

Antibacterial Activity Test of Herbal Liquid Soap Against *Streptococcus pyogenes* and *Candida albicans*

Darini Kurniawati¹

¹Pharmacy Study Program, Faculty of Health, Sari Mulia University, Banjarmasin, Indonesia

Correspondence author: darinikurniawati@gmail.com

Abstract:

Nowadays people like to use liquid soap because it is more practical and easier to store. Antiseptic liquid soap circulating in the market uses chemical compounds such as triclosan, hexachlorophen and bithional whose raw materials are from abroad. The government encourages the use of its own natural raw materials so that natural materials will become the mainstay of Indonesian industry. The purpose of this research was knowing the antibacterial effectiveness of herbal liquid soap against *Streptococcus pyogenes* and *Candida albicans*. This research used true experimental method which the process was started by making herbal liquid soap products using the hot; and cold press method for evaluating pH, organoleptic, foam stability, water content, free fatty acids and free alkali, testing germ numbers with the intervention group (washing hands with herbal liquid soap). and control group (washing hands with running water), followed by testing the inhibitory power of herbal liquid soap against *Streptococcus pyogenes* and *Candida albicans* microbes. The results of the evaluation test of herbal liquid soap have a liquid form, lemon odor and yellow color, foam height 15-70 mm, pH 10, water content 52%, free fatty acids 0.05%, free alkali 0.091%, according to SNI soap standards. required liquid. Antibacterial test by dilution method and continued with microbial inhibition results obtained MIC against *Streptococcus pyogenes* at a concentration of 20% and MBC at a concentration of 20% of 22mm. Against *Candida albicans*, the results of MIC at a concentration of 25% and MBC at a concentration of 25% were 15 mm.

Keywords: Antibacterial; Herbal; Liquid soap

Introduction

During this Covid-19 pandemic, the government requires the public to comply with health protocols, one of which is frequent hand washing with soap to break the chain of the spread of Covid-19¹. Sheaths made of lipids are easily damaged by fat solvents (soap, detergent, alcohol > 60%, chloroform, ether), making the covid-19 virus no longer able to infect and reproduce. Antiseptic liquid soap on the market usually contains chemical compounds such as triclosan, hexalorofen and bithional which are raw materials from abroad. The government encourages the use of its own natural raw materials so that natural materials will become the mainstay of the industry.²

The use of betel leaf for antiseptic is due to the presence of saponins by damaging the cytoplasmic membrane and killing microbial cells. Flavonoid compounds work by denaturing bacterial cell proteins and damaging cell membranes.³

Chemical compounds in lime peel also contain saponins and flavonoids such as hesperidin which can damage viral cells.⁴

Empirically Bundung plant (*Actinoscirpus Grossus*) is used as an antimicrobial. The phytochemical test of Noval et al's research also found flavonoid and saponin compounds that have an antibacterial mechanism of action.⁵

The results of the research formulation of 50% w/v polyherbal lime peel, betel leaf and bundung have the same effectiveness of the positive control of antiseptic hand sanitizer liquid against the microbes *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Candida albican*.⁶

The purpose of the study was to determine the ability to inhibit or kill the bacteria *Streptococcus pyogenes* and *Candida albican* from three mixtures of betel leaf infusion extract (*Piper betle*), ethanol extract of lime peel (*Citrus aurantifolia*) and ethanolic extract of weeds (*Actinoscirpus Grossus*) using the disk dilution method. against *Streptococcus pyogenes* and *Candida albicans* bacteria from the formulations of 15%, 20%,

25% and 30% herbal liquid soap 50% w/v using a positive control liquid soap.

Research Method

Materials

Fresh betel leaf (*Piper betle*), lime peel (*Citrus aurantifolia*), lime juice (*Citrus aurantifolia*), Bundung plant (*Actinoscirpus Grossus*), antiseptic hand sanitizer, distilled water, 950% ethanol, SDA (*Saboroud Dextrose Agar*), Nutrient Agar, HMA (*Hilton Muller Agar*), 0.9% NaCl, disc paper, plastic wrap, propyl paraben, methyl paraben, ice cubes.

