Vol. 9 No. 3 December 2022

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia

E-ISSN: 2580-8303
P-ISSN: 2406-9388

PUBLISHED BY:
FACULTY OF PHARMACY UNIVERSITAS AIRLANGGA in collaboration with
INDONESIAN PHARMACISTS ASSOCIATION (IAI) OF EAST JAVA

Accredited SINTA 2
No: B/1796/E5.2/KI.02.00/2020

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia

Faculty of Pharmacy, Universitas Airlangga
Jl. Dr. Ir. H. Soekarno, Mulyorejo,
Surabaya, East Java – 60115, Indonesia
Phone. (6231) 5933150, Fax. (6231) 5932594
Website:
http://e-journal.unair.ac.id/index.php/JFIKI
Email: jfiki@ff.unair.ac.id

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia (Pharmacy and Pharmaceutical Sciences Journal) P-ISSN: 2406-9388; E-ISSN: 2580-8303 is an official journal published by the Faculty of Pharmacy, Universitas Airlangga in collaboration with Indonesian Pharmacists Association (IAI) of East Java which the articles can be accessed and downloaded online by the public (open-access journal).

This journal is a peer-reviewed journal published three times a year on topics of excellence of research outcomes in the fields of pharmacy service and practise, community medicine, pharmaceutical technology, and health science disciplines that are closely related. This journal only accepts English-language submission. The following are the research areas that this journal focuses on

1. Clinical Pharmacy
2. Community Pharmacy
3. Pharmaceutics
4. Pharmaceutical Chemistry
5. Pharmacognosy
6. Phytochemistry

This journal receives a manuscript from the research results, systematic reviews and meta analyses that are closely related to the health sector, particularly the pharmaceutical field. Selected manuscripts for publication in this journal will be sent to two reviewers, experts in their field, who are not affiliated with the same institution as the author(s). Reviewers are chosen based on the consideration of the editorial team. Manuscripts accepted for publication are edited copies checked for the grammar, punctuation, print style, and format. The entire process of submitting manuscripts to make a final decision for publishing isconducted online.

Table of Content

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 209-219
DOI: 10.20473/jfiki.v9i32022.209-219
Available online at https://e-journal.unair.ac.id/JFIKI/

Isoprinosine along with Favipiravir or Oseltamivir in Patients with Moderate Covid-19 at RSD Dr. Soebandi Jember

Chrysnanda Maryska[1], Didik Hasmono[2]*, Sofiati Diah Baisuni[3], Ayunda Nur Hidayatiningsih[3], Arina Dery Puspitasari[2], Retna Dwi Puspitarini[4], Budi Suprapti[2]

[1]Master Program of Clinical Pharmacy, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[3]Department of Pharmacy, RSD dr. Soebandi Teaching Hospital, Jember, Indonesia
[4]Department of Pulmonology, RSD dr. Soebandi Teaching Hospital, Jember, Indonesia

*Corresponding author: didik-h@ff.unair.ac.id

Submitted: 12 February 2022
Accepted: 23 March 2022
Published: 9 December 2022

Abstract

Background*: Isoprinosine is an immunomodulator that is now being used to treat Covid-19 patients.* ***Objectives****: To evaluate Isoprinosine with Favipiravir or Oseltamivir in moderate Covid-19.* ***Methods****: In a retrospective observational analysis, in-hospital moderate Covid-19 patients treated between June 2020 and June 2021 were included.* ***Results****: Inclusion criteria for 364 patients were met, with 135 receiving Favipiravir-Isoprinosine (Group 1) and 229 receiving Oseltamivir-Isoprinosine (Group 2). In group 1, the majority of patients (58.50%) were female (35.60%), had no comorbidities (71.60%), were discharged with a positive PCR (74.80%), did not require a breathing apparatus (99.26%), had leukocyte levels between 4,5-11,0 (82.22%), lymphocyte levels between 25-33 (34.07%), and were discharged with no ground-glass opacity (34.07%) (54.10%), LOS was 9-13 days (50.37%), while the mortality rate was 0.70%. In group 2, the majority of patients were male (54.10%), with the highest age range being 42-56 years (35.80%), without comorbidities (69.0%), discharged with a positive PCR (72.50 %), and without the need for a breathing apparatus (99.13%), with leukocyte levels ranging from 4.5 – 11.0 (81.22 %), with lymphocyte levels ranging from 25.0 – 33.0 (26.20 %), and were discharged with no ground-glass opacity (49.34 %), LOS was 9 - 13 days (34.06 %), and the mortality rate was 0.87%.****Conclusion****: In this trial, it was determined that combining isoprinosine with antivirals favipiravir or Oseltamivir could produce significant clinical improvement.*

Keywords*: isoprinosine, covid-19, moderate, immunomodulator*

How to cite this article:
Maryska, C., Hasmono, D., Baisuni, S. D., Hidayatiningsih, A. N., Puspitasari, A. D., Puspitarini, R. D. & Suprapti, B. (2022). Isoprinosine along with Favipiravir or Oseltamivir in Patients with Moderate Covid-19 at RSD Dr. Soebandi Jember. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 9(3), 209-219. http://doi.org/10.20473/jfiki.v9i32022.209-219

P-ISSN: 2406-9388
E-ISSN: 2580-8303

INTRODUCTION

From March 2020 to the present, a virus has caused a global pandemic. SARS-CoV-2 is the virus, and the World Health Organization has named the disease coronavirus disease 2019 (Covid-19) (WHO) (Yuki *et al*., 2020). The first case in Indonesia was discovered in March 2020, consisting of two individuals, and the second case was discovered on March 6, 2020, consisting of two individuals. Thus far, Covid-19 cases have continued to rise (Burhan *et al.*, 2020). Coronavirus is a virus with a single-stranded RNA genome isolated from various animal species. The number of cases continues to grow over time, confirming that this virus can be transmitted from human to human (Burhan *et al.*, 2020).

Covid-19 is an infectious disease that primarily affects respiratory and other organs. Covid-19's clinical manifestations and severity vary considerably. Asymptomatic, mild, moderate, severe, and critical were used to classify the severity. As is the case with infectious diseases in general, the immune system plays a crucial role in the fight against viruses (Yazdanpanah *et al*., 2020). SARS-CoV-2 can suppress the host immune system by inducing programmed cell death (apoptosis) (Mortaz *et al.*, 2020).

Numerous drugs, including lopinavir-ritonavir, remdesivir, hydroxychloroquine, and Azithromycin, have been tested in clinical trials to treat Covid-19 and be curative. Additional numerous medicines are undergoing clinical trials to determine whether they can be used as a definitive or adjunctive therapy (Yuki *et al*., 2020). PDPI (Perhimpunan Dokter Paru Indonesia-Indonesian pulmonary doctors association) has established guidelines for managing Covid-19 patients with varying degrees of severity in Indonesia. It has been updated several times in response to scientific advances (Burhan *et al.*, 2020). Favipiravir, Oseltamivir, and remdesivir are currently available and widely used antivirals for Covid-19 patients. However, this study is limited to favipiravir and Oseltamivir.

Favipiravir is an antiviral prodrug. Intracellularly, favipiravir is ribosylated and phosphorylated to form the active form, favipiravir ribofuranosyl-5-triphosphate (favipiravirRTP) (Instiaty *et al.*, 2020). Favipiravir's mechanism of action as a selective inhibitor of RNA-dependent RNA polymerase (RdRp) from SARS-CoV-2, where the RdRp in SARS-CoV-2 is tenfold more active than that in other viruses (Shannon *et al.*, 2020). FavipiravirRTP inhibits the RdRp virus by binding to it and inhibiting viral genome transcription and replication (Instiaty *et al.*, 2020). Thus, favipiravir can be used as an antiviral in treating Covid-19.

Oseltamivir is available in oseltamivir phosphate, a prodrug metabolized by plasma and hepatic esterase to oseltamivir carboxylate, the active form. Oseltamivir is approved for influenza A and B treatment and prevention and is available in oral formulations. After the replication cycle, influenza viruses require the enzyme neuraminidase to generate new viruses from infected cells. The active metabolite of Oseltamivir, oseltamivir carboxylate, interacts with neuraminidase, altering its conformation and inhibiting its activity. Inhibiting neuraminidase causes viral aggregation on the cell surface, reducing virus spread in the respiratory tract. Oseltamivir is in vitro activity against SARS-CoV-2 is unknown. In contrast to influenza, coronavirus lacks neuraminidase. Oseltamivir was used as an empirical therapy during the Covid-19 pandemic in China before the identification of a causative agent of the SARS-CoV-2 virus and because the pandemic occurred during the influenza season in China (Instiaty *et al.*, 2020)

Immunomodulators are also used in patients with Covid-19. Isoprinosine is an immunomodulator used in patients with Covid-19 (Dluholucky, 2020). Isoprinosine, also known as Inosine Pranobex or Methisoprinol, is a synthetic purine derivative with immunomodulatory and antiviral properties. Based on in vivo studies, it is known that the use of isoprinosine results in an increase in immunity or immunityIsoprinosine enhances immunity by stimulating T lymphocyte differentiation into cytotoxic T cells and T helper cells and increasing lymphokine production. Additionally, isoprinosine enhances the natural killer function of cells. Isoprinosine boosts humoral immunity by stimulating B lymphocyte differentiation into plasma cells, increasing antibody production. It increases the IgG and complement surface markers, and enhances the chemotaxis and phagocytosis of neutrophils, monocytes, and macrophages (Petrova *et al.*, 2010). Numerous studies have demonstrated that administering isoprinosine as adjuvant therapy may benefit specific subgroups of patients, particularly those who frequently experience immune dysfunction due to viral infections (Lasek *et al.*, 2015). According to a study conducted in nursing homes in the Czech Republic, the case fatality rate was significantly reduced by up to 11.9 percent, with an odds ratio (OR) of 2.8 (Beran *et al.*, 2020)

There is a dearth of research on the antiviral and Isoprinosine effects of Covid-19. Hence, this study

P-ISSN: 2406-9388
E-ISSN: 2580-8303

evaluates Isoprinosine with Favipiravir or Oseltamivir in moderate Covid-19.

MATERIALS AND METHODS

Materials

Study design

This retrospective observational study used medical records from patients with moderate Covid-19 at the RSD dr. Soebandi Teaching Hospital in Jember. It took place between June 2020 and June 2021.

Study population and setting

This study enrolled adolescent (12 years), adult, and elderly patients with a moderate diagnosis of Covid-19 who were hospitalized at RSD dr. Soebandi.

Data source and data extraction

This study analyzed data from 13 months of medical records (June 2020 – June 2021) with a time-limited sampling. The Faculty of Pharmacy at Universitas Airlangga has approved this study ethically. The demographic information about the patient, the medication, the laboratory data, the clinical data, the PCR test, the Photo Thorax, the length of stay, and the death rate were all collected. Gender, age, residence, and comorbidities are all included in demographic profiles. Medication data are records of medications prescribed to patients while undergoing treatment at the hospital. Laboratories data includes leukocyte and lymphocyte counts. Clinical data are records of patients' oxygen demand levels, which are classified as mild (1 – 6 lpm), moderate (7 – 10 lpm), or severe (11 – 15 lpm). Patients receiving isoprinosine therapy, the standard treatment for moderate Covid-19 patients, adolescent patients, adult patients, and older adults, were included in this study.

Tools

The data collection process was documented in Microsoft Excel spreadsheets. Patient identifiers have been used in place of initials to maintain patient confidentiality. A total of 364 patients were sampled. The data analysis was then presented in a table.

Statistical examination

In this study, both parametric and non-parametric statistical tests were used.

RESULTS AND DISCUSSION

Descriptive analysis

All data were subjected to descriptive analysis. A therapy group then classified the data. Group 1 comprises patients who are receiving treatment with Favipiravir – Isoprinosine. While Group 2 contains patients who received Oseltamivir – Isoprinosine therapy. The analysis of the description yielded the conclusions that can be seen in Table 1.

Based on the data in Table 1, It is known that the majority of members of group 1 are female (58.50%), the majority are between the ages of 18-60 years (88.89%), and the majority are free of comorbidities (71.60%). Meanwhile, in group 2, the majority of participants were male (54.10%), the median age range was 18-60 years (85.59%), and the majority did not have any comorbidities (69.00%).

A descriptive analysis of clinical data, laboratory data, supporting examination data, LOS data, and patient mortality rates were also carried out. Table 2 summarizes the analysis's findings.

As shown in Table 2, KRS patients with clinical conditions in groups 1 and 2 do not require oxygen. Prior to receiving combination therapy, most patients in both groups had an oxygen demand of 1-6 lpm.

Table 1. The use of isoprinosine in combination with antivirals in the treatment of patients with moderate Covid-19

Characteristics		Favipiravir-Isoprinosine Combination	Oseltamivir-Isoprinosine Combination
Gender	Man	56 (41.50%)	124 (54.10%)
	Woman	79 (58.50%)	105 (45.90%)
Age	12 - 17	5 (3.70%)	4 (1.75%)
	18 - 60	120 (88.89%)	196 (85.59%)
	> 60	10 (7.41%)	29 (12.66%)
	Mean ± SD	39.6 ± 12.92	42.00 ± 14.14
Comorbid	No Comorbid	91 (71.60%)	147 (69.00%)
	Hypertension	25 (19.70%)	34 (16.00%)
	Diabetes Mellitus	11 (8.70%)	32 (15.00%)

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 2. Clinical data on isoprinosine in combination with favipiravir or oseltamivir

Characteristics			Favipiravir-Isoprinosine Combination	Oseltamivir-Isoprinosine Combination
Oxygen Consumption Level	Pre	Mild	69 (51.10%)	151 (66.00%)
		Moderate	49 (36.30%)	55 (24.00%)
		Severe	17 (12.60%)	23 (10.00%)
	Post	No Requirement for Oxygen	134 (99.26%)	227 (99.13%)

Table 3. Laboratory data on isoprinosine in combination with favipiravir or oseltamivir

Characteristics			Favipiravir-Isoprinosine Combination	Oseltamivir-Isoprinosine Combination
Leukocytes	Pre	< 4.5	13 (9.63%)	18 (7.86%)
		4.5 – 11.0	104 (77.04%)	177 (77.29%)
		>11.0	18 (13.33%)	34 (14.58%)
		mean ± SD	mean 7.50 ± 3.35	mean 7.85 ± 3.69
	Post	< 4.5	6 (4.45%)	4 (1.75%)
		4.5 – 11.0	111 (82.22%)	186 (81.22%)
		>11.0	18 (13.33%)	39 (17.03%)
		mean ± SD	mean 8.52 ± 3.66	mean 8.90 ± 3.75
Lymphocytes	Pre	< 25.00	86 (63.70%)	141 (61.57%)
		25.00 – 33.00	25 (18.52%)	50 (21.83%)
		>33.00	24 (17.78%)	38 (16.60%)
		mean ± SD	mean 22.72 ± 11.51	mean 22.19 ± 10.76
	Post	< 25.00	52 (38.52%)	109 (47.60%)
		25.00 – 33.00	46 (34.07%)	60 (26.20%)
		>33.00	37 (27.41%)	60 (26.20%)
		mean ± SD	mean 27.10 ± 9.84	mean 25.79 ± 11.34

According to Table 3, leukocyte levels in group 1 were between 4.5 -11.0 (82.22%), and lymphocyte levels were between 25.0-33.0. (34.07%). Meanwhile, in group 2, the leukocyte count was between 4.5 - 11.0 (81.22%), and the lymphocyte count was between 25.0 - 33.0 (26.2%). Normal leukocytes and lymphocytes levels increased in both combinations before and after the patient received therapy.

According to Table 4, the LOS for group 1 was between 9 and 13 days (50.3%), and the percentage of patients who died was 0.70%. While in group 2, the median LOS was 9 - 13 days (34.0%), and the mortality rate was 0.87%.

In Table 5, it is stated that in group 1, patients discharged with positive PCR (74.80%) and no infiltrates on chest X-rays were included (54.10%). In group 2, patients discharged with a positive PCR (72.50%) and no infiltrates on chest X-rays were included (49.34%). Following treatment, the number of patients developing pneumonia decreased significantly in the two combination groups. Similarly, the number of patients with positive PCR results decreased in both combination groups.

Parametric difference tests were performed on data with interval or ratio types, such as leukocyte counts, lymphocyte counts, and patient length of stay. The parametric difference test was also performed on normally distributed and homogeneous data. The Paired t-test was used to determine the parametric difference in this study. The non-parametric difference test can be used with data that are not normally distributed or homogeneous; it can also be used with ordinal or interval/ratio scales. The Wilcoxon test is used to determine non-parametric differences in this study. If the p-value for the paired t-test or Wilcoxon test is less than 0.05, the test is considered significant. Because there is no data prior to and following the procedure, the LOS variable and the patient's mortality rate cannot be tested independently. Table 6 contains the results of statistical tests conducted in this study.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 4. Characteristics of LOS data and mortality rates in isoprinosine-favipiravir- or oseltamivir-combination

Characteristics		Favipiravir-Isoprinosine Combination	Oseltamivir-Isoprinosine Combination
Length of Stay	< 9 hari	3 (2.22%)	10 (4.37%)
	9 - 13 hari	68 (50.37%)	78 (34.06%)
	> 13 hari	64 (47.41%)	141 (61.57%)
	mean ± SD	mean 13.0 ± 3.27	mean 17.22 ± 11.45
Death		1 (0.70%)	2 (0.87%)

Table 5. Other test data characteristics of isoprinosine in combination with favipiravir or oseltamivir

Characteristics			Favipiravir-Isoprinosine Combination	Oseltamivir-Isoprinosine Combination
PCR	Pre	Positive	135 (100%)	229 (100%)
	Post	Positive	101 (74.80%)	166 (72.50%)
		Negative	34 (25.50%)	63 (27.50%)
chest photograph	Pre	Pneumonia	87 (64.40%)	159 (69.43%)
		No Ground Glass Opacity	48 (35.60%)	70 (30.57%)
	Post	Pneumonia	62 (45.90%)	116 (50.66%)
		No Ground Glass Opacity	73 (54.10%)	113 (49.34%)

When a patient is hospitalized for a suspected infection, isoprinosine is given. After confirming the diagnosis of Covid-19 with two PCR tests, isoprinosine was discontinued, and standard Covid-19 therapy was initiated according to the applicable Clinical Pathway at RSD dr. Soebandi. The standard therapy is Azithromycin iv 1 x 500 mg, Oseltamivir po 2 x 75 mg (for 7-14 days)/Lopinavir 400 mg-Ritonavir 100 mg p.o 2 x 2 tabs for 7-14 days/Favipiravir p.o loading dose of 2 x 1600 mg (first day) and then 2 x 600 mg on days 2 - 5, Vitamin C p.o 1 x 500 mg, and other medications as indicated by the patients. After antiviral therapy was completed, Isoprinosine was continued; it was given on day six following favipiravir in group 1 and on day eight following favipiravir in group 2. There was no group of patients receiving Lopinavir/Ritonavir in this study. Lopinavir/Ritonavir was used in several Covid-19 patients at dr. Soebandi, but the government has made the case that the antiviral should be reserved for HIV patients only.

As is the case with infectious diseases in general, the immune system plays a critical role in the fight against viruses (Yazdanpanah *et al*., 2020). SARS-CoV-2, on the other hand, can impair the host immune system by suppressing T cell function and inducing programmed cell death (apoptosis) (Mortaz *et al.*, 2020). Thus, immunomodulatory agents may be considered in the treatment of Covid-19 patients. Isoprinosine is one of the immunomodulators currently being used in Covid-19 patients.

The study included 760 medical records of moderate Covid-19 patients as a total sample. Nevertheless, only 364 medical records met the criteria for inclusion. The following parameters were examined in this study: supporting data in the form of PCR and thorax photograph; laboratory data in the form of leukocyte and lymphocyte; clinical data from patients in the form of oxygen demand level; length of stay patient mortality.

Gender is recognized as a significant factor in the epidemiology of various diseases. This also holds for Covid-19. According to studies conducted in China and Europe, men die at a higher rate from Covid-19 infection than women and are independently associated with poor Covid-19 disease progression. This is because women and men have different immune responses. Additionally, male-specific behaviours such as smoking, drinking alcohol, or consuming alcoholic beverages increase the risk of death from Covid-19 (Vahidy *et al.*, 2021). Women are predisposed to autoimmune diseases, whereas men are predisposed to infectious diseases. Acceptable explanations include the X chromosome's protection and the female hormone estrogen. Estrogen is known to have the potential to mitigate the severity of SARS-CoV-2 infection. Several studies indicated that women had superior anti-inflammatory, antiviral, and humoral system responses during the infection process compared to men. It is well-established that testosterone can impair a man's response to vaccines (Ciarambino *et al.*, 2021). The majority of sexes in group 1 were female, while most in group 2 were male.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 6. Analysis of variables with dependent variables

No.	Variable	Different Test	Test Type	Significance	Sig. of Favipiravir and Isoprinosine in Combination	Sig. of Oseltamivir and Isoprinosine in Combination
1.	PCR	Non-Parametric	Wilcoxon	p-value < 0.05	0.000	0.000
2.	Oxygen Consumption Level	Non-Parametric	Wilcoxon	p-value < 0.05	0.000	0.000
3.	Leukocytes	Non-Parametric	Wilcoxon	p-value < 0.05	0.010	0.000
4.	Lymphocytes correlation coefficient	Parametric	Paired t-test	p-value < 0.05	0.000 0.318	0.000 0.520
5.	chest photograph	Non-Parametric	Wilcoxon	p-value < 0.05	0.000	0.000
6.	LOS	-	-	-	-	
7.	Death	-	-	-	-	

In contrast to numerous previous studies, the number of male patients infected with Covid-19 is significantly greater than that of female patients. This could have occurred due to the study's focus on moderate Covid-19 patients. Consequences of men's vulnerability to disease worsening severe or critical disease. This, however, requires additional research.

In this study, the average age of patients with moderate COVID-19 infection was 42 years, with a range of 12-81 years and a standard deviation of 14. In general, increasing age is associated with increasing the severity of Covid-19. Children are generally less susceptible to Covid-19 due to the still-functioning general system and lack of exposure to the surrounding environment during the Pandemic (Davies *et al.*, 2020). A study conducted in Italy shows that higher age is one of the independent factors associated with the risk of death (Aksel *et al.*, 2021). Another study confirmed that Covid-19 patients aged 50 years were 15.4 times more likely to die than confirmed Covid-19 patients aged 50 years (Biswas *et al.*, 2021).

Comorbidity is a predictor of Covid-19 patient progression. The percentage of samples without comorbidities was highest in both groups, namely group 1 (71.60%) and group 2 (69.00%). In group 1, hypertension was the most prevalent comorbidity (19.70%), while in group 2, hypertension was the least prevalent (16.00%). Diabetes Mellitus is the most common comorbid condition, with a percentage of 8.70% in group 1 in group 2 15.00%. Age and pre-existing comorbidities such as hypertension, diabetes, obesity, heart disease, chronic kidney disease, and liver disease worsen Covid-19 progression. According to studies conducted in China, North America, and Europe, the patient's age and comorbidities influence the mortality rates associated with worsening Covid-19 progression (Surendra *et al.*, 2021). This is based on existing research data, which indicates that group 1 has a mortality rate of 0.7% and group 2 has a mortality rate of 0.87%. According to a study, the mortality rate at age 15 is 0.6%, at age 50 is 39.5%, and at all age groups is 6% (Beran *et al.*, 2021).

All patients in this study had been discharge in a non-oxygen-requiring state, with the highest percentages in group 1 (96.26%) and group 2 (96.26 percent) (99.13%). No studies demonstrate Oseltamivir's efficacy in reducing the need for breathing apparatus in Covid-19 patients. According to one systematic review study, there is no evidence that favipiravir can reduce patients' need for breathing apparatus (Özlüşen *et al.*, 2021). However, Hassanipour et al. found a clinically significant improvement in oxygen demand between the favipiravir and the control groups 7 days after hospitalization (RR = 1.24, 95 percent CI = 1.09-1.41; P = 0.001). After 14 days of hospitalization, viral clearance occurred in the favipiravir group but was not statistically significant (RR = 1.11, 95 percent CI = 0.98-1.25; P = 0.0094). The favipiravir group had a 7% lower oxygen demand than the control group (RR = 0.93, 95% CI = 0.67-1.28; P = 0.664). The WHO recommends oxygen therapy for patients with respiratory distress, hypoxemia, or shock with a target SpO2 of greater than 94%. The favipiravir group had a 7% lower oxygen demand than the control group (RR = 0.93, 95% CI = 0.67-1.28; P = 0.664). Appropriate oxygen therapy allows for increased oxygenation of thickened and inflamed lung tissue, effectively treating hypoxemia (Long *et al.*, 2021). Shortness of breath is caused by SARS-CoV-2-mediated mitochondrial

P-ISSN: 2406-9388
E-ISSN: 2580-8303

damage in smooth muscle cells of the pulmonary arteries, resulting in impaired pulmonary hypoxic vasoconstriction (Shianata *et al*., 2021). Isoprinosine is administered following antiviral therapy, hoping the virus-cleansing process will be accelerated by boosting the body's immune system. Although there is no literature directly related to the level of patient oxygen demand, the KRS profile of patients who no longer require oxygen is one of the clinical conditions that can describe a patient's lung function improvement.

The significance of groups 1 (0.010) and 2 (0.000) is well established. On this basis, it can be concluded that each combination results in a difference in leukocyte levels prior to and following therapy, both in group 1 and group 2. The normal leukocyte count ranges from 4.5 to 11.0 in RSD dr. Soebandi. According to existing clinical reports, there are changes in the leukocyte portion of the circulation in patients with mild and severe degrees. These changes can take the form of values falling below or exceeding normal levels (Wang *et al.,* 2020). Between before and after therapy, the percentage of patients with normal leukocyte counts increases.

The combination of Isoprinosine Favipiravir and Isoprinosine Oseltamivir is known to have a significant value of 0.000. Thus, it can be concluded that each combination results in differences in lymphocyte levels prior to and following treatment with Isoprinosine Favipiravir and Isoprinosine Oseltamivir. The correlation values for each combination must be compared to determine which combination has a greater effect on the patient's lymphocyte levels. The correlation coefficient between isoprinosine and favipiravir is 0.318, while the correlation coefficient between isoprinosine and Oseltamivir is 0.520. The greater the correlation coefficient, the greater the impact on the patient's lymphocyte count..

However, additional research using a control group with and without isoprinosine is necessary to determine which drug affects lymphocyte levels. Quantitative and functional lymphocyte changes can impair the immune response to the virus and may develop an immunopathological response. Lymphopenia is a prevalent immunological disorder in severe Covid-19 patients, accounting for 96.1% of cases. The percentage of lymphocytes in the blood is a laboratory measurement significantly associated with the development of Covid-19 disease (Jafarzadeh *et al.*, 2021). Yusra and Natasha report that common 2020 laboratory data abnormalities in Covid-19 include a decrease in absolute lymphocyte and albumin counts, as well as an increase in lactate dehydrogenase (LDH) and c-reactive protein (CRP), but normal procalcitonin (PCT). COVID-19 is classified as severe when LDH, CRP, D-dimer, and IL6 levels increase while platelets and lymphocytes decrease. The percentage of lymphocytes in the blood can be considered a reliable and accurate indicator for grading Covid-19 patients. Low lymphocytes can express the primary SARS-CoV-2 receptor, the enzyme angiotensin II (ACE2). SARS-CoV-2 can also enter lymphocytes via an ACE2-independent pathway. Both SARS-CoV-2 and immune-mediated immunity have mechanisms that can result in lymphopenia by impairing lymphocyte production, survival, and redistribution of lymphocytes. In Covid-19 patients, metabolic and biochemical changes may also affect lymphocyte production and survival. Lymphopenia can result in immunosuppression and initiate a cytokine storm; these factors contribute significantly to viral persistence, replication, multi-organ failure, and death (Beran *et al.*, 2021).

There is a bias in the LOS variable due to the Covid-19 management guidelines in Indonesia, according to PDPI. At the pandemic's start, patients were permitted to KRS if their PCR results were negative for two consecutive days. Then, in PDPI ed 2, the criteria changed to allow patients to KRS if they have been free of symptoms (requiring treatment for both Covid-19 and comorbid diseases) for three days even though the PCR results remain positive (Burhan *et al.*, 2020). A different test cannot be conducted on the LOS variable because there is no prior and subsequent data.

Given that groups 1 and 2 produced statistically significant results, it can be concluded that there is a difference or influence between the two combinations on the PCR variable. The expected PCR result is negative. The Favipiravir Isoprinosine combination had a negative PCR result rate of 25.29%, while the Oseltamivir Isoprinosine combination had a negative PCR result rate of 27.50%. By examining this percentage, it is clear that Oseltamivir Isoprinosine provides superior results. Oseltamivir is no longer recommended for Covid-19 patients who do not exhibit flu-like symptoms. This may have occurred because the study's findings indicated that the data obtained were more favuorable to Oseltamivir Isoprinosine than to the use of Favipiravir Isoprinosine.

Additionally, the criteria for patient discharge in managing Covid-19 patients who change affect. Covid-19 patient management PDPI ed.1, valid from April

P-ISSN: 2406-9388
E-ISSN: 2580-8303

2020 to July 2020, states that patients may be discharged home if their two-time polymerase chain reaction (PCR) results are negative. As of August 2020, it is known that patients with PDPI ed.2 Covid-19 can be discharged with several requirements even if the PCR results remain positive. If the PCR follow-up reveals a positive result after clinical improvement and a three-day fever-free period, a persistent positive condition caused by detecting inactivated virus fragments or particles may exist. The Cycle Threshold (CT) value can be used to determine whether or not a patient's condition is infectious, as the cut-off value varies according to the reagents and tools used. According to several hypotheses, persistent positive results result from the device detecting inactivated virus components. Numerous studies have discovered that asymptomatic patients can still have positive RT-PCR results a few weeks after symptoms have resolved (Burhan *et al.*, 2020). According to PDPI edition 3 of 2020, patients may be discharged from hospital care if they meet the criteria for isolation completion and clinical criteria, namely the results of a thorough clinical study, including radiological images demonstrating improvement and blood tests demonstrating improvement, conducted by a physician stating the patient is allowed to go home. Whether related or unrelated, the patient requires no further action/treatment (Burhan *et al.*, 2020).

Because favipiravir will not be available at RSD dr. Soebandi Jember until November 2020, the magnitudes of the two percentages in the PCR results cannot be compared to determine which combination is superior. Oseltamivir lacks sufficient empirical evidence to recommend it as a treatment for Covid-19 infection associated with dyspnea or hypoxia in Wuhan. However, Oseltamivir may be used in Covid-19 patients to prevent further deterioration of the senses of taste and smell (Chiba, 2021). Favipiravir therapy resulted in a more rapid viral clearance, as indicated by a more rapid negative PCR result, compared to the group that did not receive Favipiravir (Finberg *et al.*, 2021). Isoprinosine has been shown to enhance the immune system by increasing the number of Th1 cells and the activity of natural killer cells (Sliva *et al*., 2019). With an enhanced immune system, it is hoped that the process of virus clearance in patients will be accelerated.

X-ray of the chest Each combination has a Sig value of 0.000. Thus, isoprinosine Favipiravir or isoprinosine Oseltamivir provides a significant difference in the patient's chest X-ray results. The combination of isoprinosine and favipiravir has a 54.10 percent success rate in preventing infiltrates on chest x-rays, while isoprinosine Oseltamivir has a 49.34 % success rate. In a study of the efficacy of Favipiravir therapy in Covid-19 patients, it was discovered that administering Favipiravir increased the improvement in chest X-rays by 91.43 %t in patients with negative PCR results following seven days of Favipiravir administration (Özlüşen *et al.*, 2021). This could also be related to the antibiotic Azithromycin administered to patients with moderate-grade Covid-19 at the RSD dr. Soebandi Jember.Azithromycin 5 days or Levofloxacin seven days daily is equally effective and safe for acute bacterial bronchitis exacerbations (ABECB). Although macrolide agents continue to be an effective treatment option for patients with acute bacterial exacerbations of ABECB and other community-acquired respiratory tract infections (Amsden *et al.*, 2003).

Along with patients discharged from the hospital with no visible infiltrate on the chest X-ray, there were also patients discharged with pneumonia. In Covid-19 patients who do not experience severe respiratory distress during hospitalization, abnormal lung examinations will occur ten days after the first Covid-19 symptoms appear. After two weeks, the lung lesions are absorbed, leaving behind ground-glass opacities (GGO) and subpleural parenchymal bands. Clinically cured patients of Covid-19 can still be found to have GGO (Pan *et al.*, 2020). The improvement of the immune system can also be seen in the patient's lung function, and the addition of isoprinosine to the treatment regimen is expected to aid in the immune system's improvement so that it can fight existing viruses.

Isoprinosine has long been recognized for its ability to treat various viral infections, including influenza and other influenza-like illnesses. Isoprinosine contains an immunomodulatory mechanism that promises an overall therapeutic effect against various viral pathogenic variants. While immunomodulators are unlikely to result in complete recovery, early intervention can alter the course of the disease. Clinical and immunological analytical studies conducted over the last six years have established that isoprinosine can treat most viral infections via natural killer cells and cytotoxicity; this efficacy is expected to be transferred to the acute respiratory infection caused by the Covid-19 virus that is currently spreading. Early isoprinosine therapy has been shown to reduce early viral immunosuppression and lymphopenia, which are

P-ISSN: 2406-9388
E-ISSN: 2580-8303

strongly associated with the progression of Covid-19, hospitalization, and death.

