrheal activity of the ethanol extract of sembung rambat leaf was tested using the intestinal transit method in mice-induced Oleum Ricini. Before being used as test animals, male Swiss mice were adapted to the research environment for 1 week and then fasted for 18–24 h before the test. The purpose of fasting the mice is that no food intake will affect the antidiarrheal testing process. Next, the mice were weighed to determine the dose administration, and they were divided into five groups with four mice each. The division of the group was: (1) Group I as control (+), (2) Group II as control (-), (3) Group III as a test I at a dose of 100 mg/kg BW, (4) Group IV as test II at a dose of 150 mg/kg BW, and (5) Group V as the third test with a dose of 200 mg/kg BW. All groups were induced with Oleum Ricini suspension at a dose of 0.2 ml/20 g mice. This treatment has met ethical clearance with the issuance of ethical approval with the number KE/AA/VII/10578/EC/2021.

At t = 30 min after being induced with Oleum Ricini, each group was given oral treatment, namely, (1) Group I was given Loperamide suspension at a dose of 16 mg/70 Kg BW as control (+), (2) Group II was given CMC Na suspension 0.5% as control (-), (3) Group III was given a suspension of curd extract at a dose of 100 mg/kg BW, (4) Group IV was given a suspension of sembung propagation extract at a dose of 150 mg/kg BW, and (5) Group V was given a suspension extract curdling at a dose of 200 mg/kg BW. At t = 45 min after the oral treatment, all groups were given norit per-oral as a marker, continued at t = 30 min after administration of norit. The mice's neck dislocation was performed until the mice died. Afterward, the mice's abdomen was carefully dissected. The mice's intestines were taken, and the length of the intestine through which the norit marker passed and the total length of the intestine were measured. The calculation was made to measure the ratio of the track passed by the marker to the total bowel length. The data obtained were analyzed statistically using analysis of variance (ANOVA) [10]. The data obtained were statistically analyzed to determine whether there were significant differences between each group [11].

Results

Plant determination results

The determination was carried out at the Biology Laboratory of the Faculty of Applied Science and Technology, Ahmad Dahlan University, Yogyakarta. The results indicated that the plant used was *M. micrantha* Kunth, Flora of Java [12].

Determination code

- 1b–2b–3b–4b–12b–13b–14b–17b–18b–19b–20b–21b–22b–23b–24b–25b–26b–27b–799a *Asteraceae*
- 1b–3a–4b–5a–6b–15b–16a–17b–18b–13a *Mikania*
- 1b–2a–3a *M. micrantha* Kunth.

Synonyms

Conyza balsamifera (L.) DC.

Extraction results

Sembung rambat leaf extraction using 96% ethanol solvent obtained a yield of 11.50%. The percentage of this yield was obtained from the calculation of the weight of the thick extract (g) divided by the weight of the Simplicia powder (g) multiplied by 100%. The extraction obtained a yield of 11.50%. The percentage of the yield was obtained by calculating the thick extract's weight (g), dividing by the weight of the simplicia powder (g), and multiplying by 100%. The extraction results are shown in Table 1.

Table 1: Results of ethanol extract of sembung rambat leaves

Materials and Yield	Amount
Weight of Simplicia powder	478 g
The volume of 96% ethanol	6 L
Thick extract weight	55 g
Yield	11.50%

Phytochemical screening results of Simplicia and extracts

The results of the phytochemical screening test are shown in Figure 1. The list in Table 2 describes the phytochemical screening using conventional methods or chemical reagents to determine the content of secondary metabolites of alkaloids, tannins, flavonoids, and saponins.

The results of phytochemical screening showed that sembung *rambat* (*M. micrantha)* leaves were positive for alkaloids, flavonoids, tannins, and saponins.