Beaker, erlenmeyer glass, magnetic stirrer, vortex, rotary evaporator, stir bar, measuring cup, water bath, analytical scale, maceration bottle, funnel, evaporating dish, object glass, oven, refrigerator, autoclave, BSC (*Bacteriology Safety Cabinet*), pH meter, viscosimeter, scissors, knife, cutting board, washbasin, infusion pan, stove, elpigi gas, blender, ose, triangular stem

True research design experimentally, making herbal liquid soap products using the hot and cold press method was evaluated for pH, organoleptic, foam stability, moisture content, free fatty acids and free alkali followed by the inhibition test of herbal liquid soap against microbes *Streptococcus pyogenes* and *Candida albican*.

Methods

This research was conducted in the presence of ETHICAL CLEARANCE No. 154/KEP-UNISM/IV/2022 from the Research Ethics Commission of the University of Sari Mulia Banjarmasin and a Letter of Assignment from the Research and Service Institute of the University of Sari Mulia Banjarmasin number 851/ST-Penelitian/LPPM/UNISM/XI/2021.

The steps taken are:

Preparation of Lime Peel (*Ciprus Aurantifolia*) Ethanol Extract Extraction of lime peel using maceration method. Production of ethanol extract of the bund plant (*Actinoscirpus grossus*) Extraction of the bund plant using the maceration method.

Preparation of betel leaf extract (Piper betle) using the infusion method. Formulation of 50% w/v combination of betel leaf infusion extract (Piper betle), ethanol extract of lime peel (Citrus aurantifolia) and ethanol extract of bundung (Actinuscirpus Grossus). Preparation of Herbal Antiseptic Liquid Soap with 50% w/v formulation of betel leaf, lime peel and bund plant using cold and hot press methods DanaBanaransoap, 2016: Stage 1. Make Soap Base with 1140 grams of distilled water, 900 grams of pure coconut oil, 600 mg of olive oil and 380 grams of KOH by heating. Stage 2. Heating the Soap Base until it is clear and neutral by checking for phenolphthalein. Step 3. Dissolving the Soap Base with aquades. Step 4. Neutralize Liquid Soap and add a 50% w/v formulation of betel leaf, lime peel and bund plant. Evaluation of herbal antiseptic liquid soap with 50% w/v formulation of betel leaf, orange peel and bund plant, carried out for 4 weeks, including organoleptic, foam stability, pH, water content, fat free, free alkali.

Antibacterial test of antiseptic liquid soap with a formulation of 50% w/v betel leaf, lime peel and bund plant by dilution method. [28]. The research sample was liquid soap herbal antiseptic formulation 50% w/v betel leaf, lime peel and bund plant, made in several concentrations (10%, 15%, 20%, 25% and 30%) with aquades negative control and soap positive control. 10% antiseptic liquid. Culture of *Streptococcus pyogenes* and *Candida albicans* bacteria. incubated in an aerobic atmosphere at 37°C for 24 hours. Then take 1 ose suspended in 1 ml of 0.9% NaCl solution in a sterile test tube, incubated for 24 hours at 37°C. The test samples, positive control and negative control were each added with 1 ml of bacterial suspension, then incubated for 24 hours at 37°C. On the second day, the MIC was seen on the clarity and turbidity of the test tube. To determine the KBM, a single ose tube was scratched from the second day of incubation on HMA media (Making Nutrient Agar media, weighing 9.8 g with an analytical balance, put in a glass beaker, added 250 ml of hot water, dissolving assisted by a magnetic