However, retrospective studies and studies conducted in Ecuador and India indicate that isoprinosine may be beneficial in the treatment of Covid-19 patients. Since the summer of 2020, two multicenter randomized clinical trials with IP in patients with moderate COVID-19 have been ongoing in India. The first was a 60-patient open-label proof-of-concept study. It examined the effect of IP in COVID-19 patients when used in conjunction with standard of care versus when used alone. Subgroup analysis of patients in this study revealed that when inosine pranobex was added to standard of care containing Azithromycin and Hydroxychloroquine with or without Ivermectin, it resulted in a significantly higher clinical response at Day-14 (100.00 percent vs. 69.23 percent; p = 0.03). At Day 7, 14, and 21, there was a trend toward a (numerically) greater clinical response in the IP + standard of care group compared to the Standard of care group; however, statistical significance was not reached. This could be due to the study's small sample size or to variation in the current standard of care across sites. IP was generally well-tolerated. According to a randomized controlled trial conducted in Ecuador from March to April 2020, 60 Covid-19 patients were randomly assigned to one of two groups, with 30 patients receiving Isopsinosine therapy and the remaining 30 patients receiving placebo. From the start of the study, it was known that patients in the Isoprinosine group improved clinically within 15 days, as measured by swab PCR being negative faster, reaching the SaO2 target > 90 percent faster, and chest x-rays showing a greater number of no lesions in patients in the Isoprinosine group. Additionally, isoprinosine is used in the Czech Republic and possibly elsewhere. The analysis of healthcare reimbursement data for patients treated with Isoprinosine in combination with other standard therapies and mortality data reveals essential information about Isoprinosine's effectiveness in Covid-19 patients (Beran *et al.*, 2021).

CONCLUSION

RSD dr. Soebandi Jember obtained the results from a study evaluating the combination of isoprinosine and antiviral Favipiravir or Oseltamivir in treating moderate-grade Covid-19 patients with RSD. The two of varieties improved clinical conditions as measured by PCR parameters, oxygen demand levels, leukocyte counts, lymphocyte counts, and thorax photos. Both combinations have a mortality rate of less than 1%.

STUDY LIMITATION

This study is limited to the availability of antivirus in RSD dr. Soebandi, causing an imbalance in the number of participants between the two groups. Additional research with a control group devoid of isoprinosine is required.

ACKNOWLEDGMENT

This study involves many authors who have an essential role in realizing this journal. Each author has expertise that is very useful in the research process from beginning to end. All authors declare that this study was accomplished without any funding or support.

AUTHOR CONTRIBUTIONS

Conceptualization, C. M., D. H., B. S., R. D. P.; Methodology, C. M., D. H., B. S., R. D. P.; Software, C. M., S. D. B., A. N. H.; Validation, D. H., B. S., R. D. P., A. D. P.; Formal Analysis, C. M., D. H., B. S.; Investigation, C. M., D. H., B. S., R. D. P.; Resources, C. M., D. H., B. S., R. D. P.; Data Curation, C. M., D. H., B. S.; Writing - Original Draft, C. M., D. H., B. S., R. D. P.; Writing - Review & Editing, C. M., D. H., B. S., R. D. P.; Visualization, C. M.; Supervision, D. H., B. S., R. D. P.; Project Administration, D. H., B. S., R. D. P., Funding Acquisition, C. M.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Aksel, G., İslam, M. M., Algın, A., Eroğlu, S. A., Yaşar, G. B., Ademoğlu, E. & Dölek, U. C. (2021). Early Predictors of Mortality for Moderate to Severely Ill Patients with Covid-19. *American Journal of Emergency Medicine*; *45*; 290–296. doi: 10.1016/j.ajem.2020.08.076.

Amsden, G. W., Baird, I. M., Simon, S. & Treadway, G. (2003). Efficacy and Safety of Azithromycin Vs Levofloxacin in the Outpatient Treatment of Acute Bacterial Exacerbations of Chronic Bronchitis. *Chest*; *123*; 772–777. doi: 10.1378/chest.123.3.772.

Beran, J., Špajdel, M., Katzerová, V., Holoušová, A., Malyš, J., Rousková, J. F. & Slíva, J. (2020). Inosine Pranobex Significantly Decreased the Case-Fatality Rate Among PCR Positive Elderly

with SARS-CoV-2 at Three Nursing Homes in the Czech Republic. *Pathogens*; *9*; 1-10. doi: 10.3390/pathogens9121055.

Beran, J., Špajdel, M. & Slíva, J. (2021). Inosine Pranobex Deserves Attention as a Potential Immunomodulator to Achieve Early Alteration of the Covid-19 Disease Course. *Viruses*; *13*; 1-13. doi: 10.3390/v13112246.

Biswas, M., Rahaman, S., Biswas, T. K., Haque, Z. & Ibrahim, B. (2021). Association of Sex, Age, and Comorbidities with Mortality in COVID-19 Patients: A Systematic Review and Meta-Analysis. *Intervirology*; *64*; 36–47. doi: 10.1159/000512592.

Burhan, E., Susanto, A. D., Isbaniah, F., Nasution, S. A., Ginanjar, E., Pitoyo, C. W., Susilo, A. *et al.* (2020). Pedoman Tatalaksana Covid-19 Edisi 3. In Erlina Burhan, Agus Dwi Susanto, Fathiyah Isbaniah, Sally Aman Nasution, E. *et al.* (eds). Jakarta: Perhimpunan Dokter Paru Indonesia (PDPI), Perhimpunan Dokter Spesialis Kardiovaskular Indonesia (PERKI), Perhimpunan Dokter Spesialis Penyakit Dalam Indonesia (PAPDI), Perhimpunan Dokter Anestesiologi dan Terapi Intensif Indonesia (PERDATIN), Ikatan Dokter Anak Indonesia (IDAI).

Chiba, S. (2021). Effect of Early Oseltamivir on Outpatients without Hypoxia with Suspected COVID-19. *Wiener Klinische Wochenschrift*; *133*; 292–297. doi: 10.1007/s00508-020-01780-0.

Ciarambino, T., Para, O. & Giordano, M. (2021) Immune System and COVID-19 by Sex Differences and Age. *Women's Health*; *17*; 1-6. doi: 10.1177/17455065211022262.

Davies, N. G., Klepac, P., Liu, Y., Prem, K., Jit, M., CMMID COVID-19 working group & Eggo, R. M. (2020). Age-Dependent Effects in the Transmission and Control of COVID-19 Epidemics. *Nature Medicine*; *26*; 1205–1211. doi: 10.1038/s41591-020-0962-9.

Dluholucky, S. (2020). Inosine Pranobex (IP) - Possibilities of Its Use in the Treatment of COVID19. *Archives of Community Medicine and Public Health*; *6*; 200–201. doi: 10.17352/2455-5479.000106.

Finberg, R. W., Ashraf, M., Julg, B., Ayoade, F., Marathe, J. G., Issa, N. C., Wang, J. P., Jaijakul, S., Baden, L. R. & Epstein, C. (2021). US201 Study: A Phase 2, Randomized Proof-of-Concept Trial of Favipiravir for the Treatment of COVID-19. *Open Forum Infectious Diseases*; *8*; 1-8. doi: 10.1093/ofid/ofab563.

Instiaty, Darmayani, I. G. A. A. P. S., Marzuki, J. E., Angelia, F., William, Siane, A., Sary, L. D., Yohanes, L., Widyastuti, R., Nova, R., Simorangkir, D. S., Lonah, Safitri, Y., Aliska, G. & Gayatri, A. (2020). Antiviral Treatment of COVID-19: a Clinical Pharmacology Narrative Review. *Medical Journal of Indonesia*; *29*; 332–345. doi: 10.13181/mji.rev.204652.

Jafarzadeh, A., Jafarzadeh, S., Nozari, P., Mokhtari, P. & Nemati, M. (2021). Lymphopenia an Important Immunological Abnormality in Patients with COVID-19: Possible Mechanisms. *Scandinavian Journal of Immunology*; *93*; 1–16. doi: 10.1111/sji.12967.

Lasek, W., Janyst, M., Wolny, R., Zapała, L., Bocian, K. & Drela, N. (2015). Immunomodulatory Effects of Inosine Pranobex on Cytokine Production by Human Lymphocytes. *Acta Pharmaceutica*; *65*; 171–180. doi: 10.1515/acph-2015-0015.

Long, L., Wu, L., Chen, L., Zhou, D., Wu, H., Lu, D., Li, H., Duan, X., Han, Y., Li, X., Wang, Q. & Zhang, J. (2021). Effect of Early Oxygen Therapy and Antiviral Treatment on Disease Progression in Patients with COVID-19: A Retrospective Study of Medical Charts in China. *PLoS Neglected Tropical Diseases*; *15*; 1–15. doi: 10.1371/journal.pntd.0009051.

Mortaz, E., Tabarsi, P., Varahram, M., Folkerts, G., Adcock, I. M. (2020). The Immune Response and Immunopathology of COVID-19. *Frontiers in Immunology*; *11*; 1–9. doi: 10.3389/fimmu.2020.02037.

Özlüşen, B., Kozan, S., Akcan, R. E., Kalender, M., Yaprak, D., Peltek, I. B., Keske, S., Gönen, M. & Ergönül, O. (2021). Effectiveness of favipiravir in COVID-19: A Live Systematic Review. *European Journal of Clinical Microbiology and Infectious Diseases*; *40*; 2575–2583. doi: 10.1007/s10096-021-04307-1.

Pan, F., Ye, T., Sun, P., Gui, S., Liang, B., Li, L., Zheng, D., Wang, Z., Hesketh, R. L., Yang, L. & Zheng, C. (2020). Time Course of Lung Changes at Chest CT During Recovery from Coronavirus Disease 2019 (COVID-19). *Radiology*; *295*; 715–721. doi: 10.1148/radiol.2020200370.

Petrova, M., Jelev, D., Ivanova, A. & Krastev, Z. (2010). Isoprinosine Affects Serum Cytokine

Levels in Healthy Adults. *Journal of Interferon and Cytokine Research*; *30*; 223-228 doi: 10.1089/jir.2009.0057.

Shannon, A., Selisko, B., Le, N-T-T., Huchting, J., Touret, F., Piorkowski, G., Fattorini, V., Ferron, F., Decroly, E., Meier, C., Coutard, B., Peersen, O. & Canard, B. (2020). Rapid Incorporation of Favipiravir by the Fast and Permissive Viral RNA Polymerase Complex Results in SARS-CoV-2 Lethal Mutagenesis. *Nature Communications*; *11*; 1–9. doi: 10.1038/s41467-020-18463-z.

Shianata, C. M., Engka, J. N. A. & Pangemanan, D. H. C. (2021). Happy Hypoxia pada Coronavirus Disease. *Jurnal Biomedik: Jbm*; *13*; 58–66. doi: 10.35790/jbm.13.1.2021.31743.

Sliva, J., Pantzartzi, C. N. & Votava, M. (2019). Inosine Pranobex: a Key Player in the Game Against a Wide Range of Viral Infections and Non-Infectious Diseases. *Advances in Therapy*; *36*; 1878-1905. doi: 10.1007/s12325-019-00995-6.

Surendra, H. Elyazar, I. R. F., Djaafaraa, B. A., Ekawati, L. L., Saraswati, K., Adrianf, V., Widyastuti, Oktaviaf, D., Salamaf, N., Lina, R. N., Andrianto, A., Lestari, K. D., Burhang, E., Shankar, A. H., Thwaites, G., Bairda, K. & Hamers, R. L. (2021). Clinical Characteristics and Mortality Associated with COVID-19 in Jakarta, Indonesia: a Hospital-Based Retrospective Cohort Study. *The Lancet Regional Health - Western Pacific*; *9*; 1-9. doi: 10.1016/j.lanwpc.2021.100108.

Vahidy, F. S., Pan, A. P., Ahnstedt, J., Munshi, Y., Choi, H. A., Tiruneh, Y., Nasir, K., Kash, B. A., Andrieni, J. D. & McCullough, L. D. (2021). Sex Differences in Susceptibility, Severity, and Outcomes of Coronavirus Disease 2019: Cross-Sectional Analysis from a Diverse US Metropolitan Area. *PLoS ONE*; *16*; 1–14. doi: 10.1371/journal.pone.0245556.

Wang, J., Jiang, M., Chen, X. & Montaner, L. J. (2020). Cytokine Storm and Leukocyte Changes in Mild Versus Severe SARS-CoV-2 Infection: Review of 3939 COVID-19 Patients in China and Emerging Pathogenesis and Therapy Concepts. *Journal of Leukocyte Biology*; *108*; 17–41. doi: 10.1002/JLB.3COVR0520-272R.

Yazdanpanah, F., Hamblin, M. R. & Rezaei, N. (2020). The Immune System and COVID-19: Friend or Foe?. *Life Sciences*; *256*; 1-5.

Yuki, K., Fujiogi, M. & Koutsogiannaki, S. (2020). COVID-19 Pathophysiology: a Review Koichi. *Clinical Immunology Journal*; *215*; 1-7.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 220-228
DOI: 10.20473/jfiki.v9i32022.220-228
Available online at https://e-journal.unair.ac.id/JFIKI/

The Effect of Decyl Glucoside on Stability and Irritability of Nanostructured Lipid Carriers-Green Tea Extract as Topical Preparations

Rufaidah Azzahrah[1], Noorma Rosita[2]*, Djoko Agus Purwanto[2], Widji Soeratri[2]
[1]Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: noorma-r@ff.unair.ac.id

Submitted: 28 Februari 2022
Accepted: 30 March 2022
Published: 9 December 2022

Abstract

Background*: Green Tea Extract (GTE) is a natural antioxidant compound that can protect the skin from photocarcinogenesis (DNA damage due to ultraviolet exposure). GTE has low stability, which needs a delivery system such as Nanostructured Lipid Carriers (NLC) with decyl glucoside (DG) as a natural surfactant that at the right concentration can produce a significantly small particle size which can improve the stability of the NLC.* ***Objective****: To determine the effect of DG usage on the characteristics, physical stability, and irritability of NLC-GTE preparation.* ***Methods****: NLC-GTE preparation used the High Shear Homogenization (HSH) method with three formulas, which contained DG 2%, 2.5%, and 3% consecutively. Afterwards, the characteristic and physical stability tests were conducted using the thermal cycling method for three cycles with two different temperatures (48 hours/cycle, 2 - 8°C and 40°C). The irritability test used Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) method.* ***Results****: Characteristic test of organoleptic showed that all formulas were white, odorless, and had a semi-solid consistency. However, the pH, particle size, and polydispersity index values from all formulas were within the normal range of values. The physical stability test result showed that 3% DG was the most stable formula. This formula was within the non-irritating range of values in HET-CAM.* ***Conclusion****: NLC-GTE with an increased concentration of DG as a surfactant can improve the characteristics and physical stability of the preparation. F3 (3% DG) is the best formula compared to other formulas and indicates non-irritating in the HET-CAM test.*

Keywords*: nanostructured lipid carriers, green tea extract, decyl glucoside, thermal cycling, Hen's Egg Test on the chorioallantoic membrane*

How to cite this article:
Azzahrah, R., Rosita, N., Purwanto, D. A. & Soeratri, W. (2022). The Effect of Decyl Glucoside on Stability and Irritability of Nanostructured Lipid Carriers-Green Tea Extract as Topical Preparations. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 9(3), 220-228. http://doi.org/10.20473/jfiki.v9i32022.220-228

INTRODUCTION

Green tea extract (GTE) is obtained from the tea plant or Camellia sinensis (Wang *et al*., 2020). GTE has the highest phenolic and flavonoid content compared to other tea extracts, with an IC50 value of 0.487µg/mL (Widowati *et al*., 2015). In addition, GTE can also be used for UVA and UVB protection (Saini *et al*., 2019) and anti-photoaging (Siyahpoosh *et al*., 2022). Katiyar *et al*. (in Aaron & Robert, 2009) explained that GTE could increase penetration and absorption into the skin when used topically. This statement is supported by Dal Belo *et al*. (2009) who conducted an in vitro penetration test on GTE cosmetic preparations, using the Franz diffusion cell method. The method used in this test, namely the Franz diffusion cell method, uses fresh human skin (Caucasian) as the sample application medium, then measured using the High-Performance Liquid Chromatography (HPLC) instrument. Based on these tests, the results showed that this preparation has good skin penetration and retention capabilities. It is characterized by detecting GTE in all skin layers, especially in the dermis layer (9.2%). In addition, epigallocatechin gallate (EGCG) the main phenolic compound in GTE, turns out to have poor stability because it is very susceptible to oxidation due to several factors (exposure to light, temperature, pH, and others), so it must be overcome by using a suitable delivery system (Sugihartini *et al*., 2016; Shi *et al*., 2018).

NLC or nanostructured lipid carriers is a delivery system suitable for active antioxidant ingredients (Chen-yu *et al*., 2012) and topical use (Souto *et al*., 2020). Manea *et al*. (2014) stated that green tea extract could be developed in topical preparations using an NLC delivery system. In addition, Tamjidi *et al*. (2013) explained that NLC consists of solid lipids and liquid lipids that can form a matrix and control the entrapment of active substances to increase the stability of a preparation. In addition to the various advantages described above, the preparation stage of NLC has several problems. During the crystallization process, the surface area of the particles will increase rapidly; thus, the whole system is unstable. Some literature states that it can be overcome by adding surfactants (Han *et al*., 2008) which are needed to improve the quality of the nanoparticle interface (Han *et al*., 2008). The quality of the nanoparticle interface can affect the system's characteristics and ability to absorb the active substance. Furthermore, the surfactant will also affect the formed nanoparticles' physical stability (Averina *et al*., 2009). Besides that, surfactants also have disadvantages that can cause irritation and various skin disorders by damaging the lipid membrane, which is the external protective layer on the skin, to disrupt its function (Kosswig, 2012). This problem can be solved by selecting a surfactant that has low irritability.

Decyl glucoside (DG) is a type of natural surfactant derived from plants, which is produced by the reaction of glucose (from corn starch) and fatty alcohol decanol (from coconut). Mehling *et al*. (2007) conducted the tests for DG irritability. It showed that this surfactant did not show irritation in various irritability testing methods. Furthermore, based on the research of Chaiyana *et al*. (2020), surfactant DG can produce significantly small particles in NLC preparations containing an active substance in the form of an extract, namely Ocimum sanctum Linn. In contrast, the small NLC particle size can affect various aspects, including increasing the stability of the preparation (Müller-Fischer *et al*., 2007). The problem lies in the concentration of DG used. By the explanation, Lasoń *et al*. (2018) stated in their research article that too high a DG concentration could result in foam formation during the NLC preparation process using forskolin as the active substance. Based on these considerations, several DG concentrations were compared to determine their effect on the characteristics (organoleptic, pH, particle size, polydispersity index), physical stability, and irritability of the NLC-GTE preparation. It aims to obtain the most optimal preparation.

MATERIALS AND METHODS

Materials

The materials used in this research were *green tea extract* (PT. Angler BioChemLab, Surabaya, Indonesia), *cetyl palmitate* (BASF, Indonesia), *glyceryl stearate* (BASF, Indonesia), *grape seed oil* (NHR Organic Oils, UK), *decyl glucoside* (Dow Chemical Pacific, Singapore), *synperonic*F68 (PT Megasetia Agung Kimia, Jakarta, Indonesia), and *lecithin* (Solae™, Amerika) were cosmetic grade. *Aquademineralisata* (Indonesia) was a technical grade and fertile chicken eggs (chicken farm, West Java & East Java, Indonesia).

Method

Preparation of NLC-GTE

The method used in the preparation of NLC-GTE was a modification of the high shear homogenization (HSH) method by Manea *et al*. (2014). This method was carried out by preparing two different phases: the oil and water phases (Table 1). The oil phase consists of cetyl palmitate, glyceryl stearate, and grape seed oil.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 1. Design formula of NLC-GTE

Materials		Function	Formula (%)		
			F1	F2	F3
Green tea extract (GTE)		Active substance	0.1	0.1	0.1
Lipid phase	Cetyl palmitate	Solid lipid	3.5	3.5	3.5
	Glyceryl stearate	Solid lipid	3.5	3.5	3.5
	Grape seed oil	Liquid lipid	3	3	3
Water phase	Decyl glucoside	Surfactant	2	2.5	3
	Lecithin	Cosurfactant	0.5	0.5	0.5
	Synperonic F68	Cosurfactant	0.5	0.5	0.5
	Aquademineralisata	Solvent	Ad 100	Ad 100	Ad 100

Description:
F1 = NLC-GTE + DG 2%
F2 = NLC-GTE + DG 2.5%
F3 = NLC-GTE + DG 3%

Meanwhile, the aqueous phase consisted of surfactant DG, synperonic F68, lecithin (1%, 1:1, w/w), and aquademineralisata. Furthermore, both were heated with Thermo Scientific Cimarec^{+} at the same temperature, namely 70°C for 30 minutes, then GTE (which had been dissolved in 10mL aquademineralisata) was added in the oil phase. Before mixing the two phases, the aqueous phase was stirred using Ultra-Turax IKA®T25 Digital for 2 minutes at a speed of 15,000 rpm. Then the two phases were mixed for 7 minutes at a speed of 15,000 rpm thus the NLC-GTE preparation was obtained.

Characteristics test of NLC-GTE

Organoleptic

This organoleptic test was performed visually with several aspects of observation, in the form of smell, color, and consistency of the NLC-GTE preparation (Loreta, 2015; A'yun, 2019; Indrajaya, 2021).

pH

The pH values of the NLC-GTE preparations were measured using a pH meter SI Analytics Lab 865 electrode calibrated and inserted into the preparation. The value shown on the screen was recorded as the pH value and replicated three times (A'yun, 2019; Indrajaya, 2021). Furthermore, statistical analysis was carried out using the One Way ANOVA method.

Particle size dan polydispersity index

Particle size (PS) and polydispersity index (PI) measurements were conducted by diluting the NLC-GTE preparation first and analyzing using the Beckman Coulter® DelsaTMnano C Particle Analyzer instrument. This measurement was replicated three times, and the particle size and polydispersity index values were recorded on the monitor screen (Mayangsari, 2021). The data obtained from this test were then analyzed statistically using the One Way ANOVA method.

Physical stability test of NLC-GTE

The stability test of NLC-GTE was performed using the accelerated method, namely thermal cycling. According to Rohmah (2020), this method test procedure can be conducted with a total of three cycles at two different temperatures (48 hours at a temperature of 2 - 8°C and 40°C, respectively). Moreover, the preparation characteristics such as organoleptic observations, pH, particle size (PS), and polydispersity index (PI) would be monitored. The results of these tests (pH, PS, and PI) were then analyzed using a statistical method, namely the Paired T-test.

Irritability test of NLC-GTE

This method's test procedure began with incubating and periodically rotating chicken eggs in an incubator at 37°C. On the 10th day, the eggs were binoculars to distinguish and select fertile eggs, marked the air cavity with a pencil, and then opened using sterile scissors. The outer egg membrane was moistened with warm sterile 0.9% NaCl solution and incubated for 5 - 20 minutes to facilitate the removal of the outer egg membrane. Ensure the chorioallantoic membrane (CAM) conditions were still in good condition and place 0.2 mL NLC-GTE (the best formula based on the physical stability test) in the CAM and let stand for 20 seconds (Hagino *et al*., 1999). As an irritant control (+C), 1% sodium lauryl sulfate (SLS) and sterile 0.9% NaCl solution were used as negative controls (-C). Then the CAM was cleaned using a sterile 0.9% NaCl solution. Further observations began after the CAM was cleaned from the sample for 300 seconds. Observed and recorded the time of hemorrhage, lysis, and coagulation in the presence of CAM (Yuliani, 2016).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

RESULTS AND DISCUSSION

Characteristics test of NLC-GTE

Organoleptic

NLC-GTE preparations with varying concentrations of DG as a surfactant showed that all formulas were white, odorless, and had a semi-solid consistency (Figure 1).

Figure 1. Preparation of NLC-GTE with variations in DG concentration (F1: 2%; F2: 2.5%; F3: 3%)

pH

The pH value of the NLC-GTE preparation can be determined by measuring it using a pH meter. The following results were obtained (Table 2). The results of the measurement of the pH of the NLC-GTE preparation described in Table 2 showed that all formulas were at a normal pH range of 4 - 7 (Souto & Műller, 2008). Furthermore, statistical analysis was carried out and the results showed that the difference in DG concentration did not affect the pH value of the NLC-GTE preparation (sig. > 0.05).

Table 2. pH value of NLC-GTE preparations (n = 3)

Formula	Average ± SD (CV)
F1	6.19 ± 0.00 (0.02)
F2	6.20 ± 0.00 (0.10)
F3	6.20 ± 0.00 (0.05)

Particle size (PS)

The results of characteristic testing in the form of determining the particle size of NLC-GTE can be seen in the histogram (Figure 2). Based on the histogram in Figure 2, it can be seen that the PS values of all formulas meet the NLC particle size requirements, which were in the range of 10-500nm (Sharma & Baldi, 2018) and 10-1000nm (Faizatun *et al*., 2020). The value of each formula showed that F3 has the smallest size compared to F2 and F1. These results proved that increasing the concentration of DG can reduce the PS value. This statement was in line with Zirak & Pezeshki's (2015) opinion that a high surfactant concentration can lower surface tension. Hence, the energy obtained (stirring) would make breaking up oil droplets (melted lipids) easier, which can stabilize the newly formed surface to produce a larger size. The results of statistical analysis showed that there was a significant difference between each formula (sig. < 0.05), so it can be said that an increase in DG concentration could affect the PS value of NLC-GTE.

The polydispersity index (PI)

According to Avadi *et al*. (2010), this measurement aimed to determine several aspects of the colloidal solution, particle size and homogeneity distribution. The PI value of each formula can be seen in Table 3. Table 3 showed that all formulas have a PI value of less than 0.3 which meant that all formulas had a uniform and homogeneous particle size distribution. This result is similar to Oliveira *et al*. (2021) in that PI value of < 0.3 a homogeneous particle size distribution. F3 produces a value of 0.23 ± 0.00, the smallest PI value compared to F1 and F2, which produced a PI value of 0.26 ± 0.01 and 0.25 ± 0.00, respectively. This series of values can prove that variations in DG concentration can affect the PI value in NLC-GTE (Subramaniam *et al*., 2020).

Table 3. The polydispersity index value of NLC-GTE preparations (n=3)

Formula	Average ± SD (CV)
F1	0.26 ± 0.01 (4.19)
F2	0.25 ± 0.00 (3.16)
F3	0.23 ± 0.00 (1.51)

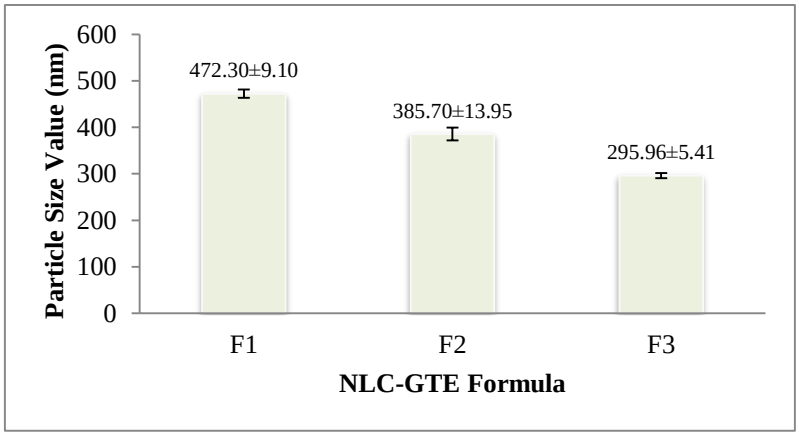


Figure 2. Histogram of the particle size value (average ± SD) of the NLC-GTE preparation (n = 3)

P-ISSN: 2406-9388
E-ISSN: 2580-8303

By Lullung & Suprapti's (2012) explanation, the smaller the surfactant concentration, the greater the PI value obtained, and vice versa. It can be caused by the DG concentration of 3% being more optimal for covering the lipid and water interface and reducing the surface tension of the new particles formed during the homogenization process so that uniform nanoparticles can be created (Witayaudom & Klinkesorn, 2017). In addition, an increase in surfactant concentration can affect the energy produced in the stirring process so that the nanoparticles formed can be distributed more efficiently (Zirak & Pezeshki, 2015). Based on the statistical analysis carried out, it showed that there was no significant difference between F1 and F2 (sig. > 0.05). At the same time, for F1 and F3, there was a significant difference between the two (sig. <0.05). Likewise, F2 and F3 showed a significant difference (sig. < 0.05). Based on these data, it can be said that the PI value in F3 was significantly different when compared to F1 and F2. While F1 and F2 have the same PI value, there was no significant difference between the two.

Physical stability test of NLC-GTE

Organoleptic

Physical stability testing of NLC-GTE was carried out using the thermal cycling method, and several things were observed, including organoleptic analysis, the results of which can be seen in Figure 3. Based on the thermal cycle test results of the NLC-GTE organoleptic preparation, which has been described in Figure 3, it can be seen that all formulas have the same color and odor, both before and after the cycle. The difference that can be seen from this observation was phase separation in F1 and F2. However, there was almost no phase separation at all in F3. These results are the opinion of Zirak & Pezeshki (2015) who state that low surfactant concentrations can cause instability and recrystallization due to insufficient amounts in the formation of nanoparticle structures. This case will trigger the formation of git, which are large particles that cannot be stabilized by surfactants and break into non-uniform particles and cause phase separation in the NLC-GTE preparation (Lullung & Suprapti, 2012). Based on these results, F3 (3% DG) showed better phase stability than F1 (2% DG) and F2 (2.5% DG). Furthermore, measurements of PS and PI were carried out in each formula to strengthen this statement.

Figure 3. Physical stability test results of NLC-GTE [(a) before cycle; (b) after cycle]

pH

The pH value was a measurement that was implemented in this physical stability test. The following was the data for each pH value before and after treatment (Table 4). The pH value of the physical stability test (thermal cycling) in all formulas was within the range of normal skin pH values, namely 4 - 7 (Souto & Műller, 2008). The pH value data were then analyzed using statistics and showed no significant difference between the pH values before and after treatment [sig.(2-tailed) > 0.05]. Thus, it can be said that the pH value of all NLC-GTE preparations remained stable after testing the physical stability using the thermal cycling method.

Table 4. The pH value of NLC-GTE preparations from physical stability tests (n=3)

Formula	Average ± SD (CV)	
	Before	After
F1	6.19 ± 0.00 (0.02)	6.20 ± 0.00 (0.05)
F2	6.20 ± 0.00 (0.10)	6.20 ± 0.00 (0.13)
F3	6.20 ± 0.00 (0.05)	6.21 ± 0.00 (0.07)

Particle size (PS)

Another measurement was applied, namely the particle size of the NLC-GTE preparation. The following are the PS values measured before and after the test cycle (Figure 4).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

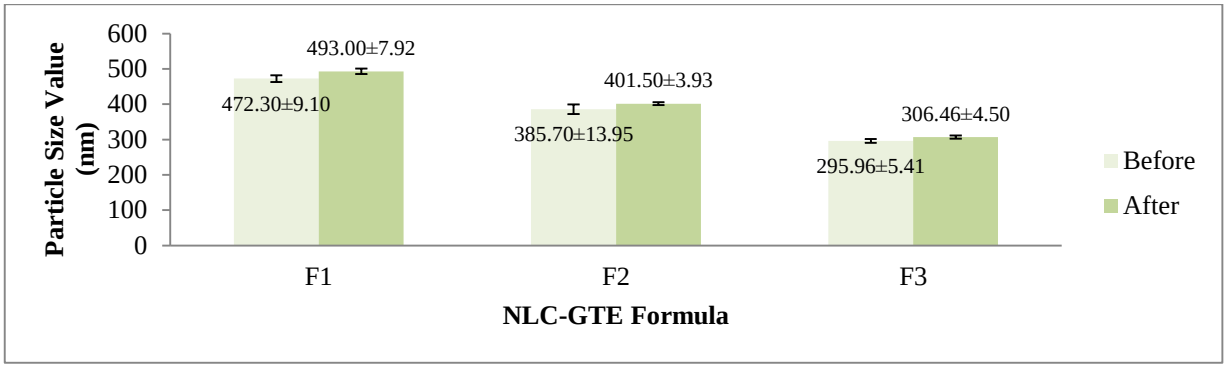


Figure 4. Histogram of particle size values (average ± SD) for NLC-GTE preparations (n = 3) from physical stability tests

PS data from each NLC-GTE formula, both before and after the cycle, showed that all formulas had particle sizes that were still within the normal range of size values, namely the range of 10-500 nm (Sharma & Baldi, 2018; Faizatun *et al*., 2020). Furthermore, statistical analysis was carried out and obtained different results for each formula. F1 showed a significant difference between before and after the cycle (sig (2-tailed) < 0.05). Meanwhile, F2 and F3 showed that there was no significant difference between before and after the cycle (sig (2-tailed) > 0.05). Based on the results of this analysis, it can be said that after testing the physical stability using the thermal cycling method on NLC-GTE preparations, F2 and F3 had PS values that were more stable than F1. These results proved that increasing the surfactant concentration of DG can affect the physical stability of NLC-GTE. Following the opinion of Han *et al*. (2008) opinion, surfactants can improve the nanoparticle interface's quality, affecting the preparation's characteristics and physical stability (Müller-Fischer *et al*., 2007).

The polydispersity index (PI)

PI values of each NLC-GTE formula obtained before and after the treatment cycle can be seen in Table 5. The results indicated an increase in the PI value when compared before and after the treatment cycle. In F1, it showed that the PI value after the cycle was more significant than 0.3, so it can be ascertained that the PI value in F1 was unstable. While F2 and F3 still produce PI values in the normal range even after testing, which was < 0.3. The data were analyzed statistically and can be interpreted that the PI values of F1 and F2 experienced a significant change (sig (2-tailed) < 0.05). Meanwhile, F3 did not change significantly between before and after treatment (sig (2-tailed) > 0.05). This analysis proved that increasing the concentration of decyl glucoside can affect the PI value in the physical stability test of NLC-GTE.

Table 5. Polydispersity index values of NLC-GTE preparations from physical tests (n = 3)

Formula	Average ± SD (CV)	
	Before	After
F1	0.26 ± 0.01 (4.19)	0.32 ± 0.00 (0.94)
F2	0.25 ± 0.00 (3.16)	0.28 ± 0.00 (0.92)
F3	0.23 ± 0.00 (1.51)	0.23 ± 0.00 (3.00)

Based on the results of the physical stability tests (organoleptic, pH, PS, and PI) described above, it can be said that 3% DG (F3) is the most optimal concentration to obtain stable NLC-GTE. These results are by the explanations of Lullung & Suprapti (2012) and Han *et al*. (2008), namely, the optimal amount of surfactant can cover the surface of nanoparticles formed in the homogenization process so that it can produce and maintain a small particle size, prevent the formation of git and can increase the stability of the nanoparticle interface in the NLC-GTE preparation. Based on these results, the test will be continue with an irritation test to determine the safety of NLC-GTE with 3% DG as a topical preparation.