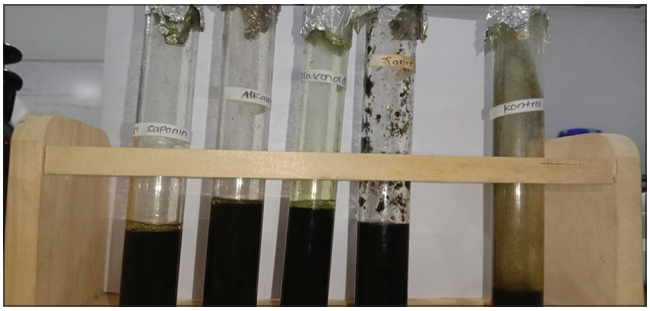

Figure 1: Phytochemical screening results

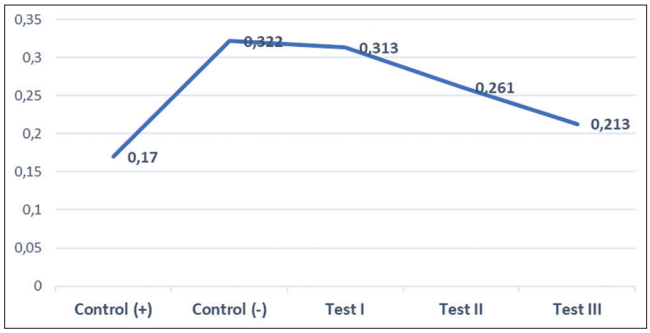

Figure 2: The average ratio of marker length to overall intestinal length

Antidiarrheal activity test

The antidiarrheal testing was carried out using the intestinal transit method, which divided the test animals into five groups. In this study, the positive control group (+) with the administration of loperamide suspension at the dose of 16 mg/70 Kg BW obtained an average ratio of 0.170 ± 0.064, smaller than the negative control group (-) with 0.5% CMC-Na solution. The average value of the ratio is 0.322 ± 0.971. Tests of antidiarrheal activity carried out in five treatment groups obtained results, as presented in Table 3.

Table 2: Results of phytochemical screening of Simplicia and extracts

Phytochemical Test	Reagent	Discoloration	Results
Alkaloids	HCl_2 N+reagent Wagner	A brown precipitate is formed	+
Tannins	FeCl3	Brownish green to blue Black	+
Flavonoids	NaOH 10%	Brownish green to green Yellowish	+
Saponins	Aquadest+Shake Strong	Foam formed	+

(+): Detected, (-): Not detected.

The ratio between the length of the intestine passed by the marker to the overall length of the intestine in each group tested is shown in Figure 2, in which test Group I, test Group II, and test Group III have a smaller ratio than the negative control group. Test Group III has an average ratio close to the positive control group.

Based on the data graph shown in Figure 2, the positive control group (+) has 0.17, the negative control (-) has a 0.322 ratio, test I has a ratio of 0.313, test II has a ratio of 0.261, and test III has a ratio of 0.213 comparisons. The positive (+) control group had the smallest ratio, which was inversely proportional to the negative (-) control group.

Table 3: Pharmacological test results data

Group	Code	Length of markers (cm)	Length of intestine (cm)	Ratio	p-value
Control(+)	K(+) 1	11.5	58	0.198	
	K(+) 2	9	63.5	0.141	0.655*
	K(+) 3	9.5	61	0.155	
	K(+) 4	10	54	0.185	
Control (-)	K(-) 1	18	60	0.300	
	K(-) 2	19.5	54	0.361	0.325*
	K(-) 3	18.5	62	0.298	
	K(-) 4	18	55	0.327	
Test I	U (1) 1	18	57.5	0.313	
	U (1) 2	19.5	59	0.330	0.406*
	U (1) 3	18	59	0.305	
	U (1) 4	18	59.5	0.302	
Test II	U (2) 1	15	56	0.267	
	U (2) 2	14	56	0.250	0.625*
	U (2) 3	15.5	55	0.281	
	U (2) 4	14	57	0.245	
Test III	U (3) 1	12	53	0.226	
	U (3) 2	13	62	0.209	0.627*
	U (3) 3	12	61	0.196	
	U (3) 4	12	54	0.222	

*Data are normally distributed (p > 0.05).

Discussion

The sample of Simplicia has been determined to ensure that the Simplicia used in this study is sembung rambat leaf (*M. micrantha)* obtained from Pangandaran district, Jawa Barat. The extraction of sembung rambat leaf utilized 96% ethanol solvent because based on the polarity level, 96% ethanol can attract or isolate secondary metabolites of alkaloids, tannins, flavonoids, and saponins, most of which have antidiarrheal properties. The thick extract resulted from the extraction of active compounds from plant Simplicia or animal Simplicia using a suitable solvent. Then, the solvent was evaporated, and a thick extract was obtained [13].