stirrer with a power of 300-300 ml). 400 rpm until dissolved, pour into an Erlenmeyer tube, then sterilize by autoclave 121°C for 30 minutes, prepare 10 sterilized petri discs, pour the media evenly divided into 10 35 ml petridiscs, let cool and ready to become Hilton Muller Agar media). then incubated for 24 hours at 37°C. KBM showed no bacterial growth at the lowest concentration. To determine the KBM, a single ose tube was scratched from the second day of incubation on HMA media (Making Nutrient Agar media, weighing 9.8 g with an analytical balance, put in a glass beaker, added 250 ml of hot water, dissolving assisted by a magnetic stirrer with a power of 300-300 ml). 400 rpm until dissolved, pour into an Erlenmeyer tube, then sterilize by autoclave 121°C for 30 minutes, prepare 10 sterilized petri discs, pour the media evenly divided into 10 35 ml petridiscs, let cool and ready to become Hilton Muller Agar media). then incubated for 24 hours at 37°C. KBM showed no bacterial growth at the lowest concentration. To determine the KBM, a single ose tube was scratched from the second day of incubation on HMA media (Making Nutrient Agar media, weighing 9.8 g with an analytical balance, put in a glass beaker, added 250 ml of hot water, dissolving assisted by a magnetic stirrer with a power of 300-300 ml). 400 rpm until dissolved, pour into an Erlenmeyer tube, then sterilize by autoclave 121°C for 30 minutes, prepare 10 sterilized petri discs, pour the media evenly divided into 10 35 ml petridiscs, let cool and ready to become Hilton Muller Agar media). then incubated for 24 hours at 37°C. KBM showed no bacterial growth at the lowest concentration. [28]. Add 250 ml of hot water, dissolving assisted by a magnetic stirrer with a power of 300-400 rpm until dissolved, pour into an Erlenmeyer tube, then sterilize with an autoclave at 121°C for 30 minutes, prepare 10 sterilized petri discs, pour the media evenly divided into 10 petridisc 35 ml, allowed to cool and ready to become Hilton Muller Agar media), then incubated for 24 hours at 37°C. KBM showed no bacterial growth at the lowest concentration. Add 250 ml of hot

water, dissolving assisted by a magnetic stirrer with a power of 300-400 rpm until dissolved, pour into an Erlenmeyer tube, then sterilize with an autoclave at 121°C for 30 minutes, prepare 10 sterilized petri discs, pour the media evenly divided into 10 petridisc 35 ml, allowed to cool and ready to become Hilton Muller Agar media), then incubated for 24 hours at 37°C. KBM showed no bacterial growth at the lowest concentration. then incubated for 24 hours at 37°C. KBM showed no bacterial growth at the lowest concentration. then incubated for 24 hours at 37°C. KBM showed no bacterial growth at the lowest concentration.

We collected data of monthly diarrhea cases visits in children under five years at public health centers from Banjarmasin City Health Office (*Dinas Kesehatan Kota*) and monthly climate data (amount of rainfall, number of rain days, temperature, humidity, sunshine duration, wind speed) from BMKG (*Badan Meteorologi, Klimatologi, dan Geofisika*) online data and also from BPS (*Badan Pusat Statistik*) Banjarmasin between January 2010 to April 2021. We divided the data into 2 periods: before (January 2010 to February 2020) and after COVID-19 pandemic (March 2020 to April 2021). Statistical analysis was carried out using Pearson correlation or Spearman's rho correlation coefficient (with IBM SPSS™ version 25 software) to determine the correlation between the average monthly data of climate factors and diarrhea cases in children under 5 years before and after the COVID-19 pandemic.

Results

Organoleptic Test

Organoleptic test is intended to see the physical appearance including shape, color and smell. This test was carried out by looking at the shape, color, and odor of the soap on storage for 2 weeks. The results of the organoleptic test for the preparation of antiseptic liquid soap with a combination of lime peel herbs, betel leaves and bund plants are smell like lemon and the color is yellow.