Irritation Test of NLC-GTE

The Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) method tests the irritation of preparation, one of which was a topical preparation. According to Chaiyana *et al*. (2020), this method did not require ethical approval because it only used animal embryos for less than half the total incubation period, making it quite convenient and easy. Based on this test, it can be seen that the highest irritation score was seen in +C, which used sodium lauryl sulfate as an irritant, which was 8.37±0.10. While the irritation scores -C (sterile NaCl 0.9%) and F3 (best formula) have the same value, namely 0.00. These values indicate that F3 can be classified as non-irritant preparation (Table 6). In contrast, the results of the +C value were classified as moderate irritation (Yuliani, 2016). The results of this test can be seen in Figure 5, namely hemorrhage

P-ISSN: 2406-9388
E-ISSN: 2580-8303

(bleeding) and lysis (loss of blood vessels) in the CAM after the application of SLS. At the same time, the -C (sterile NaCl) and F3 (best formula) groups did not show any difference between before and after the sample was applied.

Table 6. Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) test results (n = 3)

Treatment Group	Average ± SD (CV)
+C	8.37±0.10 (1.18)
-C	0.00±0.00 (0.00)
F3	0.00±0.00 (0.00)

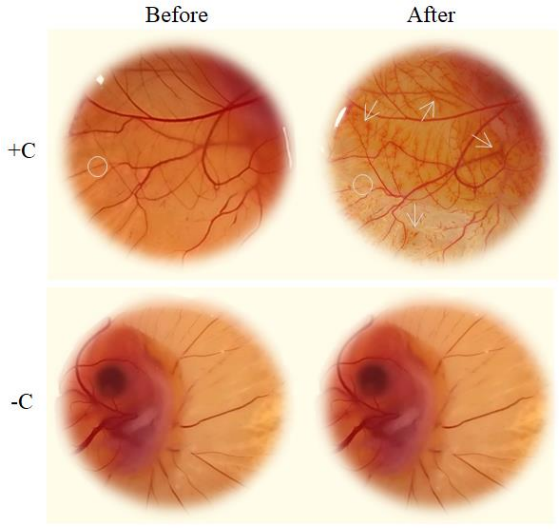


Figure 5. Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) test results

Based on the irritability test result used in the HET-CAM method, the irritability of the NLC-GTE preparation in F3 (3% DG) can be the best formula. Hence, NLC-GTE, which contained 3% DG, was considered safe to use and developed in various topical cosmetic preparations.

CONCLUSION

Based on all the tests that have been carried out, it can be concluded that increasing the surfactant DG concentration in the NLC-GTE preparation does not affect the pH of each formula. However, it can decrease PS and PI and increase the NLC-GTE preparation's physical stability. Furthermore, based on the irritability test results, F3 (3% DG) as the best formula were also proven safe because it did not show irritation characteristics in the HET-CAM test.

ACKNOWLEDGMENT

We are grateful to PT. Megasetia Agung Kimia, for providing Synperonic F68 or Poloxamer 188. In addition, the authors would like to thank all parties who have supported this research.

AUTHOR CONTRIBUTIONS

Conceptualization, R. A., N. R., W. S.; Methodology, R. A., N. R., W. S.; Validation, R. A., N. R., W. S.; Formal Analysis, R. A.; Investigation, R. A.; Resources, R. A., N. R., D. A. P.; Data Curation, R. A., N. R., W. S.; Writing - Original Draft, R. A., N. R.; Writing - Review & Editing, R. A., N. R., W. S.; Visualization, R. A., N. R., W. S.; Supervision, N. R., W. S.; Project Administration, N. R., W. S., Funding Acquisition, R. A.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

A'yun, N. Q. (2019). Pengaruh Penambahan Space Peptide terhadap Tabilitas dan Efektivitas Krim Freeze Dried Amniotic Membrane Stemcell Metabolite Product (AMSC-MP). *Tesis;* Universitas Airlangga, Surabaya.

Avadi, M. Z., Sadeghi, A. M. M., Mohammadpour, N., Abedin, S., Atyabi, F., Dinarvand, R. & Rafiee-Tehrani, M. (2010). Preparation and Characterization of Insulin Nanoparticles Using Chitosan and Arabic Gum with Ionic Gelation Method. *Nanomedicine: Nanotechnology, Biology, and Medicine; 6;* 58-63. doi: 10.1016/j.nano.2009.04.007.

Averina, E. S., Seewald, G., Muller, R. H., Radnaeva, L. D. & Popov, D. V. (2009). Nanostructured Lipid Carriers (NLC) on the Basis of Siberian Pine (Pinus sibirica) Seed Oil. *Pharmazie; 65;* 25-31.

Chaiyana, W., Anuchapreeda, S., Somwongin, S., Marsup, P., Lee, K. H., Lin, W. C., & Lue, S. C. (2020). Dermal Delivery Enhancement of Natural Anti-Ageing Compounds from Ocimum sanctum Extract by Nanostructured Lipid Carriers. *Pharmaceutics; 12*; 1-12. doi: 10.3390/pharmaceutics12040309.

Chen-yu, G., Chen-yu, Y., Qi-lu, L., Qi, T., Yan-wei, X., Wei-na, L. & Guang-xi, Z. (2012). Development of a Quercetin-Loaded Nanostructured Lipid Carrier Formulation for Topical Delivery. *International Journal of Pharmaceutics*; *430*; 292-298. doi: 10.1016/j.ijpharm.2012.03.042.

Dal Belo, S. E., Gaspar, L. R., Maia Campos, P. M. B. G. & Marty, J. -P. (2009). Skin Penetration of Epigallocatechin-3-Gallate and Quercetin from Green Tea and Ginkgo Biloba Extract Vehiculated in Cosmetic Formulations. *Skin Pharmacology and Physiology*; *22*; 299-304. doi: 10.1159/000241299.

Faizatun, Gangga, E., Anindita, S., Martati, T. & Miftahurrohmah, N. (2020). Formulation of NLC Gel from Callus Mulberry Leaf Extract Induced by NAA and BAP). *Jurnal Ilmu Kefarmasian Indonesia*; *18*; 123-129. doi: 10.35814/jifi.v18i1.814.

Hagino, S., Kinoshita, S., Tani, N., Nakamura, T., Ono, N., Konishi, K., Iimura, H., Kojima, H. & Ohno, Y. (1999). Interlaboratory Validation of In Vitro Eye Irritation Test for Cosmetic Ingredients. (2) Chorioallantoic Membrane (CAM) Test. *Toxicology in Vitro; 13;* 99-113. doi: 10.1016/s0887-2333(98)00065-4.

Han, F., Li, S., Yin, R., Liu, H., & Xu, L. (2008). Effect of Surfactant on the Formation and Characterization of a New Type of Colloidal Drug Delivery System: Nanostructured Lipid Carriers. *Colloids and Surfaces A: Physicochem. Eng. Aspect; 315;* 210-216. doi: 10.1016/j.colsurfa.2007.08.005.

Indrajaya, S. (2021). Pengaruh Carbopol940 dan CMC-Na terhadap Karakteristik, Stabilitas Fisik, Efektivitas dan Iritabilitas Ubiquinone – Nanostructured Lipid Carriers (NLC) dalam Hydrogel Sleeping Mask sebagai Kosmetik Antiaging. *Tesis;* Universitas Airlangga, Surabaya.

Katiyar, S., Elmest, C. A. & Katiyar, S. K. (2009). *Green Tea and Skin Cancer: Immunological Modulation and DNA Repair*. In: Aaron Tabor & Robert Blair (ed.) *Nutritional Cosmetics Beauty from Within;* 472-491. Oxford, USA: William Andrew.

Kosswig, K. (2012). *Surfactants*. In Ullmanns (ed.) *Encyclopedia of Industrial Chemistry; 35*; 431-505.

Lasoń, E., Sikora, E., Miastkowska, M., Escriano, E., Garcia-Celma, M. J., Solans, C., Llinas, M. & Ogonowski, J. (2018). NLCs as a Potential Carrier System for Transdermal Delivery Forskolin. *Acta Biochimika Polonica; 65;* 437-442. doi: 10.18388/abp.2018_2554.

Loreta, L. J. (2015). Pengaruh Tween 80 sebagai Emulsifying Agent dan Sorbitol sebagai Humektan dalam Sediaan Krim Ekstrak Daun Jambu Biji (Psidium guajava L.) dengan Aplikasi Desain Faktorial. *Skripsi*; Universitas Sanata Dharma, Yogyakarta.

Lullung, A. & Suprapti. (2012). Effect of Surfactant on Particle Diameter, Viscosity and the Polydispersity Index of SLN Making Cocoa Fat. *Jurnal RisetTeknologi Industri*; *6*; 1-10. doi: 10.26578/jrti.v6i12.1512.

Manea, A. M., Vasile, B. S. & Meghea, A. (2014). Antioxidant and Antimicrobial Activities of Green Tea Extract Loaded Into Nanostructured Lipid Carriers. *Comptes Rendus Chimie*; *17*; 331-341. doi: 10.1016/j.crci.2013.07.015.

Mayangsari, F. D. (2021). Efek Minyak Nilam, Pepermin dan Rosmeri pada Karakteristik Fisik, Stabilitas Fisik, Iritabilitas, dan Efektivitas NLC Coenzyme G10 Sleeping Mask sebagai Kosmetik Antiaging. *Tesis;* Universitas Airlangga, Surabaya.

Mehling, A., Kleber, M. & Hensen, H. (2007). Comparative Studies on the Ocular and Dermal Irritation Potential of Surfactants. *Food and Chemical Toxicology*; *45*; 747-758. doi: 10.1016/j.fct.2006.10.024.

Müller-Fischer, N., Suppiger, S. & Windhab, E. J. (2007). Impact of Static Pressure and Volumetric Energy Input on the Microstructure of Food Foam Whipped in a Rotor-Stator Device. *Journal of Food Engineering*; *80*; 306-316. doi: 10.1016/j.jfoodeng.2006.05.026.

Oliveira, R. N. d., Campos, P. M., Pinto, R. M. C., Mioduski, J., Santos, R. D., Justus, B., Paula, J. d. F. P. d., Klein, T., Boscardin, P. M. D., Corrêa, S. d. A. P., Allegretti, S. M. & Ferrari, P. C. (2021) The Promising Antischistosomal Activity of Oleic Acid-Loaded Polymeric Nanocapsules for Oral Administration. *Journal of Drug Delivery Science and Technology*; *63*; 1-9. doi: 10.1016/j.jddst.2021.102429.

Rohmah, N. N. (2020). Pengaruh Peppermint Essential Oil terhadap Karakteristik dan Stabilitas Fisik

(Thermal Cycle) Sistem Nanostructured Lipid Carriers Coenzyme Q10. *Skripsi*; Universitas Airlangga, Surabaya.

Saini, S., Gupta, A., Singh, N. & Sheikh, J. (2019). Functionalization of Linen Fabric Using Layer by Layer Treatment with Chitosan and Green Tea Extract. *Journal of Industrial and Engineering Chemistry*; *4811*; 1-6. doi: 10.1016/j.jiec.2019.10.005.

Sharma, A. & Baldi, A. (2018). Nanostructured Lipid Carriers: A Review. *Journal of Developing Drugs*; *7*; 1-12. doi: 10.4172/2329-6631.1000191.

Shi, M., Shi, Y. L., Li, X. M., Yang, R., Cai, Z. Y., Li, Q. S., Ma, S. C., Ye, J. H., Lu, J. L., Liang, Y. R. & Zheng, X. Q. (2018). Food-Grade Encapsulation Systems for (-)-Epigallocatechin Gallate. *Molecules*; *23*; 1-17. doi: 10.3390/molecules23020445.

Siyahpoosh, A., Sheikhi, A. R., Jafarzadeh, E. & Pipelzadeh, M. (2022). Effectiveness of Green Tea Cream in Comparison with Silver Sulfadiazine Cream in Treatment of Second Degree Burn in Human Subject. *Journal of Herbal Medicine*; *32*; 1-8. doi: 10.1016/j.hermed.2022.100533.

Souto, E. B. & Muller, R. H. (2008). Cosmetic Features and Application of Lipid Nanoparticles (SLN, NLC). *International Journal of Cosmetic Science*; *30*; 157-165. doi: 10.1111/j.1468-2494.2008.00433.x.

Souto, E. B., Baldim, L., Oliveira, W. P., Rao, R., Yadav, N., Francisco M. G. & Sheefali, M. (2020). SLN and NLC for Topical, Dermal, and Transdermal Drug Delivery. *Expert Opinion on Drug Delivery;* 1-12. doi: 10.1080/17425247.2020.1727883.

Subramaniam, B., Siddik, Z. H. & Nagoor, N. H. (2020). Optimization of Nanostructured Lipid Carriers: Understanding the Type, Design and Parameters in the Process of Formulation. *Journal of Nanoparticle Research; 22(141);* 1-29. doi: 10.1007/s11051-020-04848-0.

Sugihartini, N., Susanti, H., Zaenab, Hanifah, H. & Marlina, S. A. (2016). Stabilitas Epigalokatekin Galat dalam Krim The Hijau dengan Variasi konsentrasi Antioksidan Vitamin C 1% dan Vitamin E 1%. *Jurnal Farmasi Sains dan Komunitas*; *13*; 52-56. doi: 10.24071/jpsc.132186.

Tamjidi, F., Shahedi, M., Varshosaz, J. & Nasirpour, A. (2013). Nanostructured Lipid Carriers (NLC): A Potential Delivery System for Bioactive Food Molecules. *Innovative Food Science and Emerging Technology*; *19*; 29-43. doi: 10.1016/j.ifset.2013.03.002.

Wang, T., Zhang, F., Zhao, R., Wang, C., Hu, K., Sun, Y., Politis, C., Shavandi, A. & Nie, L. (2020). Polyvinyl Alcohol/Sodium Alginate Hydrogels Incorporated with Silver Nanoclusters via Green Tea Extract for Antibacterial Applications. *Designed Monomers and Polymers; 23(1);* 188-133. doi: 10.1080/15685551.2020.1804183.

Widowati, W., Herlina, T., Ratnawati, H., Constantia, G., Deva, I. D. G. S. & Maesaroh, M. (2015). Antioxidant Potential of Black, Green and Oolong Tea Methanol Extracts. *Biology, Medicine, & Natural Product Chemistry*; *4*; 35-39. doi: 10.14421/biomedich.2015.42.35-39.

Witayaudom, P. & Klinkesorn, U. (2017). Effect of Surfactant Concentration and Solidification Temperature on the Characteristics and Stability of Nanostructured Lipid Carrier (NLC) Prepared from Rambutan (Nephelium lappaceum L.) Kernel Fat. *Journal of Colloid and Interface Science; 505;* 1082-1092. doi: 10.1016/j.jcis.2017.07.008.

Yuliani, S. H., Rahmadani, Y. & Istyastono, E. P. (2016). Irritation Test of Wound Healing Gel of Ethanolic Extract of Binahong Leaf Using Slug Irritation Test. *Jurnal Ilmu Kefarmasian Indonesia*; *14*; 135-140.

Zirak, M. B. & Pezeshki, A. (2015). Effect of Surfactant Concentration on the Particle Size, Stability and Potential Zeta of Beta Carotene Nano Lipid Carrier. *International Journal of Current Microbiology and Applied Sciences; 4(9);* 924-932.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 229-234
DOI: 10.20473/jfiki.v9i32022.229-234
Available online at https://e-journal.unair.ac.id/JFIKI/

Effect of Alpha-Lipoic Acid on the Characteristics and Physical Stability of NLC-Green Tea Extract

Fairuz Yaumil Afra[1], Widji Soeratri[2]*, Djoko Agus Purwanto[3]
[1]Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: widjisoeratri@yahoo.com

Submitted: 28 February 2022
Accepted: 30 March 2022
Published: 9 December 2022

Abstract

Background*: The addition of alpha-lipoic acid in Nanostructured Lipid Carrier-Green Tea Extract (NLC-GTE) has potential to increase effectiveness of anti-aging preparations. It happened because alpha-lipoic acid can increase stability and antioxidant activity.* ***Objective****: Comparing the physical characteristics and stability of NLC-GTE with or without alpha-lipoic acid.* ***Methods****: NLC-GTE manufactured using the High Shear Homogenization method. NLC-GTE was divided into two formulas, without the addition of alpha-lipoic acid for F1 and with the addition of alpha-lipoic acid for F2. The characteristics and physical stability were tested, including organoleptic, pH, particle size, and polydispersity index. Stability test was held using the thermal cycling method.* ***Results****: Based on characteristic test, it was found that F2 had larger particle size value than F1. The average particle size value of F1 is 313.9 ± 0.76 nm and 423.4 ± 0.75 nm for F2. The F1 and F2 preparations had a polydispersity index value below 0.3, so they were homogeneous. The average pH value of F1 is 5.998 ± 0.01, and F2 is 4.798 ± 0.004. The physical stability test showed a difference before and after the sixth day in particle size and pH, but it was still in the range, so it was safe. However, there was a separation in F1 after day 6.* ***Conclusion****: Based on the characteristics and physical stability tests, F1 (without alpha-lipoic acid) and F2 (with alpha-lipoic acid) had differences in particle size and pH. From the physical stability test, it can be concluded that F2 is more stable than F1.*

Keywords*: nanostructured lipid carrier (NLC), green tea extract, alpha-lipoic acid*

How to cite this article:
Afra, F. Y., Soeratri, W. & Purwanto, D. A. (2022). Effect of Alpha-Lipoic Acid on the Characteristics and Physical Stability of NLC-Green Tea Extract. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 9(3), 229-234. http://doi.org/10.20473/jfiki.v9i32022.229-234

P-ISSN: 2406-9388
E-ISSN: 2580-8303

INTRODUCTION

At this time, the development of the world's population has entered a period called the aging population, an increase in life expectancy followed by an increase in the number of older people in all countries in the world. Indonesia experienced an increase in the number of older people from 18 million people (7.56%) in 2010 to 25.9 million people (9.7%) in 2019, and it is expected to increase in 2035 to 48.2 million people (15.77%) (Kemenkes, 2020). It will be the reason for health problems, including aging (Ahmad & Damayanti, 2018).

Aging is a decrease in the size and number of skin cells and changes in skin function resulting from a decline in the standard structure and function of normal skin. Physiological changes in elderly skin are shown in impaired barrier function, slowed epidermal cell turnover, decreased blood vessel network around the base of the hair and glands, decreased cell turnover function, and decreased sweat production (Anggowarsito, 2014). Cosmeceuticals are cosmetic products with active ingredients that aim to have medical or medicinal benefits and protect against deteriorating skin conditions. Cosmeceuticals claim to improve skin tone, function, and texture by promoting collagen growth and reducing wrinkles (Vaishali *et al.*, 2013).

Camellia sinensis is a plant that produces various types of tea, including green tea. Green tea extract has pharmacological effects, including antioxidants and photoaging, that have potential in developing cosmeceutical products (Ganesan & Dong, 2016). Green tea extract has a high solubility in water but very low solubility in lipids, making it difficult to penetrate the stratum corneum. Green tea extract contains the main catechins such as epicatechin gallate (ECG), epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG). The most active and abundant catechin in green tea is epigallocatechin gallate (EGCG) (Namita & Kumar, 2012).

EGCG is highly soluble in water (40 g/l at 20^0C) and has good stability at pH 3.7 (25^0C) and pH 3.9 (40^0C). EGCG has low lipophilicity, with a log P value of 1.1 at pH 4.0, while the optimal log P value for optimal penetration ranges from 2 - 3 (Rosita *et al.*, 2019). A nanocarrier system can be used to overcome this problem. Nanocarrier consists of Nanoparticle (NP), Solid Lipid Nanoparticle (SLN), and Nanoparticle Lipid Carrier (NLC). When compared in cost, SLN and NLC are more affordable than Liposomes. In addition to being more expensive, liposomes have the disadvantage of being quite complex in preparation and low instability (Manea *et al.*, 2014). Meanwhile, SLN and NLC showed high drug loading capacity (Frias *et al.*, 2016). SLN only consists of solid lipids that form perfect crystallinity. Thus, the entrapment power (EE) is lower. Meanwhile, NLC is a modification of SLN, which forms lower crystallinity so that EE is higher and has better stability than SLN (Frias *et al.*, 2016). It is because NLC contains liquid lipids, which can reduce crystallinity to accommodate more active ingredients (Mayangsari *et al.*, 2021).

Green tea extract also has a disadvantage, known as photodegradation which causes it is easy to lose active compounds, reducing stability. In the research of Chen *et al.* (2017), EGCG has a deficiency, which is proven with the presence of reactivity that causes oxidation, hydrolysis, epimerization, and polymerization reactions. To overcome this, needed the addition of co-antioxidants (Scalia *et al.*, 2013). One of the co-antioxidants that can be used is alpha-lipoic acid, where alpha-lipoic acid has antioxidant activity that effectively removes free radicals by reducing alpha-lipoic acid to dihydrolipoic acid. Alpha-lipoic acid is soluble in fat and water to reach all parts of the cell (Perricone, 2000).

Alpha-lipoic acid is a weak acid with a pKa of 4.7, therefore it will affect the characteristics of the preparation (Cichewicz *et al.*, 2013). In addition, alpha-lipoic acid is an antioxidant that can keep the preparation stable. Alpha-lipoic acid has the ability neutralize free radicals in watery and fatty areas of cells. Alpha-lipoic acid can neutralize various free radicals, such as singlet oxygen, superoxide, peroxyl and hydroxyl radicals. In the study of Hu *et al.* (2015), alpha-lipoic acid can reduce lipid oxidation in order to protecting EGCG from degradation during carrier process. EGCG can be degraded by pH so acidic state is needed to be stable. Alpha-lipoic acid is a weak acid with a pKa of 4.7, it will lower the pH and increase the stability of EGCG.

In a study by Bianchi *et al.* (2013), alpha-lipoic acid as a photo stabilizer for EGCG was much more efficient and effective at lower concentrations than vitamins E, C, and BHT (*Butylated hydroxytoluene*). Alpha-lipoic acid inhibits EGCG photodegradation and maintains functional activity under solar radiation. The addition of alpha-lipoic acid in NLC green tea extract can increase the effectiveness of anti-aging preparations. It happened because alpha-lipoic acid can increase stability and increase antioxidant activity. For the introduction in this preparation, the characteristics and physical stability tests were carried out on NLC-Green Tea Extract and NLC-Green Tea Extract with 0.5% alpha-lipoic acid.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

The preparation of this research using high shear homogenizer (HSH) method, in order to avoid the use of organic solvents that needed in the other technique.

MATERIALS AND METHODS

Materials

The ingredients used in this study were Meditea Green Tea Extract (PT. Angler BioChemab, Indonesia), cetyl palmitate (BASF, Indonesia), glyceryl stearate (BASF, Indonesia), poloxamer 188 (PT. Megasetia Agung Kimia, Indonesia), lecithin (Solae, England), grape seed oil (Brighton, UK), tween 20 (Zhang Yan, Singapore), alpha-lipoic acid (Tokyo Chemical Industry, Tokyo, Japan), NaH_2PO_4 (SAP, Indonesia), and Na_2HPO_4 (SAP, Indonesia).

Instrumentation

This research used Ultra Turrax IKA®T25 Digital High Shear Homogenizer, OHAUS analytical balance, Beckman Coulter Delsa^{TM} Nano C Particle Analyzer, Thermo Scientific Hotplate, SI Analytics Lab 865 pH meter, and other supporting tools.

Methods

NLC-GTE manufacture

The manufacture of NLC-GTE used the HSH (High Shear Homogenizer) method consist of an oil phase and a water phase mixed. The oil phase consisted of solid lipids (cetyl palmitate and glyceryl stearate) and liquid lipids (grape seed oil), melted at 70^0C for 30 minutes. In the 20th minute, the green tea extract, which was previously dissolved in a pH 5.0 buffer phosphate, was heated at the same temperature. After 30 minutes, the green tea extract liquid was added gradually into the oil phase and then stirred using a hotplate stirrer at 300 rpm for 10 minutes. The aqueous phase consisted of surfactants (tween 20), co-surfactants (Lecithin and Poloxamer 188), and buffer pH 5.0. All ingredients were put into a beaker and heated at 70^0C for 22 minutes. After that, the aqueous phase was homogenized using Ultra Turrax IKA®T25 Digital High Shear Homogenizer at 15000 rpm for 2 minutes. After finished with homogenization, the water phase was added to the oil phase and stirred using Ultra Turrax IKA® T25 Digital High Shear Homogenizer at 16000 rpm for 7 minutes. It was continued with stirring using a hotplate stirrer at 300 rpm for 12 minutes or until reaching room temperature. In F2 preparations, alpha-lipoic acid is added to the oil phase gradually while stirring using a hotplate stirrer. The formula can be seen in Table 1.

Physical characteristic testing

Organoleptic observations

Organoleptic observations were carried out by conducting color, odor, consistency, and visual phase separation tests.

Particle size and polydispersity index (PI)

Particle size and polydispersity index (PI) were measured using the Delsa^{TM} Nano C Particle Size Analyzer. The F1 and F2 preparations were diluted first by weighing 50 mg of the preparation plus 50 mL of distilled water and then stirred with a 500 rpm hotplate stirrer for 10 minutes. After that, the preparation was diluted again by taking 2 mL of the preparation and adding 8 mL of distilled water. The solution was stirred again at 100 rpm for 10 minutes. Lastly, the solution was put into a cuvette and measured using a Delsa^{TM} Nano C Particle Size Analyzer.

pH value measurement

pH testing was performed by measuring the preparation using a pH meter which was previously calibrated with a standard solution of pH 4. The name of the tool used is SI Analytics pH Meter Lab 865.

Tabel 1. F1 and F2 test formula

Composition	Material Function	Amount used (%)	
		F1	**F2**
Green tea extract	Active Ingridients	0.1	0.1
Cetyl Palmitate	Solid Lipid		
Glyceryl Stearate		1.16 : 1.16 : 1 (10%)	
Grape Seed Oil	Liquid Lipid		
Tween 20	Surfactant	2	2
Lecithin	Co-surfactant	1:1	
Poloxamer 188		(1%)	
Alpha-lipoic acid	Co- antioxidant	-	**0.5**
Water	Solvent	Ad 100	Ad 100

*F1 = NLC-GTE without Alpha-lipoic acid
F2 = NLC-GTE with Alpha-lipoic acid

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Physical stability test

Physical stability testing was carried out using the thermal cycling method. The preparation was stored in 1,5 cycles, two days in the oven at 40^0C , in the refrigerator at 2 - 8^0C, and in the oven for six days. Organoleptic, pH, particle size, polydispersity index, and the presence or absence of separation testing was carried out, before and after the physical stability testing.

RESULTS AND DISCUSSION

Physical characteristic test

Organoleptic observation

In the manufacturing process of NLC-GTE preparations with two formulas, NLC-GTE without the addition of alpha-lipoic acid for F1 and with the addition of alpha-lipoic acid for F2. The results of the characteristic test showed that the NLC-GTE preparations (F1 and F2) had white color for F1 and broken white for F2, odorless, liquid consistency, and soft texture. Visually, the homogeneous NLC-GTE preparations can be seen in Table 2.

Particle size and polydispersity index were important factors in the parameter characteristics of nanoparticle preparations. The average particle size of NLC GTE was 313.9 ± 0.76 nm for F1 and 423.4 ± 0.75 nm for F2. A statistical test was performed using the Paired-Sample T-Test with $\alpha = 0.05$, and the result was the Sig value. (2 tailed) < 0.05, it can be concluded that there was a significant difference. The particle size of F2 was larger than F1 due to the addition of alpha-lipoic acid. It was supported by Lason *et al.* (2017) research that the particle size of the NLC base without alpha-lipoic acid was smaller than the NLC preparation containing the active ingredient alpha-lipoic acid. However, the particle size of F2 was still within the range for NLC preparations, below 1000 (Mayangsari *et al.*, 2021). For the results of the polydispersity index, the results were below 0.3, i.e., 0.263 ± 0.007 for F1 and 0.272 ± 0.033 for F2. With the addition of alpha-lipoic acid, the result showed that the greater the concentration, the greater the value of polydispersity index, but it is still below the range therefore the formula is still homogeneous. The literature of Lason *et al.* (2017) stated that a polydispersity index value below 0.3 means it was homogeneous. For statistical test using Paired-Sample T-Test with $\alpha = 0.05$, the value of Sig. (2 tailed) > 0.05, it can be concluded that there was no significant difference between F1 and F2.

The average pH value of F1 was 5.998 ± 0.01, and F2 was 4.798 ± 0.004. It was in line with the literature, i.e. 4.5 - 6.8 (Mayangsari *et al*., 2021). The NLC-GTE preparation with the addition of alpha-lipoic acid has more acidic pH. After statistical testing with the Paired-Sample T-Test with $\alpha = 0.05$, information was obtained that the value of Sig. (2 tailed) < 0.05, it can be concluded that there was a significant difference. Differences in the concentration of alpha-lipoic acid between formulas affect the pH value, which make the preparation relatively stable under acidic conditions. This can protect the preparation from being degraded by pH, resulted in the preparation remains stable. The addition of alpha-lipoic acid which is a weak acid with a pKa value of 4.7 resulted in a conclusion that the greater the concentration, the lower the pH value (Cichewicz *et al.*, 2013). However, all formulas still meet the normal pH range of the skin (4.5 – 6.8) so it does not cause any side effect on the skin.

Physical stability testing

Physical stability testing used the thermal cycling method by storing the preparations in 1.5 cycles, two days in the oven, in the refrigerator, and in the oven for six days. Organoleptic, pH, particle size, polydispersity index, and the presence or absence of separation testing was carried out, before and after the physical stability testing.

Tabel 2. Characteristic observation results of F1 and F2

Observation		F1	F2
Organoleptic	Color	White	White
	Consistency	Liquid	Liquid
	Texture	Soft	Soft
	Odor	Odorless	Odorless
	Particle Size	313.9 ± 0.76	423.4 ± 0.75
	Polydispersity index	0.263 ± 0.007	0.272 ± 0.033
	pH	5.998 ± 0.01	4.798 ± 0.004

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 3. Results of observation of particle size, polydispersity index, pH, and the presence or absence of separation in the physical stability test

Formula	T = 0				T = 6 days			
	Separation	Particle Size (nm) ± SD	PDI ± SD	pH ± SD	Separation	Particle Size (nm) ± SD	PDI ± SD	pH ± SD
F1 (Without Lipoic)	-	313.9 ± 0.76	0.263 ± 0.007	5.998 ± 0.01	+	385.26 ± 2.49	0.265 ± 0.012	5.730 ± 0.007
F2 (With Lipoic)	-	423.4 ± 0.75	0.272 ± 0.033	4.798 ± 0.004	-	435.16 ± 3.65	0.260 ± 0.024	4.847 ± 0.012

Information:
- = absence of separation
+ = presence of separation

After six days, there were no significant changes in color and odor based on organoleptic testing. However, in F1, there was separation after six days, while in F2, there was no separation, so it remained homogeneous. It meant that F1 was unstable at extreme temperatures while F2 remained stable. In the physical stability test, F2 contains alpha-lipoic acid and resulted with no phase separation occurs. This is supported by the research of Lason *et al*. (2017), that NLC containing the active ingredient alpha-lipoic acid when observed with an optical microscope showed that the preparation was homogeneous and no agglomerates or crystals were found.

Observations of particle size values in F1 and F2 were conducted on days 0 and 6, resulted that there was a change in particle size but still according to the literature, which has a value below 1000 nm (Mayangsari *et al*., 2021). In F1 and F2, statistical tests were carried out using the Paired-Sample T-Test with α = 0.05. The results obtained were the Sig values (2 tailed) < 0.05, it can be concluded that there was a significant difference before and after 1.5 cycles of thermal cycling. This difference is indicated by an increase in particle size before and after storage.

The results of the observation from the F1 and F2 polydispersity indices after the sixth day were below 0.3, and this was in accordance with the literature (Lason *et al*., 2017), if the value was below 0.3, the preparation was homogeneous. The polydispersity index before and after 1.5 cycles of thermal cycling in F1 and F2 was statistically tested using the Paired-Sample T-Test, and the results obtained were Sig values. (2 tailed) > 0.05, so there was no difference and the preparation remained stable.

Related to pH observation on days 0 and 6, the results were relatively stable for F1 and F2. The pH value obtained is still in the skin pH range of 4.5 - 6.8 (Mayangsari *et al*., 2021). F1 and F2 were statistically tested with the Paired-Sample T-Test, and the results obtained were Sig values (2 tailed) < 0.05, so there was a significant difference in pH F1 and F2. The results of observations for particle size, polydispersity index, pH, and the presence or absence of separation were in Table 3. However, all formulas were still in the skin range of 4.5 - 6.8 so it still safe to be used. ALA is a weak acid with a pKa of 4.7 therefore it can lower the pH of the formulation but increase the stability of EGCG.

CONCLUSION

Based on the physical characteristics test, it was concluded that F1 (without alpha-lipoic acid) and F2 (with alpha-lipoic acid) had differences in particle size and pH. From the physical stability test, it can be concluded that F2 is more stable than F1.

AUTHOR CONTRIBUTIONS

Conceptualization, F. Y. A., W. S., D. A. P.; Methodology, F. Y. A., W. S., D. A. P.; Validation, F. Y. A., W. S., D. A. P.; Formal Analysis, F. Y. A.; Investigation, F. Y. A.; Resources, F. Y. A., W. S., D. A. P.; Data Curation, F. Y. A., W. S.; Writing - Original Draft, F. Y. A.; Writing - Review & Editing, F. Y. A., W. S., D. A. P.; Visualization, F. Y. A.; Supervision, F. Y. A., W. S., D. A. P.; Project Administration, F. Y. A., W. S., D. A. P.; Funding Acquisition, F. Y. A.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Ahmad, Z & Damayanti. (2018). Penuaan Kulit Patofisiologi dan Manifestasi Klinis. *Berkala Ilmu Kesehatan Kulit dan Kelamin-Periodical of*

Dermatology and Venereology; *30*; 208-215. doi: 10.20473/bikk.V30.3.2018.208-215.

Anggowarsito, J. L. (2014). Aspek Fisiologi Penuaan Kulit. *Jurnal Widya Medika*; *2*; 56-61. doi: 10.33508/jwm.v2i1.1652.

Bianchi, A, Valentina, T. & Santo, S. (2013). Photostability of Epigallocatechin-3- Gallate in Topical Formulation. *Monographio Supplement Series: Sun Care*. *8*; 18-22.