Before testing the ethanol extract of sembung rambat leaves, phytochemical screening was carried out to determine the secondary metabolites' content in sembung rambat. The examination of alkaloids was carried out by taking 1 ml of sembung rambat leaf extract and adding 2 N HCl and Wagner's reagent. There was a positive reaction for alkaloids because a brown precipitate was formed. Tannin examination was carried out by taking 1 ml of sembung rambat leaf extract and adding $FeCl_3$ reagent. A positive reaction for tannins occurred because a black or dark blue color was formed [14]. Examination of flavonoids was performed by taking 1 ml of sembung rambat leaf extract and adding a few drops of 10% NaOH. The reaction showed positive flavonoids with a specific color change. Saponin examination was carried out by taking 1 ml of sembung rambat leaf extract, adding distilled water, and shaking it. The positive reaction of saponins was indicated in the formation of long-lasting foam [10].

The determination of the dose variations for the test was based on the previous studies using

five variations of doses consisting of 20 mg/kg BW, 40 mg/kg BW, 80 mg/kg BW, 100 mg/kg BW, and 150 mg/kg BW. From these dose variants, the doses of 100 mg/kg BW and 150 mg/kg BW began to have an antidiarrheal effect. Therefore, this study used three variations of doses consisting of 100 mg/kg BW, 150 mg/kg BW, and 200 mg/kg BW.

In addition, loperamide was given to the positive control group as a comparison to determine whether the sembung rambat leaf extract had an antidiarrheal activity or not. The negative control group was given CMC-Na 0.5% to determine whether the solvent had an antidiarrheal effect. This step was done to ensure that the measured antidiarrheal effect was accurate – that it came from the effect of sembung rambat leaf extract and not the effect of the solvent.

Furthermore, the mechanism of loperamide as an antidiarrheal can slow the motility of the gastrointestinal tract through the circular or longitudinal muscles of the intestine, and it results in interactions with opioid receptors in the intestine [15]. Loperamide can inhibit intestinal transit time or flow rate from the intestine to the colon. It can also normalize the balance of absorption or secretion of fluids in the intestinal mucous membrane [16].

The intestinal transit method to determine the antidiarrheal activity was carried out by measuring the ratio of the length of the intestine that the marker passed at a specific time to the overall length of the intestine. A small ratio indicates antidiarrheal activity. According to the previous research [17], loperamide HCl as positive control showed an antidiarrhea effect, because the average value of the ratio of the positive control group was smaller, which is 0.667 compared to the negative control group of 0.708. In addition, the ratio of the Mindi Leaf Ethanol Extract (*Melia azedarach* Linn) administration group was smaller than the negative control group. The mechanism of action reducing intestinal peristalsis and motility will inhibit food excretion; subsequently, a smaller ratio measurement is obtained.

The results of the study were analyzed statistically to determine whether there were differences in each group. An Statistical Product and Service Solution program, ANOVA analysis, was chosen, because more than two groups were involved [10]. Before the ANOVA analysis, the data underwent a normality test. The significance of all groups is more than 0.05, which indicates that the data are normally distributed. The results of the ANOVA analysis indicate a significance of 0.00 (<0.05); therefore, it can be concluded that there is a significant difference in each group. The test results show significant differences in the antidiarrheal activity of various doses of the ethanol extract of sembung rambat (M. micrantha) leaves against male Swiss mice.

For further research, quantitative analyses could be carried out on several secondary metabolites using analytical methods such as High-Performance Liquid Chromatography. In addition, it is advisable to make herbal formulations using sembung rambat (*M. micrantha)* leaf extract that the community can consume directly by the public. *M. micrantha* extract as an antidiarrheal has the potential to be combined with porang flour as a prebiotic in the manufacture of antidiarrheal products. In a previous study, feed given glucomannan porang had a good effect in inhibiting the growth of *Escherichia coli* bacteria that cause diarrhea but had little effect on stimulating the growth of *Bifidobacteria* and *Lactobacilli* [18].

Conclusions

The screening results on the ethanol extract of sembung rambat leaves contain alkaloids, tannins, flavonoids, and saponins, in which some of these secondary metabolites have antidiarrheal properties. Ethanol extract of sembung rambat (*M. micrantha*) leaves at various doses of 100 mg/kg BW; 150 mg/kg BW; and 200 mg/kg BW has antidiarrheal activities. The most effective dose is 200 mg/kg BW as it has a ratio close to the positive control group.

Acknowledgment

This study was supported by the Ministry of Education, Culture, Research, and Technology of the Directorate General of Higher Education for the 2021 Student Creativity Program grant. In addition, we gratefully thank Alma Ata University for providing exceeding adequate research facilities.