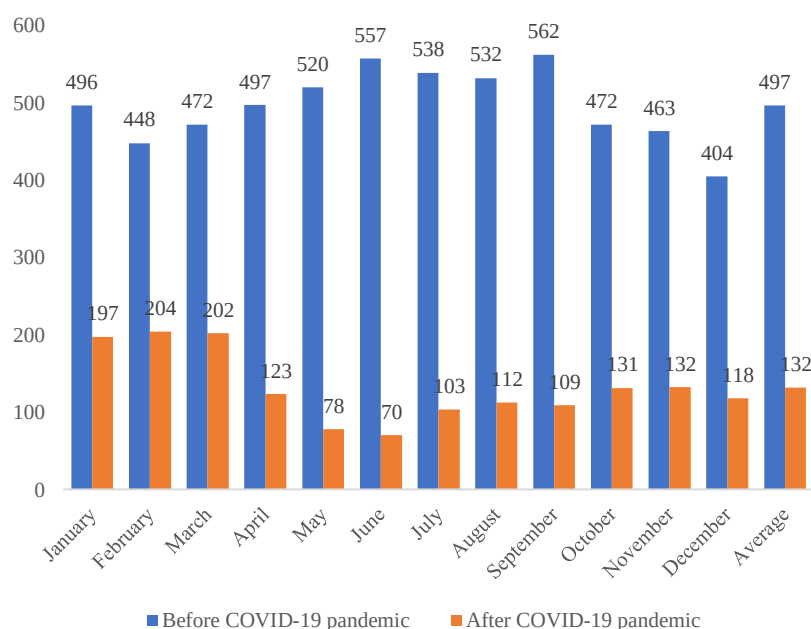
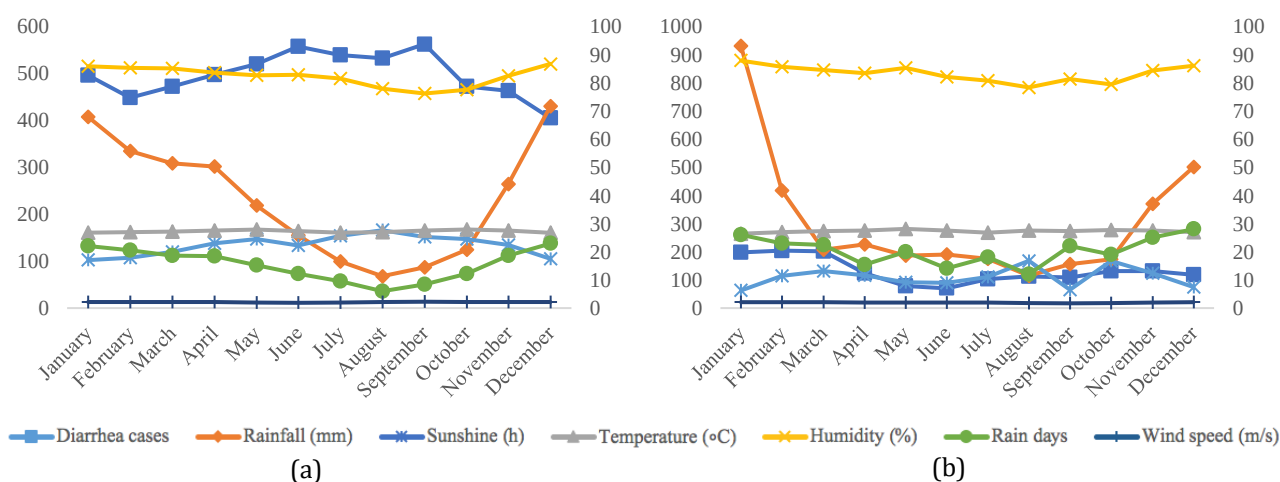
Organoleptic Test

One of the attractions of liquid soap is the foam it produces when used. Based on SNI for liquid soap, the requirements the height of the liquid soap foam is 13-220 mm. Testing the foam height using a graduated tube. From the results of the research on the height of this herbal antiseptic liquid soap foam are initial foam height is 70 mm and foam height after 5 minutes is 15 mm.