Chen, J., Ning, W., Maria, L. G., Dianna, A., Jason, L., Colin, A. & Teresa, P. (2017). Development and Evaluation of Resveratrol, Vitamin E, and Epigallocatechin Gallate Loaded Lipid Nanoparticles for Skin Care Applications. *European Journal of Pharmaceutics and Biopharmacetics*; *117*; 286-291. doi: 10.1016/j.ejpb.2017.04.008.

Cichewicz, A., Chelsea, P., Ashley, C., Martha A. H. & Luciana, B. L. (2013). Cutaneous Delivery of α-Tocopherol and Lipoic Acid Using Microemulsions: Influence of Composition and Charge. *Journal of Pharmacy dan Pharmacology*; *65*; 817-826. doi: 10.1111/jphp.12045.

Frias, I., Ana, R. N., Marina, P. & Salette, R. (2016). Design, Development, and Characterization of Lipid Nanocarriers-Based Epigallocatechin Gallate Delivery System For Preventive and Therapeutic Supplementation. *Dovepress*; *10*; 3519-3528. doi: 10.2147/DDDT.S109589.

Ganesan, P. & Dong, K. C. (2016). Current Application of Phytocoumpound-Based Nanocosmeceuticals for Beauty and Skin Therapy. *International Journal of Nanomedicine*; *11*; 1987-2007. doi: 10.2147/IJN.S104701.

Hu, C., Guodong, Z., Qiang, X. & Ria, S. (2015). Development and Characteristic of a Self-Double-Emulsifying Drug Delivery System Containing Both Epigallocatechin-3-Gallate and α-Lipoic Acid. *Journal of Materials Science*; *50*; 6567-6577. doi: 10.1007/s10853-015-9194-7.

Kementerian Kesehatan (Kemenkes). (2020). Indonesia Masuki Periode Aging Population. https://www.kemkes.go.id/article/view/19070500004/indonesia-masuki-periode-aging-population.html. Accessed : 11 March 2020.

Lason, E., Elzbieta, S., Malgorzata, M., Paulina, S. & Jan, O. (2017). NLC Delivery Systems for Alpha Lipoic Acid: Physicochemical characteristics and Release Study. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*; *6*; 1-6. doi: 10.1016/j.colsurfa.2017.06.083.

Manea, A. M, Bogdan, S. V. & Aurelia, M. (2014). Antioxidant and Antimicrobial Activities of Green Tea Extract Loaded Into Nanostructured Lipid Carriers. *Comptes Rendus Chimie*; *17*; 331-341. doi: 10.1016/j.crci.2013.07.015.

Mayangsari, F. D, Tristiana, E, Widji, S. & Noorma, R. (2021). Karakteristik dan Stabilitas Fisik NLC-Koenzim Q10 dalam *Sleeping Mask* dengan Minyak Nilam. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*; *8*; 178-186. doi: 10.20473/jfiki.v8i22021.178-186.

Namita, P. R. M & Kumar, V. (2012). *Camellia sinensis* (Green Tea): a Review. *Global Journal of Pharmacology*; *6*; 52-59.

Perricone, N. V. (2000). Topical 5% Alpha Lipoic Acid Cream in the Treatment of Cutaneous Rhytids. *Aesthetic Surgery Journal*; *20;* 208-222. doi: 10.1016/S1090-820X(00)70020-3.

Rosita, N., Vinta, A. M., Mirna, C. R., Dewi, M. H. & Andang, M. (2019). Enhancing Skin Penetration of Epigallocatechin Gallate by Modifying Partition Coefficient using Reverse Micelle Method. *Therapeutic Delivery*; *15*; 1-9. doi: 10.4155/tde-2019-0015.

Scalia, S., Nicola, M. & Anna, B. (2013). Comparative Evaluation of Different Co- Antioxidants on the Photochemical and Functional-Stability of Epigallocatechin-3-gallate in Topical Creams Exposed to Simulated Sunlight. *Molecules*; *18*; 574-587. doi: 10.3390/molecules18010574.

Vaishali, K., Chintale, A. G., Deshmukh, K. P. & Nalwad, D. N. (2013). Cosmeceuticals an Emerging Concept: A Comprehensive Review. *International Journal of Research in Pharmacy and Chemistry*; *3*; 308-316.

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 235-241
DOI: 10.20473/jfiki.v9i32022.235-241
Available online at https://e-journal.unair.ac.id/JFIKI/

Optimization Method and Stability Test to Determinate Luteolin, Quercetin, Apigenin, and Sinensetin Levels in Herbal Medicines Using TLC-Densitometry

Muhammad Hidayatullah[1], Mochammad Yuwono[2]*, Riesta Primaharinastiti[2]
[1]Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: yuwono05@yahoo.com

Submitted: 20 April 2022
Accepted: 8 September 2022
Published: 9 December 2022

Abstract

Background*: Nephrolithiasis is a condition in which there are one or more kidney stones in the pelvis or calyces. Luteolin, quercetin, apigenin, and sinensetin are marker compounds in the extracts of Plantago major, Sonchus arvensis, Strobilanthes crispus and Orthosiphon stamineus which have nephrolithiasis activity. To control the quality of herbal medicines, a TLC-Densitometry method was developed in this study using luteolin, quercetin, apigenin, and sinensetin as phytochemical markers.* ***Objective****: The present work aimed to develop optimal conditions for analyzing luteolin, quercetin, apigenin, and sinensetin.* ***Methods****: Determination of optimal conditions for analysis is carried out by determining the composition of the mobile phase, chamber saturation time, and analysis wavelength. Silica gel 60 F_{254} was used as the stationary phase. Stability tests were carried out by analyzing standards and samples at 0, 4, 8, and 24 hours.* ***Results****: The best separation that produces symmetrical peaks of herbal medicine was achieved under isocratic conditions using the composition of the mobile phase chloroform : acetone: dichloromethane : acetonitrile : formic acid (6 : 2: 2 : 0,05 : 0.05 v/v/v/v/ v) with a wavelength of 335 nm with a saturation time of 30 minutes.* ***Conclusion****: In this study, the optimal conditions for the analysis of luteolin, quercetin, apigenin, and sinensetin. Luteolin, quercetin, apigenin, and sinensetin are unstable during 8 hours of storage. Therefore, standard solutions and samples must be made fresh to maintain stability.*

Keywords*: TLC-densitometry, luteolin, quercetin, apigenin, sinensetin*

How to cite this article:
Hidayatullah, M., Yuwono, M. & Primaharinastiti, R. (2022). Optimization Method and Stability Test for Determination of Luteolin, Quercetin, Apigenin, Sinensetin Levels in Herbal Medicines Using TLC-Densitometry. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 9(3), 235-241. http://doi.org/10.20473/jfiki.v9i32022.235-241

INTRODUCTION

Over the decades, herbal plants and their active constituents have been developed and used to treat various ailments. The increasing development and use of herbal medicines are based on several reasons, namely: not all modern medicines can efficiently overcome/cure all human pathologies, there is increasing interest and attention to the guarantee and safety of synthetic medicines, and many natural products are proven to have better quality than synthetic medicines. *Plantago major*, *Sonchus arvensis*, *Strobilanthes crispus*, and *Orthosiphon stamineus*, which are empirically used to treat or prevent nephrolithiasis have been developed in many countries. Extracts of leaves, stems, and roots have long been used as a treatment for kidney stones, antifungal, bladder, antioxidant, gastrointestinal infections, diabetes, and anticancer (Kartini & Azminah, 2012; Hossain & Ismail, 2016). Several studies have proven that *Plantago major*, *Sonchus arvensis*, *Strobilanthes crispus*, and *Orthosiphon stamineus* have nephrolithiasis effects (Singh *et al.,* 2009; Alkreathy *et al.,* 2014; Chun *et al.,* 2020; Ghiasian *et al.,* 2021). Jimoh *et a*l. (2021) proved that luteolin, quercetin, apigenin, and sinensetin compounds play a role in controlling the crystallization process of kidney stones (jimoh *et al.,* 2021). Hossain & Ismail, (2012) also proved that luteolin, quercetin, apigenin, and sinensetin compounds could inhibit electrolyte reabsorption in the loop of Henle so that it has a diuretic effect (Hossain & Ismail, 2016). Diuretics effectively reduce urinary calcium excretion and kidney stone recurrence in nephrolithiasis patients by controlling urine supersaturation (Ahmed *et al.,* 2018). The structure of luteolin, quercetin, apigenin, and sinensetin can be seen in Figure 1.

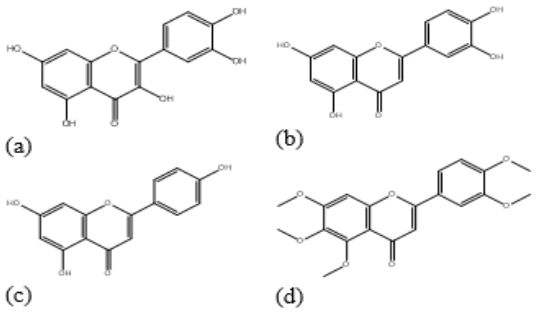


Figure 1. Structure of luteolin (a), quercetin (b), apigenin (c) dan sinensetin (d)

In developing polypharmacy herbal medicines, the selection of marker compounds and methods of identification/quantification of marker compounds play an important role in ensuring quality control of herbal medicines. The analytical method commonly used to identify marker compounds is TLC-Densitometry (Kartini & Azminah, 2012; Kim *et al.,* 2010; Bertrams *et al.,* 2013; Attarde *et al.,* 2017). Identification and quantitation of extracts in polypharmacy herbal medicines is a challenge. Therefore, developing and validating the TLC-Densitometry method are essential to obtain a simple and fast procedure applied to a quality control laboratory. The research that has been carried out is limited to determining marker compounds in single herbal plants and has not been applied to the formulation of herbal medicinal preparations using the TLC-Densitometry method (Abdullah *et al*., 2012; Hossain & Ismail, 2016; Kuppusamy *et al*., 2017). Several analytical methods for determining luteolin, quercetin, apigenin, and sinensetin have been developed using the HPLC method (Kuppusamy *et al*., 2017; Grek *et al.,* 2019; Ghiasian *et al.,* 2021). however, this instrument is expensive and require expertise specialized in instrument operation.

This study aimed to obtain an alternative method of TLC-densitometry which is cheaper and more straightforward for the simultaneous analysis of luteolin, quercetin, apigenin, and sinensetin in herbal medicines containing extracts of *Plantago major*, *Sonchus arvensis*, *Strobilanthes crispus*, and *Orthosiphon stamineus*.

MATERIALS AND METHODS

Materials

Luteolin standard (Sigma Aldrich), quercetin standard (Sigma Aldrich), apigenin standard (Sigma Aldrich), sinensetin standard (Sigma Aldrich), and Herbal medicinal products obtained from the pharmaceutical industry in Indonesia (PT. Interbat, Sidoarjo), ethanol (Merck, US/Canada), formic acid (Merck, US/Canada), chloroform (Merck, US/Canada), dichloromethane (Merck, US/Canada), acetone (Merck, US/Canada), acetonitrile (Merck, US/Canada).

Tools

Silica gel TLC plate 60 F_{254} aluminum sheet 20 x 20 cm (E, Merck, Darmstadt, Germany), Camag vessel 10 x 10 cm (Camag), TLC Scanner 3 with UV detector (Camag), winCATS software version 1.4.8.2012 (Camag).

Method

Preparation of standard solution

Standards (1.0 mg) were each dissolved in 10 mL of ethanol in a measuring flask. The stock standar solutions were then diluted to working solutions of 20 - 100 ppm.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 1. Variation of mobile phase composition tested to obtain optimal conditions

No.	Mobile phase composition	Ref.
1.	Chloroform - dichloromethane - ethyl acetate (7:4:1, *v/v/v*)	(Kartini *et al.*, 2020)
2.	Isopropyl alcohol - n-butanol (5:5, *v/v*)	(Choudhary *et al.*, 2020)
3.	Toluene - ethyl acetate - formic acid (6:4:0,15, *v/v/v*)	(Attarde *et al.*, 2017)
4.	Chloroform - acetone - dichloromethane - acetonitrile - formic acid (6:2:2:0,05:0,05, *v/v/v/v/v*)	(Singh *et al.*, 2009)

Table 2. Variations in the composition of the mobile phase composition of a mixture of luteolin, quercetin, apigenin, and sinensetin

Mobile phase	Compound	Retardation factor (Rf)	Rs	Peak
1	Luteolin	0.19	0.94	*Fronting*
	Apigenin	3.34	-	
	Sinensetin	0.82	2.4	
2	Luteolin	0.34	-	*Fronting*
	Quercetin	0.42	1.2	
	Apigenin	0.51	1.4	
	Sinensetin	0.61	2.1	
3	Luteolin	0.18	-	*Fronting*
	Quercetin	0.21	-	
4	Luteolin	0.23	2.3	*Symmetric*
	Quercetin	0.24	2.2	
	Apigenin	0.34	1.8	
	Sinensetin	0.60	3.7	

Sample preparation

Herbal medicine was weighed 1 g and dissolved 20 ethanol in a measuring flask. The solution was sonicated for 30 minutes filtered through a 0.45 µm membrane filter and spotted on a TLC plate.

Preparation of mobile phase

For optimization of the mobile phase, the mobile phase is made in several compositions as shown in Table 1.

Optimization of the analytical conditions

Optimization of analytical conditions is done by changing the composition of the mobile phase, the saturation time of the vessel, and determining the maximum wavelength. The development method uses various mobile phase processes. The mobile phase was sonicated for 15 minutes before use. Parameters observed in selecting optimal conditions are the retardation factor (Rf), and the best resolution (Rs).

Stability test of the test solution

The stability test was carried out by dividing the standard solution and the sample into four different tubes. Each tube is labeled 0 hours, 4 hours, 8 hours, and 24 hours. The test solution was analyzed according to the time specified on the label. All tubes were stored at 4^{o}C.

RESULTS AND DISCUSSION

The data in Table 2 are based on experiments conducted on variations in the composition of the mobile phase.

The composition of the mobile phase 1 shows a resolution < 1.5 so that the peaks of luteolin, quercetin, apigenin, and sinensetin are not entirely separated from the peaks of impurities. In addition, the peak is also fronting so mobile phase 1 is not selected. The composition of phase 2 also showed poor resolution between luteolin, quercetin, apigenin, and sinensetin, namely < 1.5. This could affect the analysis results, so mobile phase 2 was not selected. The composition of mobile phase 3 was unable to completely separate luteolin, quercetin, apigenin, and sinensetin, which showed a resolution < 1.5 and had a fronting peak, so the composition of mobile phase 3 was not chosen. The composition of the mobile phase 4 shows the most optimal resolution of > 1.5 and an asymmetrical peak, so this mobile phase was selected and used for further analysis. The optimal conditions obtained in this study can be used to test the stability of the pre-validation stage method. The composition of the mobile phase 4 shows the most optimal resolution of > 1.5 and an asymmetrical peak, so this mobile phase was selected and used for further analysis. The optimal conditions obtained in this study can be used to test the stability of the pre-validation stage method.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

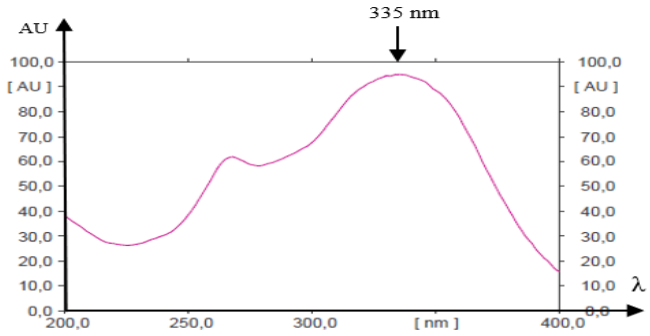


Figure 2. The Spectrum of apigenin compounds and its maximum wavelength using solvents chloroform - acetone - dichloromethane - acetonitrile - formic acid (6:2:2:0.05:0.05, *v/v/v/v/v*)

Table 3. Sample load optimization results

Concentration (ng/ µl)	Sample load (µl)	Compound	Area	Peak
12,18	4	Luteolin	6686.1	*Symmetrical*
		Quercetin	20802.7	
		Apigenin	12071.5	
		Sinensetin	9858.0	
12,18	8	Luteolin	7563.3	*Symmetrical*
		Quercetin	2187.7	
		Apigenin	1398.3	
		Sinensetin	9987.0	
12,18	14	Luteolin	10987.3	*Fronting*
		Quercetin	26234.6	
		Apigenin	16345.3	
		Sinensetin	13345.6	

The maximum wavelength of the selected analysis was observed from the peak, which gave the largest area value in the spectra of luteolin, quercetin, apigenin, and sinensetin. Besides, it did not cause interference peaks that could affect the analysis results of luteolin, quercetin, apigenin, and sinensetin compounds. At 335 nm, the maximum wavelength of apigenin compounds produced by high peaks of luteolin, quercetin, apigenin, and sinensetin and the resulting interference peak is very small, so the selected wavelength used is 335 nm (Figure 2).

Complete saturation of the vessel is required so that the elution and separation process can run smoothly good. The results of the sample load optimization are presented in Table 3. Based on the results obtained, the sample loads that give a symmetrical peak shape are 4 µl and 8 µl. At a sample load of 14 µl, the peak experienced fronting. This could be because the injection volume is too large. The optimal separation in thin-layer chromatography will be obtained if the sample is spotted with the smallest and narrowest possible spot size. As in other chromatographic procedures, the resolution will decrease if too many samples are used (Chun *et al.*, 2020).

The saturation time of the vessel has been optimized in this study. The optimization results are listed in Table 4. From the optimization results, it is found that the saturation time of the vessel can affect Rf. The optimal vessel saturation time in this study is at least 30 minutes because the area and Rf are stable after 30 minutes.

Table 4. Optimization of apigenin saturation time

Saturation time (minutes)	Area	Rf
15	956,6	0,19
30	1098,8	0,23
60	1095,0	0,23

Stability of the test solution

A stability test on standard solutions and samples is one of the tests carried out at the pre-validation stage. This stability test is carried out to evaluate the stability of the test solution at a certain storage time. Parameters observed in the selection of optimal conditions are the retardation factor (Rf), and the best resolution (Rs); a good Rf value indicates a good separation is in the range of 0.2-0.8, and a good separation if the Rs value meets the requirements, namely > 1.5 (AOAC, 2015). The results of the stability test of the test solution at several times of measurements can be seen in Table 5 and Table 6.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 5. The results of stability tests of standard solutions in the range 0 - 24 hours

Observation Time (hours)	Standard	Average Area	Area Difference (%)	Rf	Difference Rf (%)
0	Luteolin	76861	-	0.23	-
	Quercetin	218027	-	0.24	-
	Apigenin	13071.5	-	0.34	-
	Sinensetin	9958	-	0.6	-
4	Luteolin	7543.2	1.86	0.23	-
	Quercetin	21513.5	1.33	0.24	-
	Apigenin	13042.8	0.22	0.34	-
	Sinensetin	9843.4	1.15	0.61	1.64
8	Luteolin	7435.9	3.26	0.23	-
	Quercetin	20978.1	2.49	0.25	4.00
	Apigenin	12878.2	3.78	0.35	2.86
	Sinensetin	9634.7	1.48	0.62	3.23
24	Luteolin	6928.9	9.85	0.22	4.55
	Quercetin	19913.3	8.67	0.24	-
	Apigenin	11976.3	8.38	0.34	-
	Sinensetin	8967.9	9.94	0.61	1.64

Table 6. The results of stability tests of sample solutions in the range 0 - 24 hours

Observation Time (hours)	Sample	Average Area	Area Difference (%)	Rf	Difference Rf (%)
0	Luteolin	8542.2	-	0.23	-
	Quercetin	7128.3	-	0.24	-
	Apigenin	7893.4	-	0.34	-
	Sinensetin	19077	-	0.6	-
4	Luteolin	8512.1	0.35	0.23	-
	Quercetin	7098.3	0.42	0.24	-
	Apigenin	7856.9	0.46	0.34	-
	Sinensetin	18893.5	0.97	0.6	-
8	Luteolin	8467.9	0.88	0.23	-
	Quercetin	6983.2	2.08	0.25	4.00
	Apigenin	7398.4	6.69	0.35	2.86
	Sinensetin	18349.1	3.97	0.61	1.64
24	Luteolin	8119.3	5.21	0.22	4.55
	Quercetin	6723.3	6.02	0.24	-
	Apigenin	7239.9	9.03	0.34	-
	Sinensetin	18122.1	5.27	0.62	3.23

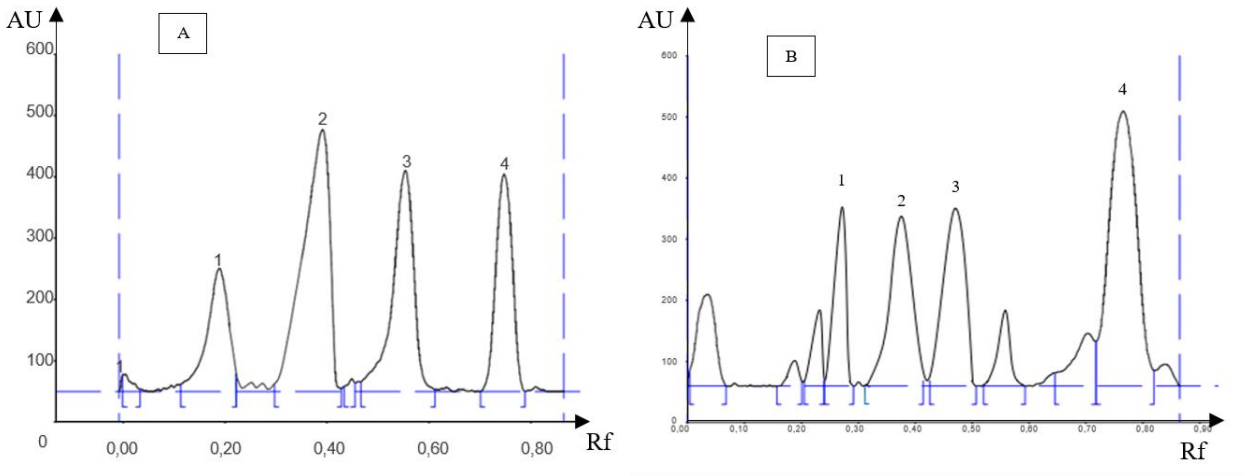


Figure 3. Chromatogram of standard (1) luteolin (2) quercetin (3) apigenin and (4) sinensetin (A) and herbal drug sample (B) with mobile phase composition of chloroform : acetone : dichloromethane : acetonitrile : formic acid (6 : 2 : 2 : 0.05 : 0.05 *v/v/v/v/v*)

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Based on the data obtained, the test solution is known to be unstable after 8 hours of storage. Then an analysis was carried out using SPSS to determine whether there was a significant difference between the area and factor retardation at each observation time. The data obtained showed a normal and homogeneous distribution so two-way ANOVA tested it. The results of the analysis show that there are significant differences in the area mean and Rf at each time of observation. This indicates that the longer the standard solution is stored, the lower the concentration will be (Figure 3).

CONCLUSION

The optimal conditions obtained in this study for philanthropic analysis were the composition of the mobile phase chloroform: acetone: dichloromethane: acetonitrile: formic acid (6 : 2 : 2 : 0.05 : 0.05 *v/v/v/v/v*), with saturation time 30 minutes, wavelength analysis 335 nm. The stability of the solution was tested in this study as a pre-validation stage. The stability of the test solution decreased with storage for a certain time, indicated by a decrease in area and a retardation shift in the chromatogram. It is recommended that the test solution be made fresh when the analysis is carried out. The optimal conditions obtained can be continued with method validation for further research.

ACKNOWLEDGMENT

Thanks to ULP Universitas Airlangga who has provided the means for this research to run, and thanks to PT. Interbat Sidoarjo provides research-related samples.

AUTHOR CONTRIBUTIONS

Conceptualization, M. H., M. Y., R. P.; Methodology, M. H., M. Y.; Software, M. H.; Validation, M. H., M. Y., R. P.; Formal Analysis, M. H., M. Y., R. P.; Investigation, M. H., M. Y., R. P.; Resources, M. H., M. Y., R. P.; Data Curation, M. H.; Writing - Original Draft, M. H.; Writing - Review & Editing, M. H., M. Y., R. P.; Visualization, M. H., M. Y., R. P.; Supervision, M. H., M. Y., R. P.; Project Administration, M. H., M. Y., R. P.; Funding Acquisition, M. H., M. Y., R. P.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Abdullah, S., Shaari, A. R. & Azimi, A. (2012). Effect of Drying Methods on Metabolites Composition of Eisai Kucing (*Orthosiphon stamineus*) Leaves. *Apcbee Procedia*; *2*; 178-182. doi: 10.1016/j.apcbee.2012.06.032.

Ahmed, S., Hasan, M. M., Khan, H., Mahmood, Z. A. & Patel, S. (2018). The Mechanistic Insight of Polyphenols in Calcium Oxalate Urolithiasis Mitigation. *Biomedicine & Pharmacotherapy*; *106*; 1292-1299. doi: 10.1016/j.biopha.2018.07.080.

Alkreathy, H. M., Khan, R. A., Khan, M. R. & Sahreen, S. (2014). CCl4 Induced Genotoxicity and DNA Oxidative Damages in Rats: Hepatoprotective Effect of *Sonchus arvensis*. *BMC complementary and Alternative Medicine*; *14*; 1-7. doi: 10.1186/1472-6882-14-452.

Attarde, D. L., Pal, S. C., & Bhambar, R. S. (2017). Validation and Development of HPTLC Method for Simultaneous Estimation of Apigenin and Luteolin in Selected Marketed Ayurvedic Formulations of 'Dashmula' and in Ethyl Acetate Extract of *Premna integrifolia* L. *Journal of Analytical & Bioanalytical Techniques*; *8*; 1-9. doi: 10.4172/2155-9872.1000343.

AOAC. (2015). Guidelines for Dietary Supplements and Botanicals. Rockville: AOAC International.

Bertrams, J., Kunz, N., Müller, M., Kammerer, D. & Stintzing, F. C. (2013). Phenolic Compounds as Marker Compounds for Botanical Origin Determination of German Propolis Samples Based on TLC and TLC-MS. *Journal of Applied Botany and Food Quality*; *86*; 143 - 153. doi: 10.5073/JABFQ.2013.086.020.

Chun, S. C., Gopal, J., Iyyakannu, S., & Muthu, M. (2020). An Analytical Retrospection of Mass Spectrometric Tools Established for Plant Tissue Culture: Current Endeavours and Future Perspectives. *TrAC Trends in Analytical Chemistry*; *126*; 1-16. doi: 10.1016/j.trac.2020.115843.

Choudhary, N. K., Gupta, K., Jain, A. K. & Pal, A. (2020). Quantitative Estimation of Marker Compound (Lupeol and Diosgenin) in Polyherbal Formulation by HPTLC Method. *Plant Archives*; *20*; 4337-4342.

Ghiasian, M., Niroomandi, Z., Dastan, D., Poorolajal, J., Zare, F. & Ataei, S. (2021). Clinical and Phytochemical Studies of *Plantago major* in Pressure Ulcer Treatment: a Randomized Controlled Trial. *Complementary Therapies in Clinical Practice*; *43*; 1-6. doi: 10.1016/j.ctcp.2021.101325.

Grek, O., Chubenko, L., Kumar, A., Khareba, V. Tymchuk, A., & Onopriichuk, O. (2019). Polyphenolic Compounds Transition into Protein-Plant Concentrates During the Deposition of Milk Proteins by *Plantago major* L. *Ukrainian Food Journal*; *8*; 745-754.

Hossain, M. A. & Ismail, Z. (2016). Quantification and Enrichment of Sinensetin in the Leaves of *Orthosiphon stamineus*. *Arabian Journal of Chemistry*; *9*; S1338-S1341. doi: 10.1016/j.arabjc.2012.02.016.

Jimoh, S. O., Ha, M., Arowolo, L. A., Badmos-Oladapo, R. B. & Akinlade, R. Y. (2021). Invitro Activity of *Phyllanthus amarus* Extract on Nephrolithiasis and Urea-Splitting Bacteria. *Fountain Journal of Natural and Applied Sciences*; *10*; 25-32.

Kartini & Azminah. (2012). Chromatographic Fingerprinting and Clustering of *Plantago major* L from Different Areas In Indonesia. *Asian Journal of Pharmaceutical and Clinical Research*; *4*; 191-195.

Kartini, K., Jayani, N. I. E., Hadiyat, M. A. & Avanti, C. (2020). Thin Layer Chromatography Fingerprinting and Clustering of *Orthosiphon stamineus* Benth from Different Origins. *Pharmacognosy Journal*; *12*; 1683-1691. doi: 10.5530/pj.2020.12.257.

Kim, H. J., Jee, E. H., Ahn, K. S., Choi, H. S. & Jang, Y. P. (2010). Identification of Marker Compounds in Herbal Drugs on TLC with DART-MS. *Archives of Pharmacal Research*; *33*; 1355-1359.

Kuppusamy, P., Lee, K. D., Song, C. E., Ilavenil, S., Srigopalram, S., Arasu, M. V. & Choi, K. C. (2018). Quantification of Major Phenolic and Flavonoid Markers in Forage Crop *Lolium multiflorum* using HPLC-DAD. *Revista Brasileira de Farmacognosia*; *28*; 282-288. doi: 10.1016/j.bjp.2018.03.006.

Singh, B., Mungara, P., Nivsarkar, M. & Anandjiwala, S. (2009). HPTLC Densitometric Quantification of Glycyrrhizin, Glycyrrhetinic Acid, Apigenin, Kaempferol and Quercetin from *Glycyrrhiza glabra*. *Chromatographia*; *70*; 1665-1672.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 242-251
DOI: 10.20473/jfiki.v9i32022.242-251
Available online at https://e-journal.unair.ac.id/JFIKI/

The Effect of Insulin Administration on Medication Adherence in Type 2 Diabetes Mellitus Patients with Neurological Complications

July[1], Rani Sauriasari[2]*, Nadia Farhanah Syafhan[2], Hadijah Tahir[1]
[1]National Brain Center Hospital, Jakarta, Indonesia
[2]Department of Pharmacy, Faculty of Pharmacy, University Indonesia, Jakarta, Indonesia

*Corresponding author: rani@farmasi.ui.ac.id

Submitted: 1 June 2022
Accepted: 17 October 2022
Published: 9 December 2022

Abstract

Background*: Medication adherence is essential to achieving controlled blood sugar in diabetic patients. Insulin generally provides better glycemic control but is considered painful and requires special techniques. Insulin administration in patients with neurological complications requires particular consideration because these complications can cause physical and cognitive barriers.* ***Objective****: This study analyses the effect of insulin administration on medication adherence in diabetic patients with neurological complications and the influence of various confounding variables (baseline characteristics, medical and medication history).* ***Methods:*** *This observational study was conducted with a cross-sectional design at a government hospital in East Jakarta from September 2021 to January 2022. The sample was type 2 diabetes mellitus patients with neurological complications who received antidiabetics for at least six months. The neurological complications include central nervous disorders (such as stroke) and peripheral nervous disorders (such as neuropathy). The independent variable was insulin administration, while the dependent variable was adherence, measured using subjective methods [Adherence to Refills and Medications Scale (ARMS)] and objective methods (Medication Refill Adherence (MRA) and HbA1c].* ***Results****: Of 175 respondents, based on the three methods (MRA, ARMS, HbA1c), 13 respondents (7.4%) were adherent, namely one respondent (1.8%) in the insulin group and 12 respondents (10.1%) in the non-insulin group. Insulin administration affects adherence to antidiabetics by 0.123 times (95% CI: 0.015 - 1.024), or patients who use insulin have 87.7% lower adherence controlled by antidiabetic changes and the total number of medicines used.* ***Conclusion****: Insulin administration significantly affects medication adherence in diabetes mellitus patients with neurological complications.*

Keywords*: diabetes mellitus, insulin, adherence, neurological complications*

How to cite this article:
July, Sauriasari, R., Syafhan, N. F. & Tahir, H. (2022). The Effect of Insulin Administration on Medication Adherence in Type 2 Diabetes Mellitus Patients with Neurological Complications. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 9(3), 242-251. http://doi.org/10.20473/jfiki.v9i32022.242-251

P-ISSN: 2406-9388
E-ISSN: 2580-8303

INTRODUCTION

Diabetes mellitus is among the ten leading causes of death globally, with a 70% increase since 2000. Around 2.3 million women and 1.9 million men aged 20 – 79 are estimated to die from diabetes and its complications in 2019 (WHO, 2020). Indonesia had the seventh-highest number of people with diabetes in the world in 2019; the number of people with diabetes is around 10.7 million (International Diabetes Federation, 2019).

Pharmacological therapy in patients with type 2 diabetes mellitus consists of oral antidiabetic and injection antidiabetic (insulin) or a combination. Insulin is given if the target blood glucose level has not been achieved with two types of oral antidiabetics (Perkeni, 2021).

Medication adherence is essential in the effective management of diabetes mellitus. Adherence to antidiabetic use is associated with improved control of blood sugar levels (Doggrell & Warot, 2014). Uncontrolled blood sugar levels increase the risk of recurrent stroke by 1.45 times compared to patients with controlled sugar levels (Shou *et al*., 2015). Insulin generally provides better glycemic control, improving quality of life and reducing diabetic complications. On the other hand, it causes discomfort, pain, and aggravation and limits the patient's daily activities, affecting adherence and ultimately the success of therapy. In particular, the administration of drugs with special techniques, such as insulin, requires special consideration in patients with neurological complications because these complications can cause the patient to have physical and cognitive barriers and limitations due to disorders of the central and/or peripheral nerves. This may be will affect medication adherence. Complications are secondary diseases or conditions that develop during the primary disease or condition (Complication Definition & Meaning - Merriam-Webster, 2022). In patients with diabetes mellitus, neurological complications can include central nervous disorders (such as stroke) and peripheral nerve disorders (such as neuropathy) (Ireland *et al*., 2010).