References

1. Dos Santos Negreiros P, da Costa DS, da Silva VG, de Carvalho Lima IB, Nunes DB, de Melo Sousa FB, *et al*. Antidiarrheal activity of α-terpineol in mice. Biomed Pharmacother. 2019;110:631-40. https://doi.org/10.1016/j.biopha.2018.11.131
   PMid:30540974
2. Triwulan. Health Information and Data Window Bulletin. Buletin Jendela Data dan Informasi Kesehatan; 2011.
3. World Health Organization. WHO Monitoring Health for the SDGs, Sustainable Development Goals. Ganeva: World Health Organization; 2019. p. 77.
4. World Health Organization. Diarrheal Disease. Geneva: World Health Organization; 2017. Available from: https://www.who.int/news-room/fact- sheets/detail/diarrhoeal-disease [Last accessed on 2021 Nov 22].
5. Indonesian Ministry of Health. Ministry of Health Strategic Plan Year 2015-2019. Rencana Strategis Kementerian Kesehatan

Tahun 2015-2019. J Kementeri Kesehatan RI. Jakarta: Indonesian Ministry of Health; 2019.

6. Fithri NK, Zelfino. Overview of Maternal Hygiene Behavior and The Incidence of Diarrhea In Toddlers in The Working Area of The Grogol Petamburan Health Center in 2018 [Gambaran Perilaku Hygiene Ibu dan Kejadian Diare pada Balita di Wilayah Kerja Puskesmas Grogol Petamburan Tahun 2018]. Indones Health Inf Manag. 2018;6:91-5.
7. Polakitan IR, Leman MA. The inhibitory power of sembung rambat (*Mikania micrantha*) leaf extract against the growth of *Streptococcus mutans*. J Ilm Farm. 2017;6(1):1-8. https://doi.org/10.22219/jpbi.v2i3.3863
8. Ningrum R, Purwanti E, Sukarsono S. Alkaloid compound identification of *Rhodomyrtus tomentosa* stem as biology instructional material for senior high school x grade. Indones J Biol Educ. 2017;2(3):231-6. https://doi.org/10.22219/jpbi.v2i3.3863
9. Damanik DD, Surbakti N, Hasibuan R. Extraction of catechins (*Uncaria gambir* Roxb) By maceration method. USU J Chem Eng. 2014;3(2):10-4. https://doi.org/10.1007/978-1-4419-0823-0
10. Fauzi R, Fatmawati A. Anti-diarrhea effect of *Moringa* leaf ethanol extract (*Moringa oleifera* L.) on male white mice. Pharm J Indonesia. 2020;6(1):35-9. https://doi.org/10.21776/ub.pji.2020.006.01.6
11. Rambe R, Gultom ED, Ginting OS, Diana S. Antidiarrheal effectiveness test of noni leaf ethanol extract (*Morinda citrifolia* L.) against male, mice using intestinal transit method. Forte J. 2021;1(1):1-11. https://doi.org/10.51771/fj.v1i1.34
12. Backer CA, Van Den Brink BR. Flora of Java (Spermatophytes Only). Vol. 2. Netherlands: NVP Noordhoff-Groningen; 1963. p. 138.
13. Department of Health. Indonesian Pharmacopoeia. 4th ed. Jakarta: Ministry of Health of the Republic of Indonesia; 1995.
14. Fatmawati A, Aji NP. Determination of Total Flavonoid Level of *Moringa* Leaf (*Moringa oleifera* Lam) Ethanol Extract Using Densitometric thin Layer Chromatography Method. Proceedings Conference Public Online; 2019. p. 1-7.
15. Tjay TH, Rahardja K. Important Drugs, Efficacy, Use and Side Effects. 5th ed. Jakarta: Komputindo Media Effects; 2002.
16. Sari CP, Febrianti Y, Indriani HY. Treatment response of diarrhea specific inpatients at private hospital Banten Province. J Ilm Farm. 2018;14:35-45. https://doi.org/10.20885/jif.vol14.iss1.art4
17. Wahyuni T, Nurinda E, Fauzi R. Potential antioxidants of wuluh (*Averrhoa Bilimbi* L.) Leaves ethanol extract and their effect on blood sugar levels in male Wistar rats induced by streptozotocin (Stz). Inpharnmed J. 2021;5(1):921. https://doi.org/10.21927/inpharnmed.v5i1.1676
18. Harmayani E, Aprilia V, Marsono Y. Characterization of glucomannan from *Amorphophallus oncophyllus* and its prebiotic activity *in vivo*. Carbohydr Polym. 2014;112:47-9. https://doi.org/10.1016/j.carbpol.2014.06.019