There was a significant decrease in the number of average monthly visits of children under five years with diarrhea before (497 ± 47.4) and after (132 ± 45.8) the COVID-19 pandemic (see figure 1). Among climate factors, amount of rainfall ($r=-0.753$; $p=0.005$) and number of rain days ($r=-0.774$; $p=0.003$) were strong and negatively correlated, while humidity ($r=-0.590$; $p=0.044$) was moderate and negatively correlated, and sunshine duration ($r=0.674$; $p=0.016$) was strong and positively correlated with diarrhea cases in children under 5 years in the period before the COVID-19 pandemic. We found no significant correlation between any climate factor and diarrhea cases in children under 5 years in the period after the COVID-19 pandemic (see table 1).

Table 1 Correlation between monthly average data of climate factors and diarrhea cases in children under 5 years before and after the COVID-19 pandemic

	Diarrhea under 5 before pandemic		Diarrhea under 5 after pandemic	
	r	p	r	p
Amount of rainfall	- 0.753	0.005	0.545	0.067
Temperature	0.190	0.554	- 0.518	0.084
Humidity	- 0.590	0.044	0.483	0.112
Sunshine duration	0.674	0.016	0.071	0.827
Number of rain days	- 0.774	0.003	0.505	0.094
Wind speed	- 0.372	0.233	0.481	0.113

**Figure 1** Number of monthly average visits of diarrhea cases under 5 years before COVID-19 pandemic (January 2010 – February 2020) and after COVID-19 pandemic (March 2020 – April 2021)**Figure 2** Trend of monthly average diarrhea cases under 5 years and monthly average climate data components: (a) Before COVID-19 pandemic (January 2010 – February 2020); and (b) After COVID-19 pandemic (March 2020 – April 2021)

Discussion

Each region has its own unique characteristics of climate and weather variabilities that may influence the pattern of diseases, especially infectious disease such as diarrhea. Banjarmasin as our research site located in wetland area with tropical savanna climate, with rainy season during November to April and often a long dry period with low humidity and longer sunshine duration during the dry season, especially from August to October. Heavy rainfall occurs from November to June and moderate rainfall from July to October, and the hottest months are between March and September.⁷ This weather pattern can be seen in figure 2. There was extreme rainfall in January 2021 which caused major floods in Banjarmasin.

Diarrhea still a major problem in Banjarmasin, with major burden especially in under 5 years old group. From figure 1 we can see that after the COVID-19 pandemic there was a significant decrease in numbers of monthly visits of diarrhea cases. This trend was similar to another study that compare the healthcare visits before and after the COVID-19 pandemic in primary health care (Rhatomy, 2020) that found the decline especially in early pandemic period for children aged 0-9 (71%) and females (46%), also in tertiary teaching hospital (Prabowo, 2021) related to people stress and COVID-19 fear, and in pediatric emergency department (Dopfer, 2020) for both communicable and non-communicable diseases.^{8, 9, 10} One study also found 60.4% reduction in overall hospital emergency and admission department visits, particularly for non-severe illnesses, but a 9% increase of hospitalization rate during pandemic¹¹. This trend apparently also occurred in Banjarmasin after the COVID-19 pandemic, that also might be influenced by the government regulations regarding social restrictions.