Various studies in Indonesia have previously been undertaken to examine medication adherence with oral antidiabetics without considering the patient's comorbidities and generally use one or two measuring instruments (Salistyaningsih *et al*., 2011; Alfian, 2015; Adikusuma & Qiyaam, 2017; Nanda *et al*., 2018; Bulu *et al*., 2019). The purpose of this study was to analyse the effect of insulin administration on medication adherence in diabetes mellitus patients with neurological complications by using subjective methods [Adherence to Refills and Medications Scale (ARMS)] and objective methods [Medication Refill Adherence (MRA) and HbA1c measurement], as well as the effect of a various confounding variable on medication adherence.

MATERIALS AND METHODS

This observational study used a cross-sectional design and was conducted at a government hospital in East Jakarta from September 2021 to January 2022. The sampling technique used was the consecutive sampling method. The inclusion criteria were typed 2 diabetes mellitus patients with neurological complications who had received at least six months of antidiabetes with a payment system by Badan Penyelenggara Jaminan Sosial (BPJS)/Indonesian Universal Covered Health Insurance and willingness to be respondents in this study.

Neurological complications are divided into central and peripheral nerve disorders. Central nervous disorders were divided into patients with and without stroke, while peripheral nervous disorders were divided into patients with and without. Both of these conditions are determined based on the doctor's diagnosis in the medical records.

Patients who redeemed antidiabetics outside the hospital where the study was conducted, whose medical record data were incomplete, and who did not fully answer the ARMS questionnaire were excluded from this study.

Using the Lemeshow formula (Lachenbruch *et al*., 1991) and previous research data (Osborn & Gonzalez, 2016), 95% confidence interval, and 70% test power, the minimum number of samples required is 53 samples for each group.

Data were collected in two ways, interviews with outpatients who had received medication for at least six months based on data obtained from the patient's medical records. Secondary data were medical history, medicine, and HbA1c from medical records.

Measurement of adherence with the subjective method was carried out using the Indonesian version of the Adherence to Refills and Medications Scale (ARMS) questionnaire, in which validity and reliability were determined. This questionnaire contains twelve questions: eight items from the drug use subscale to assess the patient's adherence to using prescribed drugs as directed, and four items from the prescription refill subscale to assess the patient's adherence to refilling his prescription. The ARMS questionnaire has been

P-ISSN: 2406-9388
E-ISSN: 2580-8303

translated and validated in several countries, such as Iran, Korea, China, Spain, and Poland (Jin *et al*., 2016; Kim *et al*., 2016; González-Bueno *et al*., 2017; *Barati et al*., 2018; Lomper et al., 2018; Park *et al*., 2018 Chen *et al*., 2020; Kripalani *et al*., 2009; Zairina *et al*., 2022).

Measurement of adherence with the objective method was carried out based on changes in HbA1c values in the last two HbA1c examinations with a minimum distance of three months and using the percentage of Medication Refill Adherence (MRA) with the formula:

$$\text{MRA} = \frac{\text{Number of prescribed treatment days for each refills}}{\text{Number of days between the refills}} \times 100 \quad (1)$$

Respondents are considered to be adherent if they met the criteria for adherence in all tests (overall), namely having an ARMS score of 12 (Kripalani *et al*., 2009), an MRA percentage of 80 - 120% (Kindmalm *et al*., 2007), and a decrease in the HbA1c value in the last two measurements 0.2% (Sherwani *et al*., 2016). Data were analysed using SPSS 26, namely descriptive analysis, different proportion analysis, and logistic regression analysis using the backward method.

This study also analysed the effect of various confounding variables derived from the patient's characteristics (age, gender, education, occupation, and body mass index) as well as the patient's medical and medication history (duration of diabetes diagnosed, antidiabetic changes, number of antidiabetic drugs, number of comorbidities, diagnosis of central and peripheral nervous disorders, total number of medications, herbal consumption, allergies, prescription iterations, and family/caregiver assistance). Diagnosis of central and peripheral nervous disorders data was obtained from the patient's medical record. Central nervous disorders were categorised into respondents with a history of ischemic or hemorrhagic stroke (stroke category) and did not have a history of stroke (non-stroke category). Non-stroke respondents include patients diagnosed with Parkinson's, dementia, depression, and others. We divided it into stroke and non-stroke because more than 80% of respondents had a stroke history. Peripheral nerve disorders were divided into patients with peripheral nerve disorders and those without. Diagnosis of peripheral nerve disorders includes neuropathy and peripheral pain.

RESULTS AND DISCUSSION

Characteristics of respondents

Table 1 shows the 175 respondent characteristics, consisting of 56 respondents who used insulin (insulin group) and 119 respondents who did not use insulin (non-insulin group). Patients taking insulin can take insulin alone or in combination with oral antidiabetics.

Table 1. Characteristics of respondents

Characteristics		Insulin n (%)[a]	Non-Insulin n (%)[a]	Total [b]	p-value (inter-group)
Age group	1 (35 - 44 years)	3 (5.4)	5 (4.2)	8 (4.6)	0.951 [c]
	2 (45 - 54 years)	10 (17.9)	23 (19.3)	33 (18.9)	
	3 (55 - 64 years)	25 (44.6)	47 (39.5)	72 (41.1)	
	4 (65 - 74 years)	14 (25.0)	33 (27.7)	47 (26.9)	
	5 (75+)	4 (7.1)	11 (9.2)	15 (8.6)	
Gender	Man	30 (53.6)	74 (62.2)	104 (59.4)	0.179 [c]
	Woman	26 (46.4)	45 (37.8)	71 (40.6)	
Education	Did not finish elementary school/did not go to school	1 (1.8)	2 (1.7)	3 (1.7)	0.478 [c]
	Elementary	4 (7.1)	9 (7.6)	13 (7.4)	
	Junior High School	4 (7.1)	8 (6.7)	12 (6.9)	
	Senior High School	14 (25.0)	46 (38.7)	60 (34.3)	
	College	33 (58.9)	54 (45.4)	87 (49.7)	
Work	Retired/not working	22 (39.3)	52 (43.7)	74 (42.3)	0.801 [c]
	PNS/TNI/POLRI	7 (12.5)	8 (6.7)	15 (8.6)	
	Self-employed/ trader	2 (3.6)	7 (5.9)	9 (5.1)	
	Private employees	5 (8.9)	9 (7.6)	14 (8.0)	
	Housewife	18 (32.1)	37 (31.1)	55 (31.4)	
	Other	2 (3.6)	6 (5.0)	8 (4.6)	
Body mass index (Kg/m^2)		25.77 ± 4.49	25.57 ± 4.15	25.64 ± 4.25	0.775 [d]

Information: [a] Value is expressed in n (%), the percentage in one category; [b] Values are expressed in n (%), percentage of all respondents; [c] Chi-squared test, [d] unpaired T-test because body mass index data is normal.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 1 shows that the respondents in this study consisted of respondents aged 55 - 64 years (41.1%), followed by 65 - 74 years (26.9%), 45 - 54 years (18.9%), over 75 years (8.6%), and 35 - 44 years old (4.6%). Male respondents were 59.4%, while female respondents were 40.6%. Most respondents were undergraduate (49.7%) and high school (34.3%). As many as 42.3% of respondents are not working or retired, and 31.4% are homemakers. Only eight respondents (4.6%) showed drug allergy but were not antidiabetic. Most respondents were overweight, whit a body mass index of 25.64 ± 4.25 Kg/m^2.

Respondent's medical and medication history

The respondent's medical and medication history is summarised in Table 2. Of the 175 respondents, 71 respondents (40.6%) had received antidiabetes for 6 - 12 months, 55 respondents (31.4%) for 12-24 months, and 49 respondents (28%) for more than 24 months. A small proportion of respondents (37.1%, n = 65) experienced changes in antidiabetics, which is the replacement or addition of antidiabetics. Sixty-five respondents (37.1%) got three antidiabetics, 59 respondents (33.7%) got two antidiabetics, and the rest received a single antidiabetic or a combination of four antidiabetics. The antidiabetic can be insulin and/or oral antidiabetic. Respondents who got four antidiabetics commonly received insulin; only one received four oral antidiabetics, which consisted of metformin, vildagliptin, gliclazide, and pioglitazone. Most respondents had stroke prevously (86.95%, n = 152). This rate is in line with the meta-analysis of 102 prospective studies involving 698782 people showed that diabetes increased the risk of ischemic stroke by 2.27 times and hemorrhagic stroke by 1.56 times. (Sarwar *et al*., 2010; Bloomgarden & Chilton, 2021). A total of 56 respondents (32.0%) were diagnosed with peripheral nerve disorders. A small proportion of respondents (20.0%, n = 35) received more than ten medicines, categorised as major polypharmacy (Kim *et al*., 2014).

Table 2. Medical and medication history of respondents

Characteristics		Insulin n (%)[a]	Non-Insulin n (%)[a]	Total[b]	p-value (inter-group)[c]
Duration of diagnosed diabetes	< 12 months	27 (48.2)	44 (37.0)	71 (40.6)	0.315
	12 - 24 months	14 (25.0)	41 (34.5)	55 (31.4)	
	> 24 months	15 (26.8)	34 (28.6)	49 (28.0)	
Antidiabetic changes in the last six months	Yes	30 (53.6)	35 (29.4)	65 (37.1)	0.002
	Not	26 (46.4)	84 (70.6)	110 (62.9)	
Number of antidiabetics (insulin and/or antidiabetic oral)	Single drug	0 (0.0)	33 (27.7)	33 (18.9)	< 0.001
	Combination of 2 drugs	9 (16.1)	50 (42.0)	59 (33.7)	
	Combination of 3 drugs	30 (53.6)	35 (29.4)	65 (37.1)	
	Combination of 4 drugs	17 (30.4)	1 (0.8)	18 (10.3)	
Number of comorbidities	≤ 3	25 (44.6)	52 (43.7)	77 (44.0)	0.517
	>3	31 (55.4)	67 (56.3)	98 (56.0)	
Central nervous system disorders	Stroke	53 (94.6)	99 (83.2)	152 (86.9)	0.027
	Non-stroke	3 (5.4)	20 (16.8)	23 (13.1)	
Peripheral nerve disorders	Yes	20 (35.7)	36 (30.3)	56 (32.0)	0.290
	Not	36 (64.3)	83 (69.7)	119 (68.0)	
Total amount of medicine	≤10	37 (66.1)	103 (86.6)	140 (80.0)	0.002
	>10	19 (33.9)	169 (13.4)	35(20.0)	
Consumption of herbs in the last six months	Yes	17 (30.4)	29 (24.4)	46 (26.3)	0.254
	Not	39 (69.6)	90 (75.6)	129 (73.7)	
Allergy	Yes	3 (5.4)	5 (4.2)	8 (4.6)	0.500
	Not	53 (94.6)	114 (95.8)	167 (95.4)	
Recipe iteration	Yes	34 (60.7)	95 (79.8)	129 (73.7)	0.007
	Not	22 (39.3)	24 (20.2)	46 (26.3)	
Family/ Caregiver support	Yes	41 (73.2)	69 (58.0)	110 (62.9)	0.037
	Not	15 (26.8)	50 (42.0)	65 (37.1)	

Description: [a] Value is expressed in n(%), percentage in one category; [b] Values are expressed in n(%), percentage of all respondents; [c] Chi-square test.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Medication adherence

Adherence to Refills and Medications Scale (ARMS)

The Indonesian version of the ARMS questionnaire in this study has obtained permission from the owner (Kripalani *et al*., 2009). Validity and reliability tests were carried out on the first 30 respondents and obtained valid results; each question on the questionnaire showed r results (correlated item-total correlation) greater than the r table ($\alpha = 0.05$, df 28 (n-2)), and reliable, means having Cronbach's Alpha above 0.6, which is 0.829. The reliability test result of the ARMS questionnaire is close to Cronbach's value in the reliability test of the questionnaire translation conducted by previous researchers, which is 0.865 (Zairina *et al*., 2018). The ARMS score of the insulin group respondents was 12 - 24, while the respondents in the non-insulin group were 12 - 30. Based on the ARMS score, respondents were declared adherent if they had a score of 12 (Kripalani *et al*., 2009); as many as 43 respondents (24.6%) were adherent, consisting of 10 respondents (17.9%) in the insulin group and 33 respondents (27.7%) in the non-insulin group.

Medication Refill Adherence (MRA)

The percentage of MRA is 20.73-114.86%. Respondents are declared adherent if the MRA is 80-120% (Kindmalm *et al*., 2007). Based on the MRA percentage, there were 87 respondents (49.7%) who were adherent, namely 25 respondents (44.6%) in the insulin group and 62 respondents (52.1%) in the non-insulin group.

Glycosylated hemoglobin (HbA1c)

Adherence based on HbA1c was determined based on changes in HbA1c values in the last two examinations, taken from medical record data. Respondents were declared adherent if they showed a 0.2% decrease in HbA1c value because it reduced mortality by 10% (Sherwani *et al*., 2016). Based on the reduction in HbA1c, there were 63 respondents (36%) who were adherent, namely 26 respondents (46.4%) in the insulin group and 37 respondents (31.1%) in the non-insulin group.

The medication adherence results using each of these measuring instruments and the combination of the three measuring instruments are summarised in Table 3.

The effect of insulin administration and confounding variables was analysed using the backward method in multivariate logistic regression. Before multivariate analysis, bivariate analysis was performed for each confounding variable (Table 4).

Confounding variables that had a p-value less than 0.25 were included in the multivariate logistic regression analysis, which included body mass index, changes in antidiabetic in the last six months, number of comorbidities, diagnosis of peripheral nerve disorders, the total number of medicines consumed by respondents, and consumption of herbs. In addition, age and gender were also included in the multivariate analysis. The effect of insulin administration and the confounding variables were analysed by logistic regression analysis using the backward method. The logistic regression analysis showed that the variables that needed to be controlled in determining the effect of insulin administration on medication adherence were antidiabetic changes in the last six months and the total number of medications used by patients (Table 5).

Table 3. Medication adherence

Characteristics		Insulin n (%)[a]	Non-Insulin n(%)[a]	Total n (%)[b]	p-value (inter-group)[c]
ARMS	Adherent	10 (17.9)	33 (27.7)	43 (24.6)	0.109
	Non-adherent	46 (82.1)	86 (72.3)	132 (75.4)	
MRA	Adherent	25 (44.6)	62 (52.1)	87 (49.7)	0.224
	Non-adherent	31 (55.4)	57 (47.9)	88 (50.3)	
HbA1c	Adherent	26 (46.4)	37 (31.1)	63 (36.0)	0.036
	Non-adherent	30 (53.6)	82 (68.9)	112 (64.0)	
Overall	Adherent	1 (1.8)	12 (10.1)	13 (7.4)	0.042
	Non-adherent	55 (98.2)	107 (89.9)	162 (92.6)	

Information: [a] Value is expressed in n(%), a percentage in one category; [b] Values are expressed in n(%), percentage of all respondents; [c] Chi-square test; Overall, adherence is measured using the three measurement methods, the patient is declared adherent if the results of the three methods show adherence.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 4. Differences in respondent adherence based on characteristics, medical history, and medication history

Confounding Variables		Adherent n (%)[a]	Non-adherent n (%)[a]	Total n (%)[b]	p-value (inter-group)[c]
Age	35 - 44 years old	0 (0.0)	8 (100.0)	8 (4.6)	0.419
	45 - 54 years old	2 (6.1)	31 (93.9)	33 (18.9)	
	55 - 64 years old	5 (6.9)	67 (93.1)	72 (41.1)	
	65 - 74 years old	6 (12.8)	41 (87.2)	47 (26.9)	
	75+	0 (0.0)	15 (100.0)	15 (8.6)	
Gender	Man	8 (7.7)	96 (92.3)	104 (59.4)	0.559
	Woman	5 (7.0)	66 (93.0)	71 (40.6)	
Education	Did not finish elementary school/did not go to school	1 (33.3)	2 (66.7)	3 (1.7)	0.406
	Elementary	1 (7.7)	12 (92.3)	13 (7.4)	
	Junior High School	0 (0.0)	12 (100.0)	12 (6.9)	
	Senior High School	5 (8.3)	55 (91.7)	60 (34.3)	
	College	6 (6.9)	81 (93.1)	87 (49.7)	
Work	Retired/ Doesn't work	6 (8.1)	68 (91.9)	74 (42.3)	0.817
	PNS/TNI/ POLRI	1 (6.7)	14 (93.3)	15 (8.6)	
	Self-employed/ trader	1 (11.1)	8 (88.9)	9 (5.1)	
	Private employees	0 (0.0)	14 (100.0)	14 (8.0)	
	Housewives	5 (9.1)	50 (90.9)	55 (31.4)	
	Other	0 (0.0)	8 (100.0)	8 (4.6)	
Body mass index	Normal	5 (14.7)	29 (85.3)	34 (19.4)	**0.081** *
	Abnormal	8 (5.7)	133 (94.3)	141 (80.6)	
Duration of diagnosed diabetes	< 12 months	7 (9.9)	64 (90.1)	71 (40.6)	0.594
	12-24 months	3 (5.5)	52 (94.5)	55 (31.4)	
	> 24 months	3 (6.1)	46 (93.9)	49 (28.0)	
Antidiabetic changes in the last six months	Yes	8 (12.3)	57 (87.7)	65 (37.1)	**0.058***
	Not	5 (4.5)	105 (95.5)	110 (62.9)	
Number of antidiabetics	Single drug	4 (12.1)	29 (87.9)	33 (18.9)	0.415
	Combination of 2 drugs	2 (3.4)	57 (96.6)	59 (33.7)	
	Combination of 3 drugs	6 (9.2)	59 (90.8)	65 (37.1)	
	Combination of 4 drugs	1 (5.6)	17 (94.4)	18 (10.3)	
Number of comorbidities	≤3	4 (5.2)	73 (94.8)	77 (44.0)	**0.242** *
	>3	9 (9.2)	89 (90.8)	98 (56.0)	
Central nervous system disorders	stroke	11 (7.2)	141 (92.8)	152 (86.9)	0.532
	Non-stroke	2 (8.7)	21 (91.3)	23 (13.1)	
Peripheral nerve disorders	Yes	6 (10.7)	50 (89.3)	56 (32.0)	**0.201** *
	Not	7 (5.9)	112 (94.1)	119 (68.0)	
Total amount of medicine	≤10	12 (8.6)	128 (91.4)	140 (80.0)	**0.223***
	>10	1 (2.9)	34 (97.1)	35 (20.0)	
Recipe iteration	Yes	10 (7.8)	119 (92.2)	129 (73.7)	0.540
	Not	3 (6.5)	43 (93.5)	46 (26.3)	
Consumption of herbs	Yes	5 (10.9)	41 (89.1)	46 (26.3)	**0.233** *
	Not	8 (6.2)	121 (93.8)	129 (73.7)	
Family/ caregiver support	Yes	8 (7.3)	102 (92.7)	110 (62.9)	0.569
	Not	5 (7.7)	60 (92.3)	65 (37.1)	

Information: [a] Value is expressed in n(%), percentage in one category; [b] Values are expressed in n(%), percentage of all respondents. * p-value < 0.25, the variable was included in the multivariate logistic regression analysis.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 5. Effect of insulin administration and confounding variables on medication adherence

Model	Confounding Variables	Category	p-value	OR	95% Confidence Interval (Min-Max)
Crude	Antidiabetic	Insulin Non-Insulin	0.084	0.162	0.021 - 1.279
Multivariate	Antidiabetic	Insulin Non-Insulin	0.054	0.113	0.012 - 1.041
	Age	35 - 44 years old 45 - 54 years old 55 - 64 years old 65 - 74 years old 75+	0.628 0.999 0.999 0.999 1.000	 0.000 0.000 0.000 1.573	 0.000 0.000 0.000 0.000
	Gender	Man Woman	0.783	0.821	0.202 - 3.335
	Body mass index	Normal Abnormal	0.045	4.345	1.031 - 18.311
	Antidiabetic changes	Yes Not	0.020	5.431	1.305 - 22.595
	Number of comorbidities	≤ 3 > 3	0.201	0.374	0.083 - 1.690
	Peripheral nerve disorders	Yes Not	0.305	2.104	0.508 - 8.719
	Total amount of medicine	≤ 10 > 10	0.208	5.008	0.408 - 61.445
	Consumption of herbs	Yes Not	0.075	3.739	0.877 - 15.942
Adjusted	Antidiabetic	Insulin Non-Insulin	0.053	0.123	0.015 - 1.024
	Antidiabetic changes	Yes Not	0.020	4.171	1.254 - 13.878
	Total amount of medicine	≤ 10 > 10	0.487	2.131	0.253 - 17.960

This study shows that respondents' characteristics (age, gender, education, occupation, and body mass index) did not impact medication adherence statistically significantly. The results shown on the variables of age and sex are following a study conducted by Sham *et al*. in Pakistan which showed that age and gender were not significantly associated with patient adherence (Shams *et al*., 2016). Regarding education, medication adherence appears to improve as the respondent's education level increases. This is in line with the review of articles and meta-analyses by Al Shaikh *et al*. (2016) that education improves patient adherence.

Bivariate analysis showed that respondents with a stroke history showed lower adherence than patients without a stroke history. The proportion of stroke respondents adherent was 7.2%, while the non-stroke respondents were 8.7%. This is in accordance with a study conducted by Bauler *et al*. (2014) which stated that adherence to medication after a stroke was influenced by various barriers and facilitators. On the other hand, respondents with peripheral nerve disorders show a higher proportion of adherence. This contrasts with previous studies showing that peripheral neuropathy was the most patient-reported complication affecting adherence (Zhang *et al*., 2021). Patients with more than three comorbidities showed a higher proportion of adherence. This result contradicts the research conducted by Saadat *et al*., which states that the more comorbidities, the lower the patient's adherence (Saadat *et al*., 2015). This is due to increased patient comorbidities followed by increased visits to different specialists, so patients' adherence is better (Capoccia *et al*., 2016). However, the effect of these three variables on medication adherence was not statistically significant. This is in line with research on the impact of comorbidities on adherence to antihypertensive use (Saadat *et al.*, 2015).

Respondents who experienced antidiabetic changes showed higher adherence than those who did not experience antidiabetic changes (12.3% vs 4.5%). The antidiabetic change in the adherent respondents was the addition of antidiabetics, which improved the patient's HbA1c.

Based on the results of measuring adherence using MRA, ARMS, and HbA1c values changes, there were 13 respondents (7.4%) who were adherent, namely one

P-ISSN: 2406-9388
E-ISSN: 2580-8303

respondent (1.8%) in the insulin group and 12 respondents (10.1%) in the non-insulin group. Based on the results of multivariate analysis, insulin administration affected patient adherence in using the antidiabetics by 0.123 times (95% CI: 0.015 - 1.024) or patients who received insulin had 87.7% lower adherence than patients who did not receive insulin after being controlled by antidiabetic changes and amount of the total drug used by the patient at the time of data collection from the medical records.

Based on the three measuring instruments, only 13 out of 175 respondents were adherent. Therefore, further research is needed to analyses variables other than insulin administration that cause low medication adherence in diabetes mellitus patients with neurological complications.

The limitations of this study include the sample of only 175 respondents with patients using insulin only 56 respondents, the sampling location was only one hospital, and using the indirect adherence measurement method. In the MRA method, the patient is assumed to use the drug every day since the antidiabetic prescription was received until the next visit. Another limitation is that this study has not analysed other variables that may affect the HbA1c value.

CONCLUSION

The results showed that the adherence of diabetes mellitus patients was low, especially in patients who used insulin. The administration of insulin significantly affects patient adherence in diabetes mellitus patients with neurological complications, which is influenced by the confounding variable of antidiabetic changes and the total number of medicines used by the patients. The results of this study are expected to help hospital decision-makers and health care providers when initiating insulin administration and improve medication adherence in patients using insulin.

ACKNOWLEDGMENT

The authors would like to thank the nurses and pharmacists at the national brain center hospital who helped carry out this research.

AUTHOR CONTRIBUTIONS

Conceptualization, J., R. S., N. F. S.; Methodology, J., R. S., N. F. S.; Software, J.; Validation, J., R. S., N. F. S., H. T.; Formal Analysis, J., R. S., N. F. S.; Investigation, J.; Resources, J., H. T.; Data Curation, J., R. S., N. F. S., H. T.; Writing - Original Draft, J.; Writing - Review & Editing, J., R. S., N. F. S., H. T.; Visualization, J., R. S., N. F. S., H. T.; Supervision, J., R. S., N. F. S., H. T.; Project Administration, J., R. S., N. F. S., H. T.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Adikusuma, W. & Qiyaam, N. (2017). Hubungan Tingkat Kepatuhan Minum Obat Antidiabetik Oral Terhadap Kadar Hemoglobin Terglikasi (HbA1c) pada Pasien Diabetes Melitus Tipe 2. *Jurnal Ilmiah Ibnu Sina*; *2*; 279–286.

Al AlShaikh, S., Quinn, T., Dunn, W., Walters, M. & Dawson, J. (2016). Predictive Factors of Non-Adherence to Secondary Preventative Medication After Stroke or Transient Ischaemic Attack: a Systematic Review and Meta-Analyses. *European Stroke Journal*; *1*; 65–75. doi: 10.1177/2396987316647187.

Alfian, R. (2015). Korelasi Antara Kepatuhan Minum Obat dengan Kadar Gula Darah pada Pasien Diabetes Melitus Rawat Jalan dI RSUD DR. H. Moch. Ansari Saleh Banjarmasin. *Jurnal Pharmascience*; *2*; 15–23.

Barati, M., Taheri-Kharameh, Z., Bandehelahi, K., Yeh, V. M. & Kripalani, S. (2018). Validation of the Short form of the Adherence to Refills and Medications Scale in Iranian Elders with Chronic Disease. *Journal of Clinical and Diagnostic Research*; *12*; 10–13. doi: 10.7860/JCDR/2018/37584.12305.

Bauler, S., Jacquin-Courtois, S., Haesebaert, J., Luaute, J., Coudeyre, E., Feutrier, C., Allenet, B., Decullier, E., Rode, G. & Janoly-Dumenil, A. (2014). Barriers and Facilitators for Medication Adherence in Stroke Patients: A Qualitative Study Conducted in french Neurological Rehabilitation Units. *European Neurology*; *72*; 262–270. doi: 10.1159/000362718.

Bloomgarden, Z. & Chilton, R. (2021). Diabetes and Stroke: an Important Complication. *Journal of Diabetes*; *13*; 184–190. doi: 10.1111/1753-0407.13142.

Bulu, A., Wahyuni, T. D. & Sutriningsih, A. (2019). Hubungan antara Tingkat Kepatuhan Minum Obat dengan Kadar Gula Darah pada Pasien Diabetes Melitus Tipe II. *Nursing News*; *4*; 181–189.

Capoccia, K., Odegard, P. S. & Letassy, N. (2016). Medication Adherence with Diabetes Medication: a Systematic Review of the Literature. *The*

Diabetes Educator; *42*; 34–71. doi: 10.1177/0145721715619038.

Chen, Y. J., Chang, J. & Yang, S. Y. (2020). Psychometric Evaluation of Chinese Version of Adherence to Refills and Medications Scale (ARMS) and Blood-Pressure Control Among Elderly with Hypertension. *Patient Preference and Adherence*; *14*; 213–220. doi: 10.2147/PPA.S236268.

Complication Definition & Meaning. (2022). Merriam-Webster. (n.d.). https://www.merriam-webster.com/dictionary/complication#learn-more. Accessed: 15 July 2022.

Doggrell, S. A. & Warot, S. (2014). The Association Between the Measurement of Adherence to Antidiabetes Medicine and the HbA1c. *International Journal of Clinical Pharmacy*; *36*; 488–497. doi: 10.1007/s11096-014-9929-6.

González-Bueno, J., Calvo-Cidoncha, E., Sevilla-Sánchez, D., Espaulella-Panicot, J., Codina-Jané, C. & Santos-Ramos, B. (2017). Spanish Translation and Cross-Cultural Adaptation of the ARMS-Scale for Measuring Medication Adherence in Polypathological Patients. *Atencion Primaria*; *49*; 459–464. doi: 10.1016/j.aprim.2016.11.008.

International Diabetes Federation. (2019). IDF Diabetes Atlas (S. Karuranga, B. Malanda, P. Saeedi, & P. Salpea (Eds.); 9th ed.). Brussels: International Diabetes Federation.

Ireland, S., Endacott, R., Cameron, P., Fitzgerald, M. & Paul, E. (2010). Association of Glycaemia with Macrovascular and Microvascular Complications of Type 2 Diabetes: Prospective Observational Study. *Resuscitation*; *35*; 18–23. doi: 10.1016/j.resuscitation.2010.10.016.

Jin, H. K., Kim, Y. H. & Rhie, S. J. (2016). Factors Affecting Medication Adherence in Elderly People. *Patient Preference and Adherence*; *10*; 2117–2125. https://doi.org/10.2147/PPA.S118121

Kim, C.-J., Park, E., Schlenk, E. A., Kim, M. & Kim, D. J. (2016). Psychometric Evaluation of a Korean Version of the Adherence to Refills and Medications Scale (ARMS) in Adults With Type 2 Diabetes. *Diabetes Educator*; *42*; 188–198. doi: 10.1177/0145721716632062.

Kim, H. -A., Shin, J. -Y., Kim, M. -H. & Park, B. -J. (2014). Prevalence and Predictors of Polypharmacy among Korean elderly. *PLoS ONE*; *9*; 1-7. doi: 10.1371/journal.pone.0098043.

Kindmalm, L., Melander, A. & Nilsson, L. (2007). Refill Adherence of Antihyperglycaemic Drugs Related to Glucose Control (HbA 1c) in Patients with Type 2 Diabetes. *Acta Diabetologica*; *44*; 209–213. doi: 10.1007/s00592-007-0006-7.

Kripalani, S., Risser, J., Gatti, M. E. & Jacobson, T. A. (2009). Development and Evaluation of the Adherence to Refills and Medications Scale (ARMS) Among Low-Literacy Patients with Chronic Disease. *Value in Health*; *12*; 118–123. doi: 10.1111/j.1524-4733.2008.00400.x.

Lachenbruch, P. A., Lwanga, S. K. & Lemeshow, S. (1991). Sample Size Determination in Health Studies: A Practical Manual. *Journal of the American Statistical Association*; 86; 1149. doi: 10.2307/2290547.

Lomper, K., Chabowski, M., Chudiak, A., Białoszewski, A., Dudek, K. & Jankowska-Polańska, B. (2018). Psychometric Evaluation of the Polish Version of the Adherence to Refills and Medications Scale (ARMS) in Adults with Hypertension. *Patient Preference and Adherence*; *12*; 2661–2670. doi: 10.2147/PPA.S185305.

Nanda, O. D., Wiryanto, B. & Triyono, E. A. (2018). Hubungan Kepatuhan Minum Obat Antidiabetik dengan Regulasi Kadar Gula Darah pada Pasien Perempuan Diabetes Mellitus. *Amerta Nutrition*; *2*; 340-348. doi: 10.20473/amnt.v2i4.2018.340-348.

Osborn, C. Y. & Gonzalez, J. S. (2016). Measuring Insulin Adherence Among Adults with Type 2 Diabetes. *Journal of Behavioral Medicine*; *39*; 633–641. doi: 10.1007/s10865-016-9741-y.

Park, H. Y., Seo, S. A., Yoo, H. & Lee, K. (2018). Medication Adherence and Beliefs About Medication in Elderly Patients Living Alone with Chronic Diseases. *Patient Preference and Adherence*; *12*; 175–181. doi: 10.2147/PPA.S151263.

Perkeni. (2021). Pedoman Pengelolaan dan Pencegahan Diabetes Melitus Tipe 2 di Indonesia. Jakarta: Perkeni.

Saadat, Z., Nikdoust, F., Aerab-Sheibani, H., Bahremand, M., Shobeiri, E., Saadat, H., Moharramzad, Y. & Morisky, D. E. (2015). Adherence to Antihypertensives in Patients with Comorbid Condition. *Nephro-Urology Monthly*; *7*; 1-6. doi: 10.5812/numonthly.29863.

Salistyaningsih, W., Puspitawati, T. & Nugroho, D. (2011). Hubungan Tingkat Kepatuhan Minum Obat Hipoglikemik Oral dengan Kadar Glukosa

Darah Pada Pasien DM Tipe 2. *Universitas Respati Yogyakarta*; *27*; 215–221.

Sarwar, N., Gao, P., Kondapally Seshasai, S. R., Gobin, R., Kaptoge, S., Di Angelantonio, E., Ingelsson, E., Lawlor, D. A., Selvin, E., Stampfer, M., Stehouwer, C. D. A., Lewington, S., Pennells, L., Thompson, A., Sattar, N., White, I. R., Ray, K. K., Danesh, J., Tipping, R. W. & Wormser, D. (2010). Diabetes Mellitus, Fasting Blood Glucose Concentration, and Risk of Vascular Disease: A Collaborative Meta-Analysis of 102 Prospective Studies. *The Lancet*; *375*; 2215–2222. doi: 10.1016/S0140-6736(10)60484-9.

Shams, N., Amjad, S., Kumar, N., Ahmed, W. & Saleem, F. (2016). Drug Non-Adherence In Type 2 Diabetes Mellitus; Predictors and Associations. *Journal of Ayub Medical College, Abbottabad*; *28*; 302–307.

Sherwani, S. I., Khan, H. A., Ekhzaimy, A., Masood, A. & Sakharkar, M. K. (2016). Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. *Biomarker Insights*; *11*; 95–104. doi: 10.4137/Bmi.s38440.

Shou, J., Zhou, L., Zhu, S. & Zhang, X. (2015). Diabetes is an Independent Risk Factor for Stroke Recurrence in Stroke Patients: A Meta-analysis. *Journal of Stroke and Cerebrovascular Diseases*; *24*; 1961–1968. doi: 10.1016/j.jstrokecerebrovasdis.2015.04.004.

WHO. (2020). The Top 10 Causes of Death. Geneva: WHO.

Zairina, E., Khotib, J., Ardianto, C. & Sulaiman, S. A. (2018). Unity in Diversity and the Standardisation of Clinical Pharmacy Services. Florida: CRC Press.

Zairina, E., Nugraheni, G., Sulistyarini, A., Mufarrihah, Setiawan, C. D., Kripalani, S. & Lestari, S. I. (2022). Factors Related to Barriers and Medication Adherence in Patients with Type 2 Diabetes Mellitus: A Cross-Sectional Study. *Journal of Diabetes and Metabolic Disorders*; *21*; 219–228. doi: 10.1007/s40200-021-00961-6.