In this study, we can see that diarrhea cases in children under 5 years before the COVID-19 pandemic tended to peak between June and September, during the dry season in Banjarmasin. This in accordance with the

findings of the climate factors that decreased in the dry season, i.e. amount of rainfall, number of rain days, and humidity which were found to be negatively correlated with number of diarrhea cases, and the sunshine duration as a climate factor increased during the dry season which was found to be positively correlated. This result is similar with a previous study in Banjarmasin (Cahyadi et al, 2019) that found diarrhea cases of all ages were negatively correlated with amount of rainfall and humidity, and positively correlated with temperature.¹² Another Indonesian study (Fachrin et al, 2020) found humidity as a determinant factor for diarrhea incidence changes on 33 province in Indonesia.¹³ Another study in Bangladesh as a 4 seasonal countries, found that humidity, temperature, and rainfall correlates with diarrhea cases and other 5 infectious diseases.¹⁴ Regarding the causes, a systematic review and meta-analysis found that rotavirus diarrhea, as the main cause of diarrhea under 5 years, was found to increase in dry season, while bacterial and parasitic diarrhea was more common in rainy season.¹⁵

In January 2021 during the period after the COVID-19 pandemic there was an extreme amount of rainfall in Banjarmasin, seen in the abnormal distribution of rainfall data (929.3 mm, 303.9 ± 229.76 , $p=0.002$), that brought major flood in this city. Extreme weather is a consequence of climate change, mainly due to global warming process, that was also expected to change the disease pattern including diarrhea. WHO estimates that between 2030 and 2050 climate change will add 48,000 deaths from diarrhea per year related to projected of increased extreme weather events including floods and droughts, both of which are associated with increased incidence of this disease. These conflicting facts were explained by the concentration-dilution hypothesis, suggests that the background level of rain affects the diarrhea incidence: rainfall following dry periods can flush pathogens into surface water and increasing diarrhea incidence, whereas

rainfall following wet periods can dilute pathogen concentrations in surface water, thereby decreasing diarrhea incidence. A study in South Africa (Ikeda et al, 2019) found that 'wetter than usual' and 'warmer than usual' conditions were anomalously associated with high number of diarrhea cases, although most of diarrhea cases under 5 years was associated with dry condition.¹⁶ Areas with a tropical climate, such as Banjarmasin, particularly like to experience changes in rainfall intensity and frequency due to climate change, where extreme rainfall following dry periods that causes flooding can increase the diarrhea incidence, seen in trending of positive correlation of rainfall and diarrhea cases during this period (see table 1). The effect of this not

As a limitation of our study, we only analyzed the correlation between monthly average data of climate factors and diarrhea in children under 5 years cases and did not take into account the spatiotemporal trends that could influence the disease pattern, and we only included the cases from primary healthcare visits. We also only observed a very short period of diarrheal disease pattern, about 14 months, after the COVID-19 pandemic.

Conclusions

Diarrhea cases in children under 5 years in Banjarmasin tend to increase in the dry season between June and September, where the amount of rainfall, number of rain days, and humidity are lower while the sunshine duration is longer. The ongoing COVID-19 pandemic is bringing some changes in society, such as working and studying from home due to social restrictions regulations and decreasing healthcare visits due to reluctance to go to health facilities, which may affect the usual trend. Lastly, the extreme weather during the COVID-19 pandemic period that might be caused by climate change such as in January 2021 can also have an impact.

Further study with a more comprehensive analysis, a wider range of subjects, and a

longer period of time is necessary to better understand the correlation between climate factors, the COVID-19 pandemic, and cases of diarrhea in children under 5 years, to prepare for the uncertainty in the future in order to improve the public health outcomes.

Acknowledgements

We acknowledge all persons and institutions that assisted us in conducting our research, especially: H.Rolli from Banjarmasin City Health Office (*Dinas Kesehatan Kota*), BPS (*Badan Pusat Statistik*) Banjarmasin, and BMKG (*Badan Meteorologi, Klimatologi, dan Geofisika*) that provided us with all the data in this study. We also acknowledge the Head of Department of Pediatrics, and the Head of Pediatrics Study Program, Faculty of Medicine, University of Lambung Mangkurat / Ulin General Hospital, Banjarmasin for all the help and support that has been given.