Zhang, Z. P., Premikha, M., Luo, M. & Venkataraman, K. (2021). Diabetes Distress and Peripheral Neuropathy are Associated with Medication Non-Adherence in Individuals with Type 2 Diabetes in Primary Care. *Acta Diabetologica*; *58*; 309–317. doi: 10.1007/s00592-020-01609-2.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 252-261
DOI: 10.20473/jfiki.v9i32022.252-261
Available online at https://e-journal.unair.ac.id/JFIKI/

The Influence of Feeling Lonely and Received Social Support on Medication Adherence in Elderly with Hypertension

Dwi Novita Sari[1], Wahyu Utami[2], Elida Zairina[2]*
[1]Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: elida-z@ff.unair.ac.id

Submitted: 11 July 2022
Accepted: 28 October 2022
Published: 9 December 2022

Abstract

Background*: Hypertension is currently a non-contagious disease that primarily affects the elderly population in Indonesia. Medication adherence is critical in managing hypertension and reducing the risk of morbidity and mortality. Previous research has found that loneliness and support received by older adults with hypertension influence medication adherence.* ***Objective****: This study aimed to examine the impact of feeling lonely and receiving social support on medication adherence in the elderly with hypertension at the Community Health Center in Surabaya.* ***Methods****: The study design of this research was a descriptive cross-sectional study from December 2021 to March 2022. A total of 235 eligible subjects fulfilled the inclusion criteria. The instruments used in the data collection were the patient's information form, UCLA-Loneliness Scale, MOS-Social Support Survey, and the ARMS (Adherence to Refill and Medication Scale).* ***Results****: The results revealed that the correlation between loneliness and social support was significantly associated with medication adherence in the elderly with hypertension ($p < 0.05$). In addition, other factors, such as occupation status, living status, comorbidity, the number of drugs taken, and antihypertensive drug therapy, showed a significant correlation with medication adherence ($p < 0,05$). The most influential factor on medication adherence was loneliness (35.5%), followed by social support (24.4%), the number of drugs taken (7.1%), antihypertensive drug therapy (monotherapy or combination therapy (2.5%), occupation status (2.4%), comorbidity (1.6%), and living status (0.2%).* ***Conclusion****: This study confirms that feeling lonely and receiving social support affect medication adherence in the elderly with hypertension at the Community Health Center in Surabaya.*

Keywords*: adherence, elderly, hypertension, loneliness, social support*

How to cite this article:
Sari, D. N., Utami, W. & Zairina, E. (2022). The Influence of Feeling Lonely and Received Social Support on Medication Adherence in Elderly with Hypertension. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 9(3), 252-261. http://doi.org/10.20473/jfiki.v9i32022.252-261

P-ISSN: 2406-9388
E-ISSN: 2580-8303

INTRODUCTION

The ageing process induces various kinds of decline in body functions, such as physical, psychological, and social conditions (Suadirman, 2011). The decreased physical condition due to the degenerative process of ageing causes non-contagious diseases like hypertension, heart disease, Diabetes Mellitus, dental problem, etc. (Kementerian Kesehatan RI, 2019). In Indonesia, the most common degenerative disease suffered by the elderly population is hypertension (Kementerian Kesehatan RI, 2019).

Long-term drug therapy is needed to treat chronic diseases such as hypertension, even for the rest of life (Dzau & Balatbat, 2019). Hypertension can be prevented and treated with treatment adherence, whether by taking medication therapy, following a diet, or having a good lifestyle (World Health Organization, 2003). Adherence to antihypertensive medication therapy should be done regularly and continuously with the result that the 'patient's blood pressure can be well controlled (World Health Organization, 2019). The therapeutic goal of medication adherence is to prevent and reduce the likelihood of morbidity and mortality in elderly patients (Corrao *et al*., 2017).

Several factors influence adherence to taking medication in the elderly with hypertension, one of which is demographic factors such as age, gender, level of education, economic status, marital status, living status, and occupation status (Gast & Mathes, 2019; Hazwan & Pinatih, 2017; Liberty *et al*., 2018; Sinuraya *et al*., 2018; Uchmanowicz *et al*., 2018). Psychosocial factors influencing medication adherence in the elderly include loneliness and social support *(Hacihasanoglu et al*., 2020; Lu *et al*., 2020). A study conducted on 1,233 elderly in Indonesia aged ≥ 60 years showed that one in five elderly lives alone, and half experience loneliness (Widhowati *et al*., 2020).

Loneliness can be caused by the lack of interpersonal relationships due to disconnection or social contact with family, friends, or partners. Those can lead a person to be unable to feel social support (Donovan & Blazer, 2020). Sufficient support has a crucial role in the treatment of hypertension, especially support from family, healthcare providers, friends, and others such as peer groups (Shahin *et al*., 2021). Elderly hypertensive patients who live with their families have better adherence than those who e live alone or in a nursing home (Uchmanowicz *et al*., 2018).

Previous research has helped us understand how loneliness and social support affect medication adherence. However, research on the impact of loneliness and social support on medication adherence in the elderly with hypertension is still limited. This study aims to see if feeling lonely and receiving social support affects medication adherence.

MATERIALS AND METHODS

Materials

This study was acquitted on the elderly with hypertension at the Community Health Centers in Surabaya. The Community Health Centers are Puskesmas Gading, Puskesmas Rangkah, and Puskesmas Mojo. The criteria for inclusion must be met: elderly aged 60 – 79 years diagnosed with hypertension and have started antihypertensive medication therapy for at least three months or more, can communicate well, can read and complete self-administered questionnaires. The exclusion criteria were: elderly hypertensive patients who also had dementia, schizophrenia, tuberculosis, tested positive for covid-19, or had physical disorders such as eye, hand movement, and hearing loss.

Tools

Patient information form, University of California Los Angeles (UCLA)-Loneliness Scale, and Medical Outcome Study-Social Support Survey (MOS-SSS) were used to collect data. Patient information forms include demographic and clinical data. The UCLA-Loneliness Scale (Version 3) was used to measure 'one's subjective feelings of loneliness (Russell, 1996). The Medical Outcome Study-Social Support Survey (MOS-SSS) determined the availability of support which was created to assess perceived social support for patients with chronic conditions (Sherbourne & Stewart, 1991). The ARMS (Adherence to Refill and Medication Scale) measured a patient's adherence to antihypertensive medication therapy (Kripalani *et al*., 2009). The ARMS was translated and validated into Indonesian by a previous study (Mubasir *et al*., 2017). The authors have obtained permission from the creators of the instruments in this study. In this study, the original English version of the UCLA-Loneliness Scale and MOS-Social Support Survey was translated and adapted into Indonesian by native Indonesian speakers who were fluent in English, using forward and backward translations according to Basic Guidelines for Translating Survey Research and Development (RAND) (RAND, 2021). The procedure is as follows: (1) forward translation, the English version of the UCLA-Loneliness Scale and MOS-Social Support Survey were translated into Indonesian by a professional translator from the Pusat Bahasa (Language Center) of

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Universitas Airlangga, (2) review, identifying the differences in items, terms, and concepts and the completion by the expert panel, (3) back-translation, the reviewed Indonesian version of the UCLA-Loneliness Scale and MOS-Social Support Survey were translated back into original English by another professional translator from the Pusat Bahasa (Language Center) of Universitas Airlangga, (4) accomplish discrepancies or problems in the translation by a committee that includes the forward translator, backward translator, and reviewer. The Indonesian version of the UCLA-Loneliness Scale and MOS-Social Support Survey were pre-tested on 70 elderly with hypertension with the same inclusion criteria in this study. Data were analyzed, and the results showed that the Corrected Item-Total Correlation for all items were greater than the r value (r UCLA-Loneliness Scale = 0.2992; r MOS-Social Support Survey = 0.3007) and the ' 'Cronbach's alpha was above 0.7 (UCLA-Loneliness Scale = 0.882; MOS-Social Support Survey = 0.937), which indicates high internal consistency. The Indonesian version of the UCLA-Loneliness Scale and MOS-Social Support Survey is valid and reliable for measuring loneliness and support in elderly patients with hypertension.

Method

Study design

This study used a descriptive cross-sectional study from December 2021 and March 2022. The research began with the recruitment of respondents. First, the researcher explained the objectives and benefits of the study to each respondent; then, the respondent was asked to sign an informed consent if they were willing to participate in the study. Second, the respondent filled out the self-administered questionnaire.

Researchers met 368 elderly patients with hypertension during the research. A total of 133 patients were excluded because of not willing to participate (n = 21), being illiterate (n = 21), did not bring glasses, so they did not read (n = 19), having physical impairment (n = 44), resigned at the time of filling out the self-administered questionnaire (n = 3), and tested for positive Covid-19 (n = 9). About 235 elderly with hypertension fulfilled the inclusion criteria and completed the self-administered questionnaire. The respondents were recruited from 3 Community Health Centers in Surabaya (Puskesmas Gading, Puskesmas Rangkah, and Puskesmas Mojo). Community health centers were chosen because they are included in the five largest health centres with the highest estimated number of hypertension patients in Surabaya (Dinas Kesehatan Kota Surabaya, 2019).

Sample size

The Lemeshow formula was used to calculate sample size at a confidence level of 95%, an error tolerance of 5%, and the estimated population proportion as the most significant proportion of data (50%). Based on the formula, it was obtained that the sample size was 235.

The ARMS (Adherence to Refills and Medication Scale)

The ARMS is a valid and reliable medication adherence scale when used in 'patients with chronic disease, with good performance characteristics, and even in patients with low literacy skills (Kripalani *et al*., 2009). This questionnaire consists of 12 question items, 8 are sub-scales for medication adherence and 4 are sub-scales for drug refill adherence. Each item was rated using a Likert scale, ranging from 1 (none of the time) to 4 (all of the time). Especially for the last question, the scale is reversed from 1 (for all of the time) to 4 (none of the time) (Kripalani *et al*., 2009). The overall adherence score may range from 12 to 48, with lower scores indicating better adherence (Kripalani *et al*., 2009).

The UCLA-loneliness scale (version 3)

The UCLA-Loneliness Scale (Version 3) is the latest version of the UCLA-Loneliness Scale developed by Russell (1996). This is a 20-item scale that has attempted to simplify the response format and the wording of the item. The scale has been used in studies of various populations, including the elderly (Russell, 1996). Each item has been rated using the Likert scale from 1 (never) to 4 (always), 11 negative words (lonely) encoded directly and nine positively (non-lonely)inverted. Higher scores indicate a higher degree of loneliness (Russell, 1996).

The MOS-social support survey

The MOS-Social Support Survey is a 19-item multidimensional instrument for patients with chronic conditions in the Medical Outcomes Study developed by Sherbourne & Stewart (1991). This questionnaire measures the availability of support in various dimensions of social support, not only the sources of social support but also overall perceived support, including emotional/informational support, tangible support, affectionate support, and positive social interaction (Sherbourne & Stewart, 1991). Each item was rated using a Likert scale from 1 (none of the time) to 5 (all of the time). A higher score indicates more support than perceived by ' 'participants (Sherbourne & Stewart, 1991).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Statistical analysis

The data were presented in the Statistical Package for the Social Sciences (SPSS) version 24.0. Statistical analyses used descriptive analysis to have the distribution of frequency of demographic and clinical characteristics. The normality of the distribution of loneliness, social support, and adherence variables was examined using the Kolmogorov-Smirnov test. The test results showed that the data were not normally distributed, so the differences in the proportion of adherence between two groups based on demographic and clinical factors examine by using the Mann-Whitney test and between three groups or more using Kruskal-Wallis. The 'Spearman's rank correlation coefficient was used to acquire the correlation between loneliness, social support, and adherence. Linear regression was used as a multivariate analysis to determine how much influence each variable has on adherence. The results are statistically significant with P-value (< 0,05).

Ethics consideration

The Human Research Ethics Committee Faculty of Nursing Universitas Airlangga has approved this study (No.: 2392-KEPK).

RESULTS AND DISCUSSION

The comparison between demographic and clinical characteristics to adherence scores

The detailed demographic and clinical characteristics of the patients are shown in Table 1. In this study, most participants were aged 60 - 79 years (74%) with the mean (SD) was 65.8 ± 8.3, female (61%), married (64%), living with family (81%), primary school graduate (36%), not working (77%), the income per month less than Rp 1,500,000.00 (88%), the duration of hypertension 1 to 5 years (51%) with a mean (SD) was (59.3 ± 67.3) (months), the number of drugs taken is one pill a day (38%), not have comorbidity (39%), and having monotherapy antihypertensive drug (84%). The results in Table 2 showed that age, gender, marital status, level of education, and the duration of hypertension showed no significant difference in adherence to antihypertensive ($p > 0.05$). Table 2 presents the mean rank of adherence level for each group. The group with a lower mean rank score indicates better adherence. However, occupation status, living status, comorbidity, antihypertensive medication, and the number of drugs taken significantly differed with adherence to hypertension medication ($p < 0.05$).

Although there are still differences between previous studies, which indicated that demographic and duration of hypertension are significantly associated with medication adherence (Agung *et al*., 2021; Gast & Mathes, 2019; Liberty *et al*., 2018; Sinuraya *et al*., 2018; Wan *et al*., 2022), there are other having similar findings to our study. The studies in Thailand, China and Korea exhibit that age, gender, marital status, education level, occupation status, the number of drugs taken, and the duration of hypertension were not statistically significant to medication adherence in the elderly with hypertension (Cho *et al*., 2018; Wan *et al*., 2022; Woodham *et al*., 2018). A meta-analysis study in the Asian region revealed that gender and education level were not associated with medication adherence in patients with hypertension (Akbar *et al*., 2021).

In this study, the first factor related to medication adherence in elderly hypertensive patients was occupation and living status. Previous research conducted in China and Indonesia (Bandung, Magelang, and Semarang) showed that occupation status had a statistically significant effect on medication adherence in adult to elderly hypertensive patients (Agung *et al*., 2021; Nurhanani *et al*., 2020; Pan *et al*., 2019; Sinuraya *et al*., 2018). Patients who are not working or retired tend to adhere less to the treatment of hypertension *(Pan et al*., 2021; Woodham *et al*., 2018). Living status in this study included living alone, with a spouse, or with family. Another study in China showed that elderly hypertensive patients with hypertension who lived with spouses and offspring had a much higher level of medication adherence than those who lived alone (Wan *et al*., 2022). Elderly hypertensive patients who live with others and have social interactions are more motivated to adhere to antihypertensive medication therapy *(Lu et al*., 2020).

Several studies revealed a significant association between the number of drugs taken and medication adherence in elderly hypertensive patients (Shareinia et al., 2020), and patients with more complicated prescriptions had better medication adherence (Thuy et al., 2020). In this study, clinical factors related to medication adherence were comorbidities, antihypertensive medication therapy, and the total number of daily drugs taken. Similar results were obtained from the studies in Romania and Korea on elderly hypertensive patients. Comorbidities such as diabetes mellitus, heart disease, kidney disease, dyslipidemia, cancer, and stroke had high medication adherence (Cho *et al*., 2018; Tilea et al., 2018). A study in Wuhu, China, conducted antihypertensive medication therapy (single or combination), and the total number of drugs taken did not affect antihypertensive medication adherence (Wan *et al*., 2022). In this study, however, the

P-ISSN: 2406-9388
E-ISSN: 2580-8303

opposite results were obtained; elderly hypertensive patients who received antihypertensive monotherapy demonstrated better adherence (Uchmanowicz *et al*., 2018). Another study found that the number of antihypertensive drugs taken was related to medication adherence; when the number of medicines taken increased, a person had better medication adherence (Pan *et al*., 2021).

Table 1. Demographic and clinical characteristics (N=235)

Variable	Category	N (%)
Age (years), mean ± SD (65.8 ± 8.3)	60 - 69	173 (74%)
	70 - 79	62 (26%)
Gender	Female	144 (61%)
	Male	91 (39%)
Marital status	Not married	2 (1%)
	Married	150 (64%)
	Divorced and not remarried	8 (3%)
	Widowed and not remarried	75 (32%)
Living status	Living alone	13 (5%)
	With spouse	32 (14%)
	With family	190 (81%)
Education	None	54 (23%)
	Primary school	84 (36%)
	Secondary school	40 (17%)
	High school	47 (20%)
	Vocational	2 (1%)
	Bachelor	8 (3%)
Occupation status	Working	54 (23%)
	Not working	181 (77%)
Income per month	< Rp 1,500,000.00	207 (88%)
	Rp 1,500,000,00 – Rp 2,500,000.00	16 (7%)
	Rp 2,500,000,00 - Rp 5,000,000.00	11 (5%)
	> Rp 5,000,000.00	1 (0%)
Duration of hypertension (in years), mean ± SD (59.3 ± 67.3) (in months)	< 1	61 (26%)
	1 – 5	120 (51%)
	6 – 10	31 (13%)
	11 – 15	6 (3%)
	16 – 20	13 (6%)
	> 20	4 (2%)
The number of drugs taken	1	89 (38%)
	2	40 (17%)
	3	68 (29%)
	4	28 (12%)
	5	8 (3%)
	> 5	2 (1%)
Comorbidities	None	92 (39%)
	Diabetes Mellitus	84 (36%)
	Heart disease	10 (4%)
	Hypercholesterolemia	6 (3%)
	Gastritis	4 (2%)
	Vertigo	4 (2%)
	Others	35 (15%)
Antihypertensive drug therapy	Monotherapy	197 (84%)
	Combination of 2 drugs	37 (16%)
	Combination of 3 drugs	1 (0%)

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 2. Medication adherence based on demographic and clinical characteristics

Variable	Category	Medication Adherence	
		Mean rank	**P-value**
Age (years)[a]	60 - 69	119.34	0.610
	70 - 79	114.27	
Gender[a]	Female	116.39	0.643
	Male	120.55	
Marital status	Not married	127.00	0.523
	Married	113.31	
	Divorced and not remarried	116.69	
	Widowed and not remarried	127.46	
Living status	Living alone	164.19	0.007*
	With spouse	94.66	
	With family	118.77	
Level of Education	None	121.07	0.461
	Primary school	109.58	
	Secondary school	111.56	
	High school	132.97	
	Vocational	151.75	
	Bachelor	120.06	
Occupation status	Working	137.67	0.014*
	Not working	112.13	
Income per month	< Rp 1,500,000.00	114.20	0.087
	Rp 1,500,000,00 – Rp 2,500,000.00	136.97	
	Rp 2,500,000,00 – Rp 5,000,000.00	161.55	
	> Rp5,000,000.00	122.50	
Duration of hypertension (years)[b]	< 1	128.76	0.739
	1 – 5	112.67	
	6 – 10	121.58	
	11 – 15	115.83	
	16 – 20	115.62	
	> 20	99.38	
The number of drugs taken	1	132.28	0.001*
	2	136.38	
	3	106.16	
	4	96.32	
	5	65.81	
	> 5	30.00	
Comorbidities	None	132.87	0.000*
	Diabetes Mellitus	113.36	
	Heart disease	57.18	
	Hypercholesterolemia	128.20	
	Gastritis	186.25	
	Vertigo	148.30	
	Others	99.03	
Antihypertensive drug therapy	Monotherapy	123.44	0.015*
	Combination of 2 drugs	93.04	
	Combination of 3 drugs	30.00	

*statistically significant (P < 0.05)

[a] using the Mann-Whitney test

[b] using the Kruskal-Wallis test

[c] the group with a lower mean rank score indicates better adherence

P-ISSN: 2406-9388
E-ISSN: 2580-8303

The relationship between loneliness and social support with adherence

The total score of the UCLA-Loneliness Scale questionnaire indicates that the lower the score obtained, the lower the perceived loneliness, and the lower ARMS-adherence score indicates that the adherence is lower, so the two scores from the two questionnaires are in line (positive correlation coefficient). There was a positive correlation coefficient value (0.618) in Table 3, which means that the two variables have a positive direction. There was a significant association between loneliness and medication adherence in this study. In our study, medication adherence is improved when loneliness is less of a concern. In addition, people who experience loneliness, especially the elderly, tend to be more at risk of hypertension, cardiovascular disease, and respiratory disease (Golaszewski *et al*., 2022).

Table 3. The connection between loneliness and social support with medication adherence

Variable	Medication Adherence	
	Correlation Coefficient	Sig.
Loneliness	0.618**	0.000
Social support	-0.558**	0.000

**statistically significant (< 0,01)

The higher score of the MOS-Social Support Survey indicates the higher support received, while the lower ARMS-adherence score indicates that the adherence is getting better, so the two scores are opposite or inverted (negative correlation coefficient). Social support has a negative correlation coefficient value (-0.558) in Table 3., it means that the more support the elderly felt, the better medication adherence. This study's findings are similar to the current study that investigated the association between loneliness and medication adherence; loneliness is statistically significantly related to medication adherence; the lower perceived loneliness, the better medication adherence (Hacihasanoglu *et al*., 2020; Jankowska-Polańska *et al*., 2020; Lu *et al*., 2020).

Social support can be defined as support from anyone, including family, friends, neighbours, and health care providers, containing emotional or informational support, tangible support, affectionate support, and positive social interaction (Sherbourne & Stewart, 1991). The results of this study revealed that social support is significantly related to medication adherence. A systematic review study explained that social support was significantly associated with medication adherence in hypertensive patients (Shahin *et al*., 2021). The existence of adequate social support from the social environment around the patient can improve medication adherence (Shahin *et al*., 2021). Low social support can lead to suboptimal medication adherence in elderly hypertensive patients with or without comorbidities (Lu *et al*., 2020). Another study on adult and elderly hypertensive patients showed that the more significant support received, the better adherence to medication (Gast & Mathes, 2019; Pan *et al*., 2021; Turan *et al*., 2019). Practical support such as medical assistance (reminders to take medication, direct instructions for drug use, and prescription) and financial support lead to better medication adherence (Adisa *et al*., 2017; Ashoorkhani *et al*., 2018; Jung & Lee, 2017; Yazdanpanah *et al*., 2019). A study that provides social support through SMS intervention (medication reminders, diet, and control schedules) can improve medication adherence and control blood pressure in patients with hypertension (Nursalam et al., 2020).

The impact of demographic, clinical, loneliness, and social support on medication adherence

This study evaluated the factors influencing medication adherence in the elderly with hypertension. The results of the linear regression test revealed the R square value on medication adherence in Table 4. The most influential factor on medication adherence based on R square values is loneliness (35.5%), followed by social support (24.4%), the number of drugs taken (7.1%), antihypertensive drug therapy (2.5%), occupation status (2.4%), comorbidity (1.6%), and living status (0.2%).

This study is inseparable from limitations; there are three limitations. First, this study only reached patients who could communicate well and read, so it did not reach patients who could not speak well and were illiterate. Second, this study excluded patients who could read but had limited physical impairment such as hearing loss, body movement disorders (history of stroke in hand; unable to hold a pen), and vision impairment (not carrying glasses, low vision, blurred eyes, cataract, glaucoma, etc.) so that patient cannot read the letters. Third, this research was conducted in the urban area; it did not reach rural or remote areas. In the future, further research is needed to find a design that can be applied to all conditions of elderly hypertensive patients.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 4. The connection between Loneliness and Social Support with medication adherence

Variable	Medication adherence		
	R	**R square**	**P value**
Loneliness	0.596	0355	0.000*
Social support	0.494	0.244	0.000*
Living status	0.040	0.002	0.545*
Occupation status	0.155	0.024	0.018*
Comorbidity	0.126	0.016	0.053*
Antihypertensive drug therapy	0.157	0.025	0.000*
The number of drugs taken	0.266	0.071	0.000*

*statistically significant (< 0.05)

CONCLUSION

Adherence to medication therapy may significantly impact treatment goals in the elderly with hypertension. Feelings of loneliness and lack of received social support may decrease medication adherence in the elderly with hypertension. In the future, strategies such as appropriate treatment or intervention are needed to overcome loneliness and increase social support to increase medication adherence among elderly patients.

ACKNOWLEDGMENT

The authors would like to thank Prof. Daniel W. Russel, Dr. Cathy D. Sherbourne, Dr. Anita L. Stewart, who have permitted them to use the UCLA-*Loneliness Scale* dan MOS-*Social Support Survey*. In addition, the authors would like to express gratitude to the Community Health Centers staff who helped during the research process.

AUTHOR CONTRIBUTIONS

Conceptualization, D. N. S., W. U., E. Z.; Methodology, D. N. S., W. U., E. Z.; Software, D. N. S.; Validation, W. U., E. Z.; Formal Analysis, D. N. S., E. Z.; Investigation, D. N. S.; Resources, D. N. S., E. Z.; Data Curation, D. N. S., E. Z.; Writing - Original Draft, D. N. S., W. U., E. Z.; Writing - Review & Editing, D. N. S., W. U., E. Z.; Visualization, D. N. S., E. Z.; Supervision, E. Z.; Project Administration, D. N. S., W. U., E. Z.; Funding acquisition, E. Z.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Adisa, R., Olamide, O. & Fakeye, T. (2017). Social Support, Treatment Adherence and Outcome among Hypertensive and Type 2 Diabetes Patients in Ambulatory Care Settings in Southwestern Nigeria. *Ghana Medical Journal*; *51*; 64–77.

Agung, W. T., Nugraheni, A., Wahyudi, F. & Ardhianto, P. (2021). Factors Associated with Medication Adherence in Hypertension in The Ngluwar Public Health Center in Magelang Regency. *Diponegoro Medical Journal*; *10*; 426-432.

Akbar, I., Isa, M., Arifin, S., Barlian & Husaini. (2021). Meta-Analysis: Relationship of Age, Gender, and Education Level with Medication Adherence of Hypertension Patients. *The International Journal of Travel Medicine and Global Health*; *7*; 20–24. doi: 10.20469/ijhms.7.3004.

Ashoorkhani, M., Majdzadeh, R., Gholami, J., Eftekhar, H. & Bozorgi, A. (2018). Understanding Non-Adherence to Treatment in Hypertension: A Qualitative Study. *International Journal of Community Based Nursing and Midwifery*; *6*; 314–323.

Cho, M. H., Shin, D. W., Chang, S. -A., Lee, J. E., Jeong, S. -M., Kim, S. H., Yun, J. M. & Son, K. (2018). Association between Cognitive Impairment and Poor Antihypertensive Medication Adherence in Elderly Hypertensive Patients Without Dementia. *Scientific Reports*; *8*; 1-7. doi: 10.1038/s41598-018-29974-7.

Corrao, G., Rea, F., Monzio Compagnoni, M., Merlino, L. & Mancia, G. (2017). Protective Effects of Antihypertensive Treatment in Patients Aged 85 Years or Older. *Journal of Hypertension*; *35*; 1432-1441. doi: 10.1097/HJH.0000000000001323.

Dinas Kesehatan Kota Surabaya. (2019). Profil Kesehatan Kota Surabaya Tahun 2019. Surabaya: Dinas Kesehatan Kota Surabaya.

Donovan, N. J. & Blazer, D. (2020). Social Isolation and Loneliness in Older Adults: Review and Commentary of a National Academies Report. *The American Journal of Geriatric Psychiatry*; *28*; 1233–1244. doi: 10.1016/j.jagp.2020.08.005.

Dzau, V. J. & Balatbat, C. A. (2019). Future of Hypertension. *Hypertension (Dallas, Tex. : 1979)*;

74; 450–457. doi: 10.1161/HYPERTENSIONAHA.119.13437.

Gast, A., & Mathes, T. (2019). Medication Adherence Influencing Factors—an (updated) Overview of Systematic Reviews. *Systematic Reviews*; *8*; 1-17. doi: 10.1186/s13643-019-1014-8.

Golaszewski, N. M., LaCroix, A. Z., Godino, J. G., Allison, M. A., Manson, J. E., King, J. J., Weitlauf, J. C., Bea, J. W., Garcia, L., Kroenke, C. H., Saquib, N., Cannell, B., Nguyen, S. & Bellettiere, J. (2022). Evaluation of Social Isolation, Loneliness, and Cardiovascular Disease Among Older Women in the US. *JAMA Network Open*; *5*; e2146461–e2146461. doi: 10.1001/jamanetworkopen.2021.46461.

Hacihasanoglu, A. R., Yildirim, A., Saglam, R., Demirturk Selcuk, E., Erduran, Y. & Sarihan, O. (2020). The Effect of Loneliness and Perceived Social Support on Medication Adherence Self-Efficacy in Hypertensive Patients: An Example of Turkey. *Journal of Vascular Nursing*; *38*; 183-190. https://doi.org/10.1016/j.jvn.2020.07.003.

Hazwan, A. & Pinatih, G. N. I. (2017). Gambaran Karakteristik Penderita Hipertensi dan Tingkat Kepatuhan Minum Obat di Wilayah Kerja Puskesmas Kintamani I. *Intisari Sains Medis*; *8*; 130–134. doi: 10.1556/ism.v8i2.127.

Jankowska-Polańska, B., Świątoniowska-Lonc, N., Sławuta, A., Krówczyńska, D., Dudek, K. & Mazur, G. (2020). Patient-Reported Compliance in Older Age Patients with Chronic Heart Failure. *PLOS ONE*; *15*; 1-16. doi: 10.1371/journal.pone.0231076.

Jung, H. & Lee, J. -E. (2017). The Impact of Community-Based eHealth Self-Management Intervention among Elderly Living Alone with Hypertension. *Journal of Telemedicine and Telecare*; *23*; 167–173. doi: 10.1177/1357633X15621467.

Kementerian Kesehatan RI. (2019). Laporan Nasional RISKESDAS 2018. Jakarta: Kementerian Kesehatan RI.

Kripalani, S., Risser, J., Gatti, M. E. & Jacobson, T. A. (2009). Development and Evaluation of the Adherence to Refills and Medications Scale (ARMS) Among Low-Literacy Patients with Chronic Disease. *Value in Health: The Journal of the International Society for Pharmacoeconomics and Outcomes Research*; *12*; 118–123. doi: 10.1111/j.1524-4733.2008.00400.x.

Liberty, I. A., Pariyana, P., Roflin, E., & Waris, L. (2018). Determinan Kepatuhan Berobat Pasien Hipertensi pada Fasilitas Kesehatan Tingkat I. *Jurnal Penelitian Dan Pengembangan Pelayanan Kesehatan*; *1*; 58–65. doi: 10.22435/jpppk.v1i1.428.

Lu, J., Zhang, N., Mao, D., Wang, Y. & Wang, X. (2020). How Social Isolation and Loneliness Effect Medication Adherence among Elderly with Chronic Diseases: An Integrated Theory and Validated Cross-Sectional Study. *Archives of Gerontology and Geriatrics*; *90*; 1-7. doi: 10.1016/j.archger.2020.104154.

Mubasir, M., Setiawan, C. D., Wathaniah, N., Rahmaniah, A., Maharani, P., Mahmudah, A. & Achmad, G. N. V. (2017). The Influence of Medication 'Days' Supply on Adherence and Cost of Hypertensive Patients at Primary Health Care Centre. *Indonesian Journal of Pharmacy*; *28*; 213 – 220. doi: 10.14499/indonesianjpharm28iss4pp213.

Nurhanani, R., Susanto, H. S. & Udiyono, A. (2020). Hubungan Faktor Pengetahuan dengan Tingkat Kepatuhan Minum Obat Antihipertensi (Studi Pada Pasien Hipertensi Essential di Wilayah Kerja Puskesmas Bandarharjo Kota Semarang). *Jurnal Kesehatan Masyarakat*; *8*; 114–121.

Nursalam, N., Dewi, L. C., & Widhiastuti, E. (2020). The Effect of Social Support Based on Short Message Service (Sms) on Compliance in Treatment and Reduction of Blood Pressure in Hypertension Patients in Betun Puskesmas and Besikama Puskesmas. *Critical Medical and Surgical Nursing Journal (CMSNJ)*; *9*; 10–16. doi: 10.20473/cmsnj.v9i1.17802.

Pan, J., Hu, B., Wu, L. & Li, Y. (2021). The Effect of Social Support on Treatment Adherence in Hypertension in China. *Patient Preference and Adherence*; *15*; 1953–1961. doi: 10.2147/PPA.S325793.

Pan, J., Wu, L., Wang, H., Lei, T., Hu, B., Xue, X. & Li, Q. (2019). Determinants of Hypertension Treatment Adherence Among a Chinese Population using the Therapeutic Adherence Scale for Hypertensive Patients. *Medicine*; *98*; 1-7. doi: 10.1097/MD.0000000000016116.

RAND. (2021). Basic Guidelines for Translating Surveys. California: RAND.

Russell, D. W. (1996). UCLA Loneliness Scale (Version 3): Reliability, Validity, and Factor

Structure. *Journal of Personality Assessment*; *66*; 20–40. doi: 10.1207/s15327752jpa6601_2.

Shahin, W., Kennedy, G. A. & Stupans, I. (2021). The Association Between Social Support and Medication Adherence in Patients with Hypertension: A Systematic Review. *Pharmacy Practice*; *19*; 1-8. doi: 10.18549/PharmPract.2021.2.2300.

Sherbourne, C. D. & Stewart, A. L. (1991). The MOS Social Support Survey. *Social Science & Medicine*; *32*; 705–714. doi: 10.1016/0277-9536(91)90150-b.

Sinuraya, R., Destiani, D., Puspitasari, I. M. & Diantini, A. (2018). Medication Adherence among Hypertensive Patients in Primary Healthcare in Bandung City. *Indonesian Journal of Clinical Pharmacy*; *7*; 124–133. doi: 10.15416/ijcp.2018.7.2.124.

Suadirman, S. P. (2011). Psikologi Usia Lanjut. Yogyakarta: Gadjah Mada University Press.

Turan, G. B., Aksoy, M. & Çiftçi, B. (2019). Effect of Social Support on the Treatment Adherence of Hypertension Patients. *Journal of Vascular Nursing*; *37*; 46–51. doi: 10.1016/j.jvn.2018.10.005

Uchmanowicz, B., Chudiak, A., Uchmanowicz, I., Rosińczuk, J. & Froelicher, E. S. (2018). Factors Influencing Adherence to Treatment in Older Adults with Hypertension. *Clinical Interventions in Aging*; *13*; 2425–2441. doi: 10.2147/CIA.S182881.

Wan, J., Wu, Y., Ma, Y., Tao, X. & Wang, A. (2022). Predictors of Poor Medication Adherence of Older People with Hypertension. *Nursing Open*; *9*; 1370-1378. doi: 10.1002/nop2.1183.