References

1. Kementerian Kesehatan Republik Indonesia. *Profil Kesehatan Indonesia Tahun 2019*. Kementerian Kesehatan Republik Indonesia; 2020.
2. Pratama, M. D. 2020. Tantangan Kita Bersama: *Menjadikan Industri Obat Herbal sebagai Industri Prioritas Andalan RI*. Webinar Kelas Medis Obat Herbal BPOM RI.
3. Amanda, S., Nyoman, M., I Gede, S. *Uji Aktivitas Antibakteri Rebusan Daun Sirih (Piper betle Linn) Terhadap Bakteri Streptococcus pyogenes*. *Meditory*. 2019: 37-43.
4. Endang TM, Jatmiko S, Siva D. K. S. Power to Dissolve Ca Oxalate by Lemon Juice (*Citrus lemon*) on Variation of Concentration and Immersion Times. Universitas Muhammadiyah Semarang. 2017: 15-19.
5. Noval, N., Yuwindry I. and Syahrina D. 2019. Phytochemical Screening and Antimicrobial Activity of Bundung Plants Extract by Dilution Methode. *Jurnal Surya Medika (JSM)*. 5 (1), pp 143-154.

6. Darini, Noval, Kunti. 2021. Formulation and Antiseptic Activity Test of Natural Lime Peel, Betel Leaf, and Bundung Plants against *Staphylococcus aureus* and *Candida albican*. *FARMASIS: Jurnal Sains Farmasi*. Volume 2 No. 1 Maret 2021, ISSN 2746-6418.
7. Wikipedia. Kota Banjarmasin - Wikipedia bahasa Indonesia, ensiklopedia bebas. Published online 2021:1-10. https://id.wikipedia.org/wiki/Kota_Banjarmasin
8. Rhatomy S, Prasetyo TE. Impact of COVID-19 on primary care visits: lesson learnt from the early pandemic period. *J Community Empower Heal*. 2020;3(2):102.
9. Prabowo NA, Apriningsih H, Dirgahayu P, et al. The Decrease in Hospital Visits at Universitas Sebelas Maret Hospital Due to the Level of Stress and Fear of COVID 19. *Proc 4th Int Conf Sustain Innov 2020–Health Sci Nurs (ICoSIHSN 2020)*. 2021;33(ICoSIHSN 2020):101-4.
10. Dopfer C, Wetzke M, Zychlinsky Scharff A, et al. COVID-19 related reduction in pediatric emergency healthcare utilization - A concerning trend. *BMC Pediatr*. 2020;20(1):1-10.
11. Muselli M, Cofini V, Mammarella L, et al. The impact of covid-19 pandemic on emergency services. *Ann Ig*. 2021;(1).
12. Cahyadi DD, Indah MF, Ishak NI. Analisis Faktor Iklim Terhadap Kejadian Diare Di Kota Banjarmasin Tahun 2014 – 2019 Analysis of Climate Factors for Diarrhea Occurrence in Banjarmasin City At 2014 - 2019. 2020;34:1-10.
13. Fachrin AA, Deityana H, Sani SR. Pengaruh Iklim Terhadap Kejadian Diare Berdasarkan Provinsi di Indonesia. *Stat J Theor Stat Its Appl*. 2020;20(1):45-65
14. Chowdhury FR, Shihab Q, Ibrahim U, et al. The association between temperature , rainfall and humidity with common climate-sensitive infectious diseases in Bangladesh. *PLoS One*. 2018;10(1371):1-17
15. Kraay ANM, Man O, Levy MC, Levy K, Ionides E, Eisenberg JNS. Understanding the impact of rainfall on diarrhea: Testing the concentration-dilution hypothesis using a systematic review and meta-analysis. *Environ Health Perspect*. 2020;128(12):126001-1-126001-126016.
16. Ikeda T, Kapwata T, Behera SK, et al. Climatic factors in relation to diarrhoea hospital admissions in rural Limpopo, South Africa. *Atmosphere (Basel)*. 2019;10(9):1-18.