Widhowati, S. S., Chen, C. -M., Chang, L. -H., Lee, C. -K. & Fetzer, S. (2020). Living Alone, Loneliness, and Depressive Symptoms among Indonesian Older Women. *Health Care for Women International*; *41*; 984-996. doi: 10.1080/07399332.2020.1797039.

Woodham, N., Taneepanichskul, S., Somrongthong, R., & Auamkul, N. (2018). Medication Adherence and Associated Factors among Elderly Hypertension Patients with Uncontrolled Blood Pressure in Rural Area, Northeast Thailand. *Journal of Health Research*; *32*; 449–458. doi: 10.1108/JHR-11-2018-085.

World Health Organization. (2003). Adherence to Long-Term Therapies: Evidence for Action. Geneva: World Health Organization.

World Health Organization. (2019). Hypertension by WHO. https://www.who.int/news-room/fact-sheets/detail/hypertension. Accessed: 13 September 201.

Yazdanpanah, Y., SalehMoghaddam, A., Mazloum, S., Hajialibeigloo, R. & Mohajer, S. (2019). Effect of an Educational Program based on Health Belief Model on Medication Adherence in Elderly Patients with Hypertension. *Evidence Based Care Journal*; *9*; 52-62. doi: 10.22038/EBCJ.2019.35215.1895.

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 262-271
DOI: 10.20473/jfiki.v9i32022.262-271
Available online at https://e-journal.unair.ac.id/JFIKI/

Sunscreen Cream Formulation of Noni Leaf Extract (*Morinda citrifolia* L.) with Emulsifier Combination of Tween 80 and Lecithin

Bianca Levie Tania, Rini Dwiastuti, Agatha Budi Susiana Lestari, Dewi Setyaningsih*
Departement of Pharmaceutical Sciences and Pharmaceutical Technology, Faculty of Pharmacy, Sanata Dharma University, Yogyakarta, Indonesia

*Corresponding author: dewi@usd.ac.id

Submitted: 3 June 2022
Accepted: 22 November 2022
Published: 9 December 2022

Abstract

Background*: Noni leaf (Morinda citrifolia L.) extract is a natural product that can be used as a sunscreen. The extract contains flavonoids which function act as an antioxidant. In this work, sunscreen cream formulated with noni leaf extract was prepared using a combination of tween 80 and lecithin.* ***Objective:*** *The purpose of this study is to evaluate how the combination of tween 80 and lecithin affects the physical qualities of the cream, such as organoleptic, homogeneity, emulsion type, spreadability, adhesion, pH, and stability over 28 days of storage at room temperature, and to find the best formula.* ***Methods:*** *This study used 10% of noni leaf extract in the cream formulation. The Simplex Lattice Design (SLD) method was used to determine the effect of different concentrations of the two emulsifiers on the cream's spreadability, adhesion, and pH. Furthermore, the SLD was used to find the best formula.* ***Results:*** *The results showed that different concentrations of the emulsifier, which are the tween 80 and lecithin combination, affected the physical properties and storage stability of cream preparations. The interaction of tween 80 and lecithin is having a significant impact on the cream's adhesion and spreadability; however, the effect of the interaction on the pH value was not significant****. Conclusion:*** *The formula containing 2.5 % tween 80 and 2.5 percent lecithin was found to be the most effective in fulfilling the cream physical properties while remaining stable during storage.*

Keywords*: noni leaf extract (Morinda citrifolia L.), sunscreen, cream, tween 80, lecithin*

How to cite this article:
Tania, B. L., Dwiastuti, R., Lestari, A. B. S. & Setyaningsih, D. (2022). Sunscreen Cream Formulation of Noni Leaf Extract (*Morinda citrifolia* L.) with Emulsifier Combination of Tween 80 and Lecithin. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 9(3), 262-271. http://doi.org/10.20473/jfiki.v9i32022.262-271

INTRODUCTION

Ultraviolet (UV) rays are one type of radiation emitted by sunlight. UV rays are classified as UV-A (320 - 400 nm), UV-B (290-320 nm), and UV-C (200-290 nm) (Morita, 2018). UV-A and UV-B radiation are important factors in skin ageing (Park *et al*., 2017). Long-term sun exposure can have negative effects on the skin, such as causing degenerative changes in skin cells that lead to premature ageing, sunburn, and skin cancer (Rohmah *et al.*, 2020). Sweating, melanin formation, and other natural mechanisms protect the skin from the harmful effects of sunlight (Putri *et al*., 2019). However, skin tissue can be damaged if the body's defense system is insufficient to protect it from such radiation.

Sunscreen can protect the skin against the harmful effect of UV radiation from the sun. The sunscreen's component(s) can absorb, reflect or scatter the sunlight (Goswami *et al*., 2013). The effectiveness of sunscreen protection against UV rays can be determined by the Sun Protection Factor (SPF) of the range 0 - 100, at which a value of more than 15 is categorized as adequate protection (Mulyani *et al.*, 2015; Suhaenah *et al*., 2019).

The molecular structure of flavonoids contains more than one phenol group (-OH and aromatic groups) and conjugated double bonds to protect against free radicals (Kamilatussaniah *et al*., 2015). The leaf of the noni tree (*Morinda citrifolia* L.) contains more flavonoids than the fruit. According to Rubens *et al*., (2018), the noni leaf has 89.1063 mg/L of rutin flavonoid. Besides flavonoids, other phytochemical compounds are present in the noni leaf extract. Phytochemical screening of noni leaf extract by Ly *et al*. (2020) resulted from maceration on 70% ethanol demonstrates the existence of alkaloids, tannins, triterpenoids, saponins, coumarins, anthraquinones, carotenoids, organic acids, and reducing agents.

In this study, the oil-in-water (O/W) cream was chosen as a sunscreen dosage form because it has advantages such as being comfortable to use, easy to apply, non-sticky, and easy to wash with water compared to ointment or paste preparations (Sharon et al., 2013). In cream preparations, an emulsifier or surfactant is required to stabilize the emulsion. Surfactant is also chemical enhancer to accelerate the absorption of active substances (Ramadon *et al*., 2021). Tween 80 is one of the most commonly used emulsifiers. Tween 80 is used because it is relatively stable to electrolytes, does not irritate the skin, is non-toxic, and can produce stable emulsions *(*Syamsuddin *et al*., 2016). However, due to its hydrophilic nature, tween 80 molecules can be desorbed from the oil droplet to the aqueous phase, leaving an uncovered region of the oil droplet at the interface and can cause flocculation or coalescence (Athas *et al*., 2014).

The use of a combination of emulsifiers, one more hydrophobic and one more hydrophilic, can improve emulsion stability (Yamashita *et al*., 2017). Tween 80, a hydrophilic emulsifier, can be combined with a more hydrophobic emulsifier, such as lecithin, to improve emulsion stability. With an HLB value of 4, lecithin is less toxic and more biocompatible than polymer surfactants (Chuacharoen *et al*., 2019; Estiasih *et al*., 2015). Lecithin is an emulsifier with two hydrophobic tails, one of which has unsaturated cis bonds, while tween 80 (HLB 15) has 1 oleyl tail with one unsaturated cis bond. The presence of unsaturated cis bonds in the tail of the emulsifier causes the tail to remain flexible and liquid at room temperature. By combining the unsaturated with the saturated bond in lecithin and Tween 80; the tails of the saturated bond tend to stiffen at room temperature; this combination renders flexibility in the molecular organization in the cream formulation. The flexible lecithin and tween 80 tails will help pack the molecules close together. The interactions between the hydrocarbon tails of tween 80 and lecithin include van der Waals forces, whose interactions become more vital when the molecules are close together (Athas *et al*., 2014). The aim of this study was to understand the effect of the combination of emulsifier tween 80 and lecithin on the cream physical properties and to obtain the optimum formulation of the cream containing noni leaf extract.

MATERIALS AND METHODS

Materials

A dry powder of the noni leaf ethanol extract was obtained from PT. Bina Agro Mandiri, Tirtonirmolo, Kasihan, Bantul, Yogyakarta. Ethanol pro analysis were obtained from Merck (Darmstadt, Germany). Liquid paraffin, distilled water, and glycerin were purchased from CV. Sentra Teknosains Indonesia. Stearic acid, cetyl alcohol, methylparaben, and tween 80 were obtained from PT. Brataco, Indonesia. Lecithin was purchased from Tokyo Chemical Industry (TCI) (Japan).

Method

Total flavonoid content

The total flavonoid content was determined using rutin as the standard compound. The measurement was carried out at Gadjah Mada University's Integrated

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Research and Testing Institute (LPPT) using a UV-Vis spectrophotometer instrument.

Determination of noni leaf extract SPF value

The noni leaf extract samples were prepared in ethanol at 5%, 10%, and 20% concentrations. The UV absorption of the samples was measured using a UV-Vis spectrophotometer at the wavelength of 290 - 320 nm with 5 nm intervals (Mugitasari & Rahmawati, 2020; Mulyani *et al*., 2015). The Mansur formula in equation (1) (Mansur *et al*., 1986), which was updated by a later researcher (Yulianti *et al*., 2015) was used to determine the Sun Protection Factor (SPF) value. The SPF formula involves the value of the constant of Effectiveness of Erythema due to light (EE x I). The value of EE x I were according to Table 1.

Calculation of SPF value

The SPF value of noni leaf extract was calculated using the Mansur formula:

$$\text{SPF} = CF \times \sum_{290}^{320} EE\ (\lambda) \times I\ (\lambda) \times Abs(\lambda) \quad (1)$$

Information:

EE : The effectiveness of erythema due to UV light at a wavelength of nm

I : Intensity of UV light at wavelength nm

Abs : Extract absorbance

CF : Correction factor (10) (Yulianti *et al*., 2015).

Table 1. EE X I value in calculation of SPF value

No	Wavelength (λ nm)	EE x I
1	290	0.015
2	295	0.0817
3	300	0.2874
4	305	0.3278
5	310	0.1864
6	315	0.0839
7	320	0.018
Total		1

The EE X I value in calculation of SPF value can be seen in Table 1.

Sunscreen cream formulation

The formula refers to Mugitasari & Rahmawati (2020) research on the formulation of noni leaf extract sunscreen cream using various extract concentrations (Table 2).

The ingredients that will be used in making the cream are weighed first. The oil phase in the (stearic acid, cetyl alcohol, liquid paraffin, and lecithin) was melted in a water bath at a temperature of 70 - 80ºC. The aqueous phase (glycerin, propylene glycol, and tween 80) was dissolved in distilled water heated at a temperature of 70 - 80ºC in a beaker glass. The oil phase is added to the water phase gradually in the stainless bowl and stirred using a mixer at 5800 rpm until a cream is formed. Noni leaf extract powder that has been weighed is put into a mortar and ground until smooth. Half of the distilled water used in the formula was added gradually into the mortar containing the extract and ground until the extract was dissolved. Noni leaf extract, which had been dissolved, was added to the base at 45ºC. The cream is stirred again and put into a closed container (Mugitasari & Rahmawati, 2020; Safitri *et al.*, 2014).

Physical properties test

Emulsion type. The dilution method conducted the test by dissolving the oil-in-water (O/W) cream in water. If the cream is soluble in water, then the cream is O/W type (Elcistia & Zulkarnain, 2018). *Spreadability test*. A total of 0.5 grams of cream was weighed, placed in a round glass, then covered with another glass, and left for 1 minute. The diameter of the spread cream was measured by taking the average diameter from several sides. Then the load is added every 1 minute, 50 grams to 250 grams, and the diameter of the spread is measured (Elcistia & Zulkarnain, 2018).

Table 2. Noni leaf extract cream formula modified from Mugitasari & Rahmawati, (2020)

Ingredients	**Concentration (%*w/v*)**				
	F1	**F2**	**F3**	**F4**	**F5**
Noni leaf extract	10	10	10	10	10
Stearic acid	4	4	4	4	4
Liquid paraffin	10	10	10	10	10
Cetyl alcohol	3	3	3	3	3
Glycerine	10	10	10	10	10
Methyl paraben	0.1	0.1	0.1	0.1	0.1
Tween 80	**5**	**3.75**	**2.5**	**1.25**	**0**
Lecithin	**0**	**1.25**	**2.5**	**3.75**	**5**
Distilled Water	ad 100 mL	ad 100 mL	ad 100 mL	ad 100 mL	ad 100 mL

P-ISSN: 2406-9388
E-ISSN: 2580-8303

The spreadability test requirements for topical preparations are 5 - 7 cm (Mugitasari & Rahmawati, 2020). *Adhesion test*. A total of 0.1 grams of cream was weighed and placed on two glass objects whose area had been determined. Then press with a load of 1 Kg for 5 minutes. The glass object is mounted on the test equipment, and a 20-gram load is dropped. The time was recorded until the two glass objects were released (Elcistia & Zulkarnain, 2018). The adhesion test requirement for topical preparations is 4 seconds (Mugitasari & Rahmawati, 2020). *pH measurement*. A total of 1 gram of cream was weighed and then diluted with 10 mL of distilled water. The pH of the preparation was measured using a pH meter. The pH requirement for good topical preparation is 4.5 - 6.5 (Lumentut *et al*., 2020).

Storage stability test

The stability test of the cream was carried out by storing the cream in a tightly-closed container for 28 days at 75% Relative Humidity and 30°C temperature (Phetmung & Sawatdee, 2019). Changes in the cream physical properties such as organoleptic (color, shape, odor), spreadability, adhesion, and pH were observed. If there is no significant change during the storage period, its characterization remains within acceptable limits, and there is no phase separation, the cream preparation is stable (Dewi *et al*., 2014; Mailana *et al*., 2016).

Data analysis

The Design-Expert programme version 13.0 Trial was used to analyse quantitative data on the physical properties of sunscreen cream, such as spreadability, adhesion, and pH. The stability test results of sunscreen cream, including spreadability, adhesion, and pH after 28 days of storage, were analysed with a 95% confidence level using paired samples T-Test on IBM SPSS Statistic version 25 Trial programme. The data was analysed on the first and 28th days. There is no significant difference during storage if the p-value is greater than 0.05.

Using Design-Expert version 13.0 Trial, the optimum emulsifier composition was determined by inserting the criteria for spreadability, adhesion, and pH that met the requirements for a good cream preparation, and then selecting the formula that approached the highest desirability.

RESULTS AND DISCUSSION

Noni leaf extract total flavonoid content test

This test aimed to determine the total flavonoid content of noni leaf extract powder. According to the results of the tests, a 70% ethanol extract of noni leaves had a total flavonoid content of 4768.18 µg/g, standardized as rutin.

Determination of extract concentration

The concentration of noni leaf extract is being determined in order to determine the concentration of the extract to be used in the preparation of sunscreen cream. According to the results, extracts with concentrations of 5%, 10%, and 20% (equal to 0.024, 0.048, 0.095% flavonoid) in ethanol solvent had SPF values of 39.46, 39.59, and 39.68, respectively. All extract concentrations are ultra-protective, with SPF values greater than 15 (Damogalad *et al*., 2013).

When large quantities of plant-based derivatives are employed to increase the product's effectiveness, the research on the interactions between the active ingredients and the vehicle components should always be considered. Finding the ideal balance between rheological characteristics, such as the usability and stability of the emulsion, and the efficacy of the product, such as the concentration of its active ingredients, is the most difficult task for cosmetic formulators Numerous literature reports discuss how plant extracts' activity decreases when they are combined with topical bases and when stored, which emphasizes the significance of thorough formulation development and optimization process (Almeida *et al*., 2014). In this study, we examined the strength of the tween 80 emulsifier and lecithin in sustaining and supporting the stability of the cream product, so we chose 10% extract rather than 5% extract for our formulation in the tween 80-lecithin emulsion system. Therefore, through this study, we convey that the carrying capacity of the tween 80-lecithin system can still produce stable emulsions at 10% extract, so we predict that this system can be used at lower concentrations.

Initially, the cream was made with extract concentrations of 10% and 20% (Table 3). The sunscreen cream with 10% extract had a smooth texture with no coarse grains. Meanwhile, grains of noni leaf extract powder feel rough on the skin when sunscreen cream with 20% extract is applied. The presence of powder granules in sunscreen cream containing 20% extract is due to the extract powders not being completely dispersed in the emulsion with the emulsifier used. Based on the preliminary results, a concentration of 10% extract was used in subsequent experiments, resulting in an SPF value of 39.57.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 3. Sunscreen cream formula using noni leaf extract 10% and 20%

Ingredients	Concentration (%*w/v*)	
	10% Extract Cream	**20% Extract Cream**
Noni leaf extract	**2**	**4**
Stearic acid	0.8	0.8
Liquid paraffin	2	2
Cetyl alcohol	0.6	0.6
Glycerin	2	2
Methyl paraben	0.02	0.02
Tween 80	0.5	0.5
Lecithin	0.5	0.5
Distilled Water	ad 20 mL	ad 20 mL

Table 4. Organoleptic test results of F1, F2, F3, F4, F5

Organoleptic	F1	F2	F3	F4	F5
Shape	Slightly liquid	Semisolid	Semisolid	Semisolid	Semisolid
Colour	Light green	Light green	Yellowish green	Yellowish green	Yellowish green
Odour	Typical noni leaves	Typical noni leaves	Typical noni leaves	Typical noni leaves	Typical noni leaves
Texture	Smooth	Smooth	Smooth	Smooth	Smooth

Table 5. Spreadability test results of F1, F2, F3, F4, F5

Spreadability	F1 (cm)	F2 (cm)	F3 (cm)	F4 (cm)	F5 (cm)
$\bar{x}$ ± SD	7.45 ± 0.05	6.66 ± 0.15	6.3 ± 0.2	5.61 ± 0.12	4.47 ± 0.13

Organoleptic test

The organoleptic test aims to determine the characteristics of the cream preparations that have been made. The organoleptic test was performed visually by observing the cream preparation's shape, color, smell, and texture (Table 4).

According to observations, all sunscreen creams have a typical noni leaf aroma, a green colour, and a smooth texture. F1 has a slightly liquid consistency. Meanwhile, F2, F3, F4, and F5 have semisolid forms, and the consistency becomes denser as the concentration of lecithin increases. This is due to a lecithin emulsifier in F2, F3, F4, and F5, but not in F1. The presence of lecithin in the oil phase reduces the size of the oil droplets and narrows the particle size distribution, allowing for increased viscosity. Some lecithin molecules can migrate to the aqueous phase and form vesicles, increasing the volume fraction of the dispersion phase in the system and the emulsion's viscosity (Luo *et al*., 2017).

There is a slight colour difference between the formulas due to differences in the composition of the lecithin concentration. Because it lacks lecithin, F1 cream is typically light green. While F5 has the highest concentration of lecithin, the cream is yellowish-green. Lecithin is a brownish-yellow coloured emulsifier (Agu *et al*., 2021). The higher the concentration of lecithin used, the more yellow the cream.

Physical appearance

Physical appearance was used to monitor whether the cream product was smooth, and free of coarse particles or lumps. According to the observations, all formulas were free of coarse particles and lumps.

Emulsion type test

The dilution test of water determines the type of emulsion used in the sunscreen cream. Based on the observations, it was determined that all formulas were soluble in water, implying that all formulas were of the O/W type.

Spreadability test

The spreadability test determines how easily the cream can be used or applied (Lumentut *et al*., 2020). The spreadability test for topical preparations is 5-7 cm (Mugitasari & Rahmawati, 2020). Table 5 shows the results of the spreadability test.

According to the spreadability test results, F2, F3, and F4 have diameters in the range of 5 - 7 cm and thus meet the requirements for good cream spreadability (Mugitasari & Rahmawati, 2020). Meanwhile, F1 does not meet the requirements for good cream spreadability because it has an average of more than 7 cm whereas F5 has an average spread of less than 5 cm. The analysis results using the Simplex Lattice Design method are given in equation (2).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 6. Adhesion test results of F1, F2, F3, F4, F5

Adhesion	F1 (seconds)	F2 (seconds)	F3 (seconds)	F4 (seconds)	F5 (seconds)
$\bar{x}$ ± SD	2.00 ± 0.00	5.00 ± 0.00	5.67 ± 0.58	6.67 ± 0.58	8.33 ± 0.58

$$Y = 7.46A + 4.49B + 1.07AB \quad (2)$$

Information:

Y : spreadability (cm)

A : concentration tween 80

B : lecithin concentration

AB : interaction between tween 80 and lecithin

In equation (2), it is discovered that tween 80 has a higher coefficient than lecithin. As a result, tween 80 has a greater effect than lecithin in increasing spreadability.

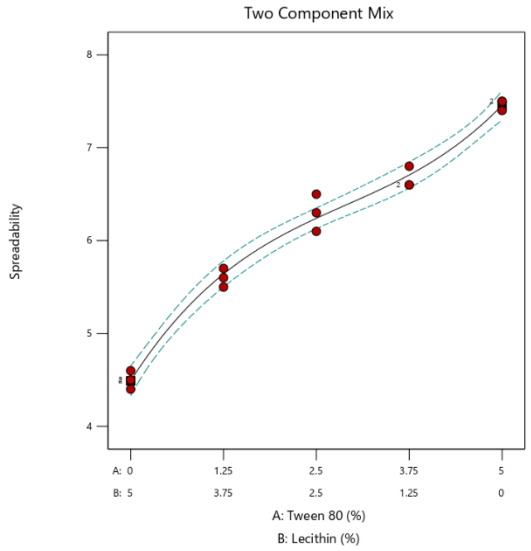


Figure 1. Spreadability test results contour plot graph of F1, F2, F3, F4, F5

According to the contour plot graph in Figure 1, the higher the spreadability value, the higher the concentration of tween 80 and the lower the concentration of lecithin. The spreadability value is inversely proportional to the viscosity; the greater the viscosity, the lower the spreadability value (Yusuf et al., 2017). Lecithin can increase cream viscosity due to the formation of phospholipid vesicles in the aqueous phase (Arancibia *et al*., 2017). As a result, the higher the concentration of lecithin, the higher the viscosity and the lower the spreadability value.

Adhesion test

The adhesion test aims to determine the cream's ability to adhere to the skin (Mailana *et al*., 2016). The adhesion test time for good topical preparations must be at least 4 seconds (Mugitasari & Rahmawati, 2020). The results of the adhesion test can be seen in Table 6.

As shown in Table 6, F2, F3, F4, and F5 have met the requirements for good cream adhesion, which are greater than 4 seconds. Meanwhile, F1 does not meet the adhesive power requirements because the stickiness is less than 4 seconds. This has to do with the spreadability of each cream formulation formula. The adhesion value is inversely proportional to the spreadability value—the greater the spreadability, the lower the adhesion value (Lumentut *et al*., 2020). The analysis results using the Simplex Lattice Design method are given in equation (3).

$$Y = 2.03A + 8.37B + 2.67AB \quad (3)$$

The tween 80 coefficient and lecithin have a positive value according to equation (3). As a result, the two emulsifiers affect the adhesion of sunscreen cream preparations. Because lecithin has a higher coefficient than tween 80, it can be said that lecithin has a more significant effect on cream adhesion than tween 80.

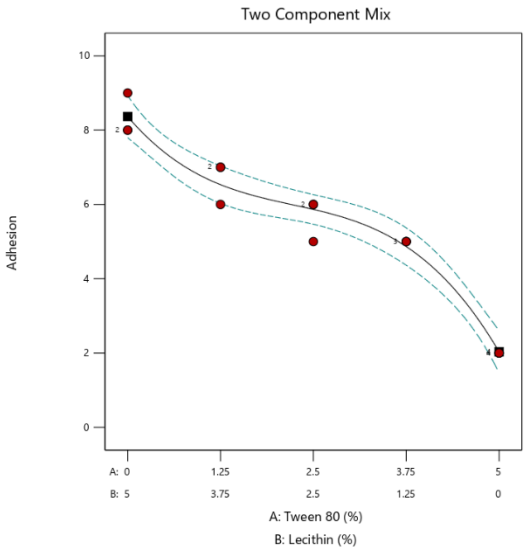


Figure 2. Adhesion test results contour plot graph of F1, F2, F3, F4, F5

The contour plot of the adhesion test results in Figure 2 shows that the higher the concentration of tween 80 and the lower the concentration of lecithin, the lower the adhesion value. The adhesion contour plot has an inversely proportional to the spreadability contour plot. This follows the statement that the greater the spreadability, the lower the adhesive power (Lumentut *et al*., 2020).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 7. pH value of F1, F2, F3, F4, F5

pH	F1	F2	F3	F4	F5
$\bar{x}$ **± SD**	5.07 ± 0.06	5.03 ± 0.06	5.03 ± 0.06	5.1 ± 0.00	5.13 ± 0.06

pH measurement

The pH measurement aims to determine the preparation's pH value in order to meet the pH requirements of topical preparations. A good topical preparation must have a pH between 4.5 and 6.5 (Lumentut *et al*., 2020). A very low pH or acidic preparation can irritate the skin, whereas a high pH or alkaline preparation can make the skin scaly (Iskandar *et al*., 2021). According to Table 7, all formulas met the requirements for the pH of the cream preparation, which ranged from 4.5 to 6.5, and there was no statistically significant difference between each formula.

Storage stability test

The appearance of F4 and F5 changes after 28 days of storage. On day 14, both formulas began to release oil on the surface of the cream preparation. This indicates that the resulting cream is unstable. Because F4 contains more lecithin than tween 80 and F5 only contains lecithin as an emulsifier, oil may be released onto the cream's surface. Excess lecithin concentration cannot be adsorbed at the oil droplet interface, resulting in a less stable emulsion. As a result, when the lecithin concentration is too high, the oil droplets are completely covered, and excess lecithin can cause droplet interactions or coalescence, resulting in the formation of an oil layer on the cream preparation's surface (Dammak & Sobral, 2017). During storage, F1, F2, and F3 have consistent physical appearances. The odour and colour of each formula did not change significantly during storage.

There were significant changes in F1 and F4 marked with a significance value of 0.05 based on the analysis of the spreadability test data on the first and 28th day. Spreadability in F1 is decreasing while spreadability in F4 is increasing. F2, F3, and F5 have significance values greater than 0.05, indicating that there is no significant change in the spreadability of the cream after 28 days of storage. F1 to F4 still had a spreadability value within the required limits of 5 - 7 cm on the 28th day, indicating that they were stable during storage, whereas F5 had a spreadability value of less than 5 cm, indicating that they did not meet the requirements for the cream spreadability value.

There was no significant difference of each formula based on the analysis of the adhesion test data on the first and 28th days. The significance value of each formula indicates this is greater than 0.05. The adhesion test results of each formula still met the requirements for good cream adhesion on the 28th day because it can stick for more than 4 seconds.

There was no significant change in each formula based on the analysis of the pH test data from the first to the 28th day. The pH of all formulas is within the required range of 4.5 - 6.5. As a result, the pH of each formula remained stable after 28 days of storage.

Interaction between lecithin and tween 80

Tween 80 and lecithin may bind strongly at the O/W cream preparation's interface and stabilize the cream. The interaction between the tail and head of each emulsifier results in this tight packaging. Both emulsifiers have hydrocarbon tails; tween 80 has one oleyl tail, and lecithin has two C16 - C18 tails. There is a van der Waals or dispersion force interaction between the hydrocarbon tails, which becomes much more potent when the tails are close. Because of the presence of unsaturated cis bonds in the emulsifier's tail, it remains flexible and liquid at room temperature. On the other hand, Saturated tails tend to stiffen or "freeze" at room temperature. The flexible lecithin and tween 80 tails will aid in packing the molecules (Figure 3a) (Athas *et al*., 2014).

Because of its low HLB, lecithin is hydrophobic and oil-soluble. As a result, when lecithin reaches the O/W cream interface, it has a low tendency to desorb into the aqueous phase and remains on the oil droplets (Figure 3b). The lecithin will cover the oil droplets, preventing the cream preparation from coalescing. Tween 80, on the other hand, provides steric repulsion on oil droplets (Figure 3c). This is due to tween 80's highly water soluble oxyethylene side chain, which prefers to be surrounded by solvent rather than interpenetrated when droplets collide (Athas *et al*., 2014).

If the O/W cream only contains lecithin, there will be only a weak barrier to prevent oil droplet coalescence. If only tween 80 is present, a steric barrier will be present initially, but desorption of tween 80 into the aqueous phase will leave the droplet uncovered, and coalescence may occur (Athas *et al*., 2014). This helps to explain why an emulsifier combination of lecithin and tween 80 is required in the emulsion to ensure the stability of the O/W cream.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

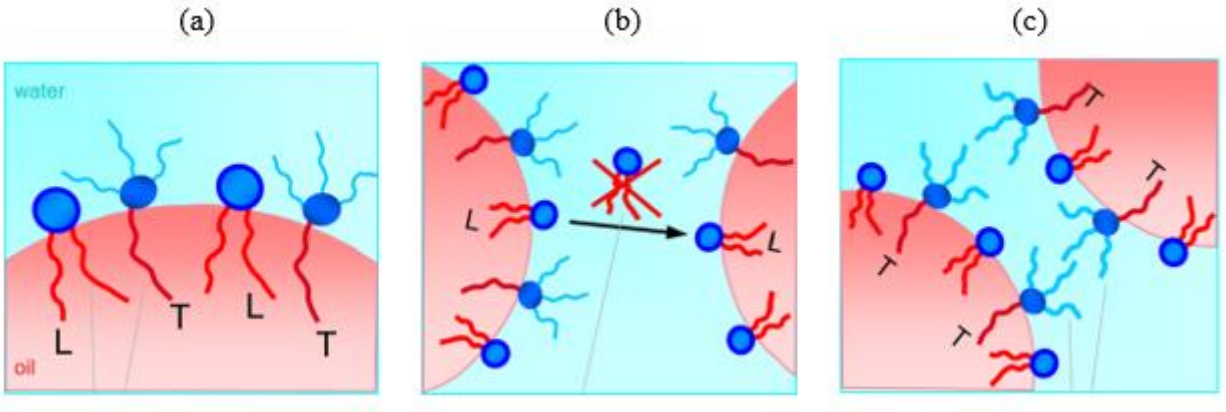


Figure 3. (a) The unsaturated cis tail of tween 80 and lecithin provide a tight packing in the oil droplet. (b) Lecithin does not desorb to the water phase because its low HLB. (c) Tween 80 prevent the droplets from coalescence by steric repulsion (Athas *et al*., 2014)

Determination of optimum composition

The highest desirability value was discovered to be between F3 and F4 based on data analysis using the Design Expert version 13.0 Trial. F3 and F4 are the best formulas based on the desirability value (Figure 4). However, according to the stability test, the oil began to leak from the F4's surface on the 14th day. This indicates that the cream preparation is unstable due to the presence of coalescence, which occurs in the O/W cream preparation, and thus F4 was not chosen as the optimum formula. F3 was selected as the best formula because it has a desirability value close to one and meets the criteria for organoleptic tests, homogeneity, emulsion type, and storage stability.

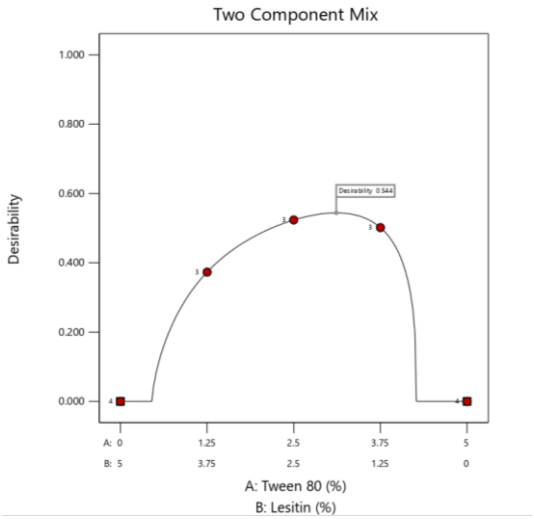


Figure 4. Desirability value of F1, F2, F3, F4, F5

CONCLUSION

Based on the findings, it can be concluded that 10% noni leaf extract is the best extract concentration, with an SPF value of 39.59. The tween 80/lecithin emulsifier combination affects the physical properties and physical stability of the noni leaf extract sunscreen cream, including spreadability and adhesion, but does not affect pH value. F3 with 2.5 % tween 80 and 2.5 % lecithin is a sunscreen cream with an optimal formula that has met the requirements for good cream preparation and good physical stability of the cream based on data analysis and storage stability for 28 days.

ACKNOWLEDGMENT

Sanata Dharma University funded the research under project number 017/Penel./LPPM-USD/IV/2022.

AUTHOR CONTRIBUTIONS

Conceptualization, D. S., B. L.T.; Methodology, D. S.; Software, B. L. T.; Validation, D. S., A. B. S. L.; Formal Analysis, D. S., B. L. T., A. B. S. L.; Investigation, B. L. T.; Resources, D. S., R. D.; Data Curation, B. L. T.; Writing - Original Draft, D. S., B. L. T., A. B. S. L., R. D.; Writing - Review & Editing, D. S.; Visualization, B. L. T.; Supervision, D. S.; Project Administration, D. S.; Funding acquisition, D. S., B. L.T.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Agu, H. O., Ejike, R. C., Obiegbuna, J. E. & Ezegbe, C. C. (2021). Production and Physicochemical Properties of Cake with Different Ratios of Production and Physicochemical Properties of Cake with Different Ratios of Soy Lecithin. *Asian Food Science Journal*; *20*; 1–9. doi: 10.9734/afsj/2021/v20i930337.

Almeida, I. F., Costa, P. C. & Bahia, M. F. (2014). Optimization of A Surfactant-free Antioxidant Formulation Using Response Surface Methodology. *Drug Development and Industrial Pharmacy 40*; 120–125. doi: 10.3109/03639045.2012.750666.

Arancibia, C., Riquelme, N., Zúñiga, R. & Matiacevich, S. (2017). Comparing the Effectiveness of Natural and Synthetic Emulsifiers on Oxidative and Physical Stability of Avocado Oil-based Nanoemulsions Comparing the Effectiveness of Natural and Synthetic Emulsifiers on Oxidative and Physical Stability of Avocado Oil. *Innovative Food Science and Emerging Technologies*; *44*; 159-166. doi: 10.1016/j.ifset.2017.06.009.

Athas, J. C., Jun, K., McCafferty, C., Owoseni, O., John, V. T. & Raghavan, S. R. (2014). An Effective Dispersant for Oil Spills Based on Food-Grade Amphiphiles. *Langmuir*; *30*; 9285–9294. doi: 10.1021/la502312n.

Chuacharoen, T., Prasongsuk, S. & Sabliov, C. M. (2019). Effect of Surfactant Concentrations on Physicochemical Properties and Functionality of Curcumin Nanoemulsions under Conditions Relevant to Commercial Utilization. *Molecules*; *24*; 1–12. doi: 10.3390/molecules24152744.

Dammak, I. & Sobral, P. J. do A. (2017). Formulation and Stability Characterization of Rutin-Loaded Oil-in-Water Emulsions. *Food and Bioprocess Technology*; *10*; 926–939. doi: 10.1007/s11947-017-1876-5.

Damogalad, V., Jaya Edy, H. & Sri Supriati, H. (2013). Formulasi Krim Tabir Surya Ekstrak Kulit Nanas (*Ananas comosus* L Merr) dan Uji *In Vitro* Nilai Sun Protecting Factor (SPF). *Pharmacon Jurnal Ilmiah Farmasi*; *2*; 39–44.

Dewi, R., Anwar, E. & Yunita, K. S. (2014). Uji Stabilitas Fisik Formula Krim yang Mengandung Ekstrak Kacang Kedelai (Glycine max). *Pharmaceutical Sciences and Research*; *1*; 194–208.

Elcistia, R., & Zulkarnain, A. K. (2018). Optimasi Formula Sediaan Krim O/W Kombinasi Oksibenzon dan Titanium Dioksida serta Uji Aktivitas Tabir Suryanya secara *In Vivo*. *Majalah Farmaseutik*; *14*; 63–78.

Estiasih, T., Ahmadi, K. & Alifatur Rizqiyah, L. (2015). Mikroemulsifikasi Fraksi Tidak Tersabunkan Distilat Asam Lemak Minyak Sawit. *Jurnal Teknologi dan Industri Pangan*; *26*; 189–200. doi: 10.6066/jtip.2015.26.2.189.

Goswami, P. K., Samant, M., Srivastava, R., Kantivan, P. & Email, G. (2013). Natural Sunscreen Agents: A Review. *Scholars Academic Journal of Pharmacy (SAJP)*; *2*; 458–463.

Iskandar, B., Sidabutar, S. E. B. & Leny. (2021). Formulasi dan Evaluasi Lotion Ekstrak Alpukat (Persea Americana) sebagai Pelembab Kulit. *Journal of Islamic Pharmacy*; *6*; 14–21. doi: 10.18860/jip.v6i1.11822.

Kamilatussaniah, Yuniastuti, A. & Iswari, R. . (2015). Pengaruh Suplementasi Madu Kelengkeng terhadap Kadar TSA dan MDA Tikus Putih yang Diinduksi Timbal (Pb). *Jurnal MIPA*; *38*; 108–114.

Lumentut, N., Jaya, H. & Melindah, E. (2020). Formulasi dan Uji Stabilitas Fisik Sediaan Krim Ekstrak Etanol Kulit Buah Pisang Goroho (Musa acuminafe L.) Konsentrasi 12.5% sebagai Tabir Surya. *Jurnal MIPA*; *9*; 42–46. doi: 10.35799/jmuo.9.2.2020.28248.

Luo, X., Zhou, Y., Bai, L., Liu, F., Zhang, R., Zhang, Z., Zheng, B., Deng, Y. & McClements, D. J. (2017). Production of Highly Concentrated Oil-In-Water Emulsions Using Dual-Channel Microfluidization: Use of Individual and Mixed Natural Emulsifiers (Saponin and Lecithin). *Food Research International*; *96*; 103–112. doi: 10.1016/j.foodres.2017.03.013.

Ly, H. T., Pham Nguyen, M. T., Nguyen, T. K. O., Bui, T. P. Q., Ke, X., & Le, V. M. (2020). Phytochemical Analysis and Wound-Healing Activity of Noni (*Morinda citrifolia*) Leaf Extract. *Journal of Herbs, Spices and Medicinal Plants*; *26*; 379–393. doi: 10.1080/10496475.2020.1748159.

Mailana, D., Nuryanti & Harwoko. (2016). Antioxidant Cream Formulation of Ethanolic Extract from Avocado Leaves (*Persea americana* Mill.). *Acta Pharmaciae Indonesia*; *4*; 7–15.

Mansur, J. de S., Breder, M. N. R., Mansur, M. C. d'Ascençäo, & Azulay, R. D. (1986). Determinaçäo do Fator de Proteçäo Solar Por Espectrofotometria. *Anais Brasileiros de Dermatologia*; *61*; 121–124.

Morita, A. (2018). Current Developments in Phototherapy for Psoriasis. *Journal of Dermatology*; *45*; 287–292. doi: 10.1111/1346-8138.14213.

Mugitasari, D. M. & Rahmawati, B. (2020). Formulasi Krim Ekstrak Daun Mengkudu (*Morinda citrifolia* L.) sebagai Sediaan Pelindung Sinar

Ultraviolet. *Jurnal Keperawatan dan Kesehatan Masyarakat*; *9*; 109–119.

Mulyani, Syamsidi, A. & Putri, P. (2015). Penentuan Nilai SPF (Sun Protecting Factor) Ekstrak N-Heksan Etanol dari Rice Bran (Oryza Sativa) secara In Vitro Dengan Metode Spektrofotometri UV-VIS. *Online Jurnal of Natural Science*; *4*; 89–95.

Park, J. H., Lee, J. E., Choi, S. S., & Park, T. H. (2017). Protective Effects of Silkworm Hemolymph Extract and Its Fractions on UV-induced Photoaging. *Biotechnology and Bioprocess Engineering*; *22*; 37–44. doi: 10.1007/s12257-016-0588-4.

Phetmung, H. & Sawatdee, S. (2019). Development of Cosmetic Cream Containing a Pharmaceutical Cocrystal of Salicylic and Phenylalanine. *International Journal of Applied Pharmaceutics*; *11*; 1–11. doi: 10.22159/ijap.2019v11i5.33869.

Putri, Y. D., Kartamihardja, H., & Lisna, I. (2019). Formulasi dan Evaluasi Losion Tabir Surya Ekstrak Daun Stevia (Stevia rebaudiana Bertoni M). *Jurnal Sains Farmasi & Klinis*; *6*; 32-36. doi: 10.25077/jsfk.6.1.32-36.2019.

Ramadon, D., McCrudden, M. T. C., Courtenay, A. J., & Donnelly, R. F. (2021). Enhancement Strategies for Transdermal Drug Delivery Systems: Current Trends and Applications. *Drug Delivery and Translational Research*; *12*; *758-791*. doi: 10.1007/s13346-021-00909-6.

Rohmah, J., Wulandari, F. E., & Rini, C. S. (2020). In Vitro: Sunscreen Activity of Red Lettuce Extract (*Lactuca sativa* var. crispa L.). *IOP Conference Series: Earth and Environmental Science*; *519*; 1-7. doi: 10.1088/1755-1315/519/1/012009.

Rubens, P. de B., Ligia, S. R., Joao, G. da C., Amanda, C. L., Isabelle, C. S. M., Claudio, G. da S., Aldenir, F. dos S., Jhonatan, D. S. das N., Adriana, G. D., Gabriela, S. V. de M., Johnnatan, D. de F., Jonas, dos S. S. & Simone, P. B. F. (2018). Bioactivity and Phenolic Composition of Extracts of Noni (*Morinda citrifolia* L., Rubiaceae) in Tomato Moth (*Tuta absoluta* Meyrick, 1917) (Lepidoptera: Gelechiidae). *African Journal of Agricultural Research*; *13*; 2063–2069. doi: 10.5897/ajar2018.13447.

Safitri, N. A., Puspita, O. E. & Yurina, V. (2014). Optimasi Formula Sediaan Krim Ekstrak Stroberi (Fragaria x ananassa) sebagai Krim Anti Penuaan. *Majalah Kesehatan FKUB*; *1*; 235–246. doi: 10.22146/jps.v1i1.23429.

Semenzato, A., Costantini, A., Meloni, M., Maramaldi, G., Meneghin, M. & Baratto, G. (2018). Formulating O/W Emulsions with Plant-Based Actives : A Stability Challenge for an Effective Product. *Cosmetics*; *5*; 1–11. doi: 10.3390/cosmetics5040059

Sharon, N., Anam, S. & Yuliet. (2013). Formulasi Krim Antioksidan Ekstrak Etanol Bawang Hutan (*Eleutherine palmifolia* L. Merr). *Online Jurnal of Natural Science*; *2*; 111–122.

Suhaenah, A., Tahir, M. & Nasra, N. (2019). Penentuan Nilai SPF (Sun Protecting Factor) Ekstrak Etanol Jamur Kancing (*Agaricus bisporus*) secara In Vitro dengan Metode Spektrofotometri UV-VIS. *Jurnal Ilmiah As-Syifaa*; *11*; 82–87. doi: 10.33096/jifa.v11i1.523.

Syamsuddin, A., Zulkarnain, I. & Hasyim, N. (2016). Formulasi Losio Emolien dari Ekstrak Etanol Sarang Semut (*Myrmecodia pendens*) dengan Menggunakan Emulgator Anionik dan Nonionik. *Journal Og Pharmaceutical and Medicinal Sciences*; *1*; 79–82.

Yamashita, Y., Miyahara, R. & Sakamoto, K. (2017). Emulsion and Emulsification Technology. In *Cosmetic Science and Technology: Theoretical Principles and Applications*; *2017*; 489-506. doi: 10.1016/B978-0-12-802005-0.00028-8

Yulianti, E., Adelsa, A. & Putri, A. (2015). Penentuan Nilai SPF (Sun Protection Factor) Ekstrak Etanol 70 % Temu Mangga (*Curcuma mangga*) dan Krim Ekstrak Etanol 70 % Temu Mangga (*Curcuma mangga*) secara *In Vitro* Menggunakan Metode Spektrofotometri. *Majalah Kesehatan FKUB*; *2*; 41–50.

Yusuf, A. L., Nurawaliah, E. & Harun, N. (2017). Uji Efektivitas Gel Ekstrak Etanol Daun Kelor (*Moringa oleifera* L.) sebagai Antijamur Malassezia furfur. *Kartika: Jurnal Ilmiah Farmasi*; *5*; 62-67. doi: 10.26874/kjif.v5i2.119.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 272-278
DOI: 10.20473/jfiki.v9i32022.272-278
Available online at https://e-journal.unair.ac.id/JFIKI/

Mannitol Production from Fructose by Using Resting Cells of Methylotrophic Yeasts

Nisa Yulianti Suprahman[1]*, Khairul Basyar[2], Herman Suryadi[2]
[1]Department of Pharmacy, Faculty of Science, Institut Teknologi Sumatera, Lampung, Indonesia
[2]Department of Pharmacy, Faculty of Pharmacy, Universitas Indonesia, Jakarta, Indonesia

*Corresponding author: nisa.suprahman@fa.itera.ac.id

Submitted: 3 June 2022
Accepted: 22 November 2022
Published: 9 December 2022

Abstract

***Background**: Mannitol is a polyol sugar widely used in pharmaceutical and food industries which can be produced by bioconversion. Using of resting cells and methanol as a carbon source are strategies to increase the efficiency of mannitol production by increasing NAD(P)H needed in the reduction process. **Objectives**: This research aimed to optimize bioconversion condition by using resting cells of methylotrophic yeasts with methanol and fructose as carbon source and substrate, respectively. **Methods**: Several isolates were used including Candida sp, Debaryomyces nepalensis and Debaryomyces hansenii and three species suspected to be yeast isolated from a local paddy field. The methylotrophic characteristic of the yeasts was screened by turbidometry. The optimization of fermentation condition was conducted by varying cultivation time (24-96 hours), resting cell concentration (30-140 mg/mL), fructose concentration (7.5-15%), ammonium sulphate concentration (0.25-0.75%) and aeration condition (50-80%). Quantitative analysis of the mannitol was conducted by HPLC with NH_2 column and Refractive Index Detector. **Results**: D. hansenii showed the highest yield value in mannitol production (23.17%), followed by D. nepalensis, Isolate A and Candida sp. (6.52%, 6.50% and 4.38%, respectively). Variation of bioconversion condition using D. hansenii showed that the highest resting cell concentration (140 mg/mL) incubated for 72 hours, moderate fructose concentration (10%), the highest ammonium sulphate concentration (0.75%) and moderate aeration condition (70%) would result in the highest yield value of mannitol (60%). **Conclusion**: This finding showed the potency of D. hansenii in mannitol production and gave preliminary information of its optimum fermentation condition.*

***Keywords**: resting cell, methylotrophic yeast, mannitol, fructose, methanol*

How to cite this article:
Tania, B. L., Dwiastuti, R., Lestari, A. B. S. & Setyaningsih, D. (2022). Sunscreen Cream Formulation of Noni Leaf Extract (*Morinda citrifolia* L.) with Emulsifier Combination of Tween 80 and Lecithin. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 9(3), 272-278. http://doi.org/10.20473/jfiki.v9i32022.272-278

INTRODUCTION

Mannitol is a polyol sugar with the lowest glycemic index (Msomi *et al*., 2021). Mannitol is used not only in the food industry but also in the pharmaceutical industry, as an active ingredient and excipient (Shi et al., 2020; Yang et al., 2021), particularly as tablet diluent. The advantages of mannitol as a diluent are its low hygroscopicity and pleasant taste (Kosugi *et al.*, 2020; Martau *et al.*, 2021). Currently, industrial production of mannitol is done with catalytic hydrogenation. However, this method provides low efficiency and comprises complex purification steps (Mérillon & Ramawat, 2018).

Bioconversion technology for producing mannitol using lactic acid bacteria, yeast and fungi has been studied to increase its efficiency on industrial scale. In microorganism cells, mannitol is produced with a reduction process from fructose, catalyzed by mannitol dehydrogenase (MDH) enzyme (Gonçalves *et al*., 2019; Lu *et al*., 2019).

Several research have been conducted to optimize the efficiency of the bioconversion by using methylotrophic yeasts, which are able to use methanol as carbon source (Yurimoto et al., 2011). Compared to sugars, methanol has higher reduction degree by which the yield value will increase. In the bioconversion process, Nicotinamide Adenine Dinucleotide (Phosphate) Hydrogen (NAD(P)H) is required to reduce fructose into mannitol. Using methanol, NAD(P)H is produced from oxidation of methanol into formaldehyde catalyzed by methanol dehydrogenase (Wang *et al*., 2019).

Another strategy to increase the efficiency of mannitol production is resting cells. Resting cells are cells that are not active in growing, but cell metabolisms are still going on. This characteristic allows minimum carbon and energy use for biomass production. Instead, those are used for oxidation-reduction (Jackson *et al*., 2019; Ng, 2020). Mannitol production by resting cell of *Escherichia coli* BL21 and *Candida magnoliae* has been studied previously and showed 82% and 45% yield value, respectively (Khan *et al.*, 2009; Koko *et al.*, 2021). However, studies on utilizing the resting cell of methylotrophic yeast for mannitol production have not been published. Therefore, this research was performed to identify the ability of resting cells from several methylotrophic yeasts to make mannitol, including optimising the fermentation condition. The change in yield value with varying fermentation conditions showed the significance of each variable in mannitol production, by which efficiency of the mannitol production could be increased, especially on an industrial scale.

MATERIALS AND METHODS

Materials

Three strain of yeasts were used in this study, including *Candida* sp. UICC Y216, *Debaryomyces hansenii* UICC Y276 and *Debaryomyces nepalensis* UICC Y328 (University of Indonesia Culture Collection). In addition, species expected to be yeast, isolated from local paddy field (Jl. Sindang Barang, Dramaga, Bogor Barat, Bogor, Indonesia), was also used.

Four types of medium were used in this study, including medium for isolation, cultivation, preculture and fermentation. An isolation medium was used for yeast isolation from paddy soil. It was a selective media, composed of 0.5 g of ammonium sulphate, 0.05 g of magnesium sulphate, 0.35 g of disodium hydrogen phosphate, 0.3 g of kalium dihydrogen phosphate, 0.05 g of chloramphenicol and 0.01 g of yeast extract (Asthana *et al*., 1971). Chloramphenicol was added to the isolation media. The composition of each 100 mL of cultivation medium was 2 mL of glucose, 2mL of peptone, 1 g of yeast extract and 1.5 g of agar (Lai *et al*., 2019).

According to Suryadi et al. (2000), the fermentation was done in two steps with some modifications. The media used in the first steps was preculture media composed of 1 mL of methanol, 1 mL of fructose, 0.5 g of ammonium sulphate, 0,05 g magnesium sulphate, 0.35 g disodium hydrogen phosphate, 0.3 g potassium dihydrogen phosphate and 0.01 g yeast extract in 100 mL of distilled water. At the second step, the media used was fermentation media composed of 1% methanol and 10% fructose.

Chemicals used in this study were methanol (JT Baker), acetonitrile (JT Baker), fructose (Merck), glucose (Merck), mannitol (Merck), ammonium sulphate (Merck), magnesium sulphate (Merck), dinatrium hydrogen phosphate (Merck), kalium dihydrogen phosphate (Merck), chloramphenicol, yeast extract (Bacto) and 1.5 g of agar (Wako), glucose (Merck), peptone (Liofilchem), yeast extract (Bacto) and 1.5 g of agar (Wako).

Instruments

Shaking bath incubator (Labline), pH meter (Eutech), microscope (Euromex), centrifuge (Kubota 6800), HPLC (Shimadzu LC-20AD), refractive index detector (Shimadzu RID-10A), degasser (Shimadzu DGU-20A5), HPLC oven column (Shimadzu CTU-

P-ISSN: 2406-9388
E-ISSN: 2580-8303

6AS), Carbohydrate Analysis NH_2 column (3,9 mm x 300 mm, 10 µm) (Waters) and Spectrophotometry UV-Vis (Shimadzu 1601).

Methods

Isolation of yeast from paddy soil

The soil was taken from 2-10 cm depth of the paddy field. Paddy soil was suspended with serial concentrations (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} g/mL). The suspension (0.1 mL) was then incubated in an isolation medium for 2-3 days. Then, the cells grown were recultivated in new isolation medium with the addition of 1% methanol, for 2-3 days in at room temperature. The isolation medium was adapted from Asthana et al., (1971).

Screening of paddy soil isolates

Isolates grown from paddy soil were screened for the methylotrophic ability as well as the ability to produce mannitol. Screening for methylotrophic ability was conducted by cultivation of the isolates in the preculture media while screening for mannitol production was done in the preculture media containing 5% of fructose.

Determination of cell concentration

Cell suspension in the preculture media was centrifuged and the supernatant was filtered through 0.22 µm filter. The biomass was determined turbidometrically at 600 mm with Spectrophotometry UV-Vis (Shimadzu UV 1601) (Suryadi *et al*., 2000). Dry cell weight was determined by centrifugation and wash of cell suspension. The supernatant was then dried at 110°C for 7 hours and then triplicate weighing was performed. From this procedure, one OD unit was shown corresponded to 2.26 mg/mL dry cells.

Fermentation

Three loopful of cells were cultivated in 100 mL of preculture media for 2 days with 175 rpm shaking in room temperature (Kasbawati *et al*., 2022). Resting cells were then made from preculture cell suspension by centrifugation at 8000 rpm, 5°C for 10 minutes. The cells were resuspended in sterile NaCl 0.9% solution and then centrifuged at 8000 rpm, 5°C for 10 minutes. This procedure was done with two replicates to get clean resting cells (Khan *et al*., 2009). The resting cells were then cultivated in the fermentation media, incubated at 175 rpm with shaking incubator in room temperature. Optimization of fermentation condition was conducted by the following factors: cultivation time (24, 48, 72 and 96 hours); resting cell concentration (300, 500, 100 and 1400 mg), fructose concentration (7,5; 10; 12.5 and 15%), ammonium sulphate concentration (0.25; 0.50 and 0.75%.) and aeration condition (varying the volume of fermentation media into 10, 15, 20 and 25 mL in 50 mL Erlenmeyer.).

Quantitative analysis of substrate and product

Fructose and mannitol calibration curves had previously been prepared. The substrate (fructose) and product (mannitol) were then quantified from 1 mL of fermentation culture. The culture was centrifuged at 6000 rpm for 10 minutes, and the supernatant was filtered through a 0.22m filter. HPLC was then used to analyze the supernatant. (Shimadzu model LC-20AD), refractive index detector (Shimadzu RID-10A), degasser (Shimadzu DGU-20A5), oven column (Shimadzu CTU-6AS), Waters Carbohydrate Analysis NH_2 column (3.9 mm x 300 mm, 10 µm), with mobile phase acetonitrile:distilled water (97:3). The column was run at a temperature of 298 K, the flow rate of 1.0 mL/min and and injection volume of 20 μL.

RESULTS AND DISCUSSION

Macroscopic and microscopic visualization of isolates were depicted in Figure 1. All isolates have glossy surfaces and circular edges. The color of isolate A, B and C colonies were yellow, bright yellow and white, respectively. Microscopically, all isolates were circular. Similar media was used by other research previously and isolated 2 yeast species and 6 Actinomycetes bacteria (Asthana *et al*., 1971).

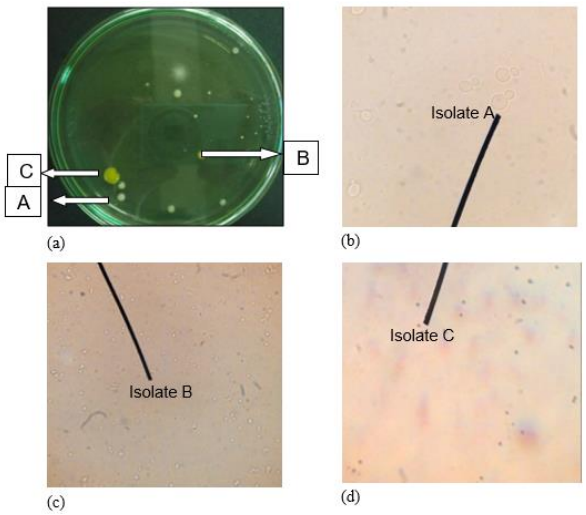


Figure 1. Yeast colonies (A) and microscopic (100 x magnification) examination of isolates (B, C, D)

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 1. Yield value of mannitol from fermentation with different yeasts

Yeast	Yield Value
Debaryomyces hansenii	23.17%
Debaryomyces nepalensis	6.52%
Isolate A	6.50%
Candida sp.	4.38%
Isolate B	Undetected
Isolate C	Undetected

The cell's ability to use methanol as a carbon source was screened based on the change in Optical Density (OD) after incubation in preculture media for three days at room temperature. It was found that all isolates were methylotrophs. However, the ability to produce mannitol was only shown by UICC strains and isolate A. The highest mannitol yield was achieved by *D. hansenii* (23.17%), followed by *D. nepalensis*, isolate A and *Candida sp* (Table 1). The *D. hansenii* has long been known for its potency in polyol production, including arabitol and xylitol (López-Linares *et al.*, 2018; Mardawati *et al.*, 2019). The yeast was reported as a higher xylitol producer than *Candida sp* (Breuer & Harms, 2006; Loman *et al.*, 2018; Rice *et al.*, 2020).

As *D. hansenii* showed the highest yield value of mannitol among the 5 other tested yeasts, variation of fermentation condition was then only conducted with this microorganism. The result of bioconversion to produce mannitol with each fermentation condition variation using *D. hansenii* was presented in Table 2. Addition of the resting cell at the optimum concentration (140 mg/mL) leads to an increase in the mannitol yield from the first to the third day. However, the yield value of mannitol was decreased on the fourth day. On the contrary, the amount of fructose decreased from the first to the third day but increased on the fourth day (Figure 2), indicating that mannitol had been re-converted into fructose on day 4.

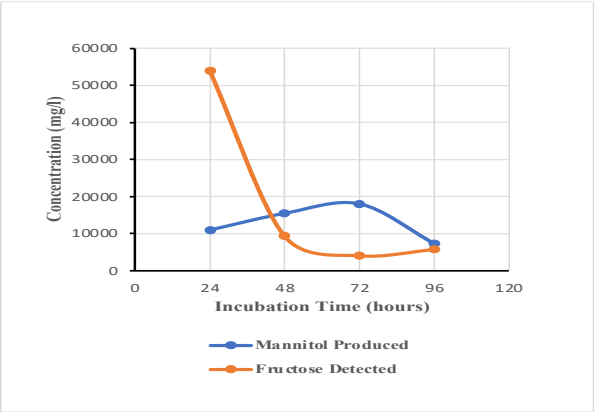


Figure 2. Mannitol and fructose concentration detected in fermentation culture of *Debaryomyces hansenii* with resting cell concentration 140 mg/mL

This is probably due to the negative feedback mechanism, in which the mannitol inhibits mannitol dehydrogenase, as reported by research (Lee *et al.*, 2003). This was supported by the different resultss obtained when less mannitol was produced in the third day of fermentation using less resting cell concentration. The addition of 30, 50 and 100 mg/mL resting cells caused the mannitol production on the third day to be smaller than the mannitol produced by 140 mg/mL resting cells. In this scenario, the mannitol yield value increased from the first to the fourth day, indicating that product inhibition was not shown with less mannitol (Figure 3).

Table 2. Yield value of mannitol from fermentation with varying resting cell concentration, fructose and ammonium concentration and aeration condition

Resting Cells Variation		**Fructose Variation**		**Ammonium Sulphate Variation**		**Aeration Variation**	
Resting Cell Concentration	Yield Value (%)	Fructose Concentration	Yield Value (%)	Ammonium Sulphate Concentration	Yield Value (%)	Aeration Concentration	Yield Value (%)
300 mg	5.24	7.50	15.65	0.25	3.47	80.00	10.67
500 mg	14.18	10.00	16.33	0.50	9.57	70.00	61.15
				0.75	40.99		
1000 mg	18.44	12.50	11.0			60.00	31.85
1400 mg	20.49	15.00	9.35	-	-	50.00	6.14

P-ISSN: 2406-9388
E-ISSN: 2580-8303

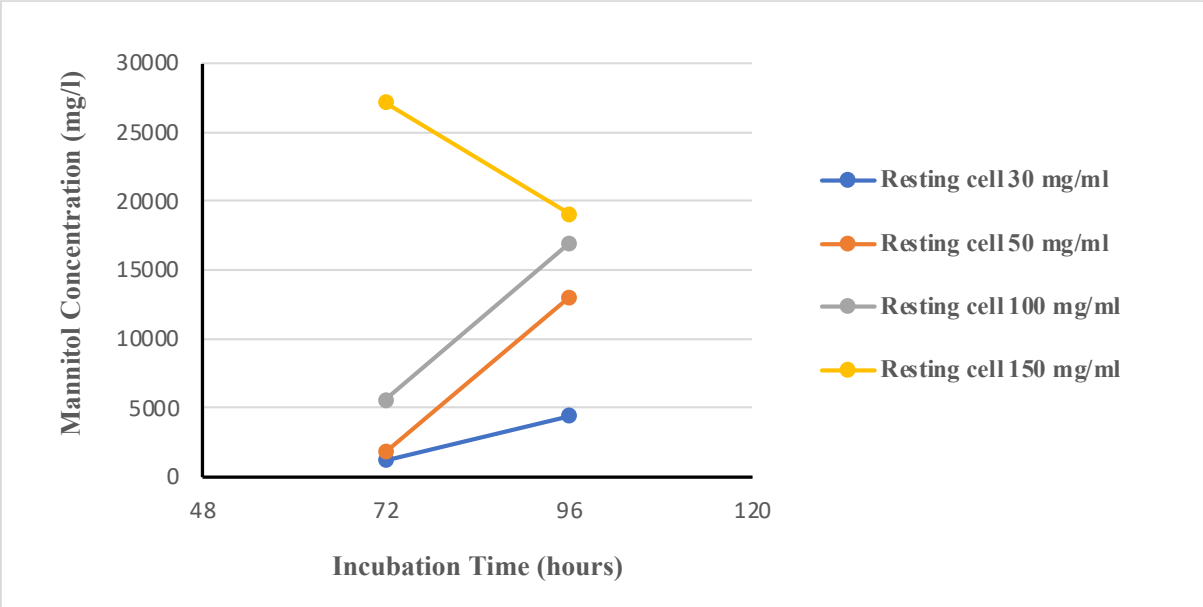


Figure 3. Mannitol concentration detected in fermentation culture of *Debaryomyces hansenii* with different resting cell concentration

The effect of biomasses variation on mannitol yield valuhas been studied previously in mannitol dehydrogenase-transfected *Escherichia coli* BL21. In this study, increasing biomass from OD (600 nm) 20 to 60% decreased mannitol production, which may have been caused by lower substrate availability with higher cell amount (Koko *et al.*, 2021). This study did not examine fermentation with resting cell concentrations greater than 140 mg/mL.. However, given the behavior of decreased mannitol yield value on the fourth day compared to the third day, increasing resting cell concentration to more than 140 mg/mL without increasing substrate concentration may not result in increasing product yield value.

The decrease in mannitol yield value was also obtained by using fructose concentration higher than 10%. This was probably because of the fructose osmotic effect on biomass production. Osmotic pressure equivalent to NaCl 3-5% w/v has been reported as the ideal osmotic condition for *D.hansenii* growth as moderate halophilic yeast (Breuer & Harms, 2006; Navarrete *et al.*, 2022). The fructose is an osmotic agent. Optimal growth will shift the use of substrate to fulfil the biomass production, therefore, the mannitol yield value was decreased. This was supported by less amount of fructose detected on the fourth day of fermentation using fructose 12.5%, compared to 7.5 and 10% fructose. Moreover, fructose detected on the fourth day of fermentation was higher than the added amount in fermentation using 15% fructose, indicating the high use of fructose caused mannitol to be converted into fructose (Figure 4). Previous research studied galacticol production by yeast *Rhodosproridium toruloides* IFO0880 with variations in galactose concentration (20, 40 and 60 g/L). This research reported that increasing galactose concentration up to 60 g/L increased biomass production. However, the yield value of galactitol was increased only by increasing galactose concentration up to 40 g/L but decreased by higher galactose. This occurrence indicated that increasing biomass production by increasing galactose concentration did not correlate with galactitol yield value (Jagtap *et al*., 2019).

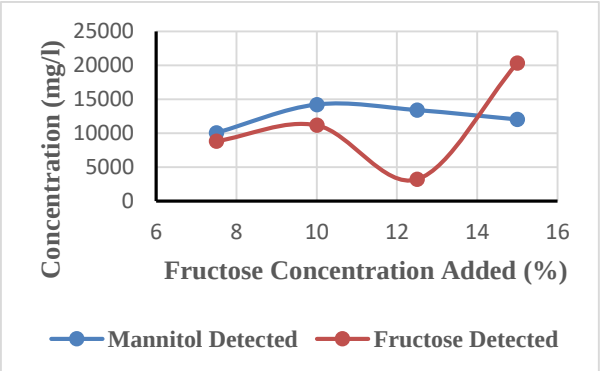


Figure 4. Mannitol and fructose concentration detected in fermentation culture of *Debaryomyces hansenii* with different resting cell concentration

Optimization of aeration level was conducted by varying the volume of culture media (10, 15, 20 dan 25 mL) with the use of the same amount of *resting cell* (500 mg), the same concentration of fructose (10%) and methanol (1%) and the same Erlenmeyer volume (50

P-ISSN: 2406-9388
E-ISSN: 2580-8303

mL). The variation in culture media volume will allow different aeration level (80%, 70%, 60% and 50% for media culture volume of 10, 15, 20 and 25 mL, subsequently). Based on this optimization, the highest mannitol yield value was achieved by the moderate aeration (70%, resulted from the use of 15 mL media in 50 mL Erlenmeyer flask).

This founding was in concordance to research conducted previously on bioconversion of xylose to xylitol by *D. hansenii* (Breuer & Harms, 2006). In the highly aerobic condition, the yield value of xylitol was decreased because the reoxidation of NADH into NAD^+ needed for biomass production was increased with the high availability of oxygen in the respiratory chain. In contrast, with moderate aeration, the available oxygen was only used for the regeneration of NADH into NAD^+ required for xylitol production. In minimal oxygen, xylitol yield value is low, owing to low NADH-dependent *Xylitol Dehydrogenase* activity (Breuer & Harms, 2006).

CONCLUSION

Debaryomyces hansenii is a promising microorganism for mannitol production. Variations in fermentation conditions will highly determine the yield value of mannitol production. The balance between cell amount and substrate will provide an optimum number of cell production and substrate availability for mannitol production. Limiting biomass production will direct the use of substrate for metabolism to produce mannitol rather than cell growth. Therefore, the use of resting cell and applying osmotic and aeration condition that is less in favor of biomass production will increase mannitol production efficiency.

ACKNOWLEDGMENT

The authors would like to acknowledge University of Indonesia for providing yeasts culture for this research and for Prof. Dr. Harmita, Apt for his expertise and assistance in quantitative analysis.

AUTHOR CONTRIBUTIONS

Conceptualization, N. Y. S., K. B., H. S.; Methodology, N. Y. S., K. B., H. S.; Software, N. Y. S., K. B., H. S.; Validation, N. Y. S., K. B., H. S.; Formal Analysis, N. Y. S., K. B., H. S.; Investigation, N. Y. S., K. B., H. S.; Resources, N. Y. S., K. B., H. S.; Data Curation, N. Y. S., K. B., H. S.; Writing - Original Draft, N. Y. S.; Writing - Review & Editing, H. S.; Visualization, N. Y. S., K. B., H. S.; Supervision, H. S.; Project Administration, N. Y. S., K. B., H. S.; Funding acquisition, N. Y. S., K. B., H. S.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Asthana, H., Humphery, A. E. & Moritz, V. (1971). Growth of Yeast on Methanol as The Sole Carbon Substrate. *Biotechnology and Bioengineering*; *13*; 923–929. doi: 10.1002/bit.260130615.

Breuer, U., & Harms, H. (2006). *Debaryomyces hansenii* - an Extremophilic Yeast with Biotechnological Potential. *Yeast*; *23*; 415–437. doi: 10.1002/yea.1374.

Gonçalves, C., Ferreira, C., Gonçalves, L. G., Turner, D. L., Leandro, M. J., Salema-Oom, M. & Gonçalves, P. (2019). A New Pathway for Mannitol Metabolism in Yeasts Suggests a Link to the Evolution of Alcoholic Fermentation. *Frontiers in Microbiology*; *10*; 1–15. doi: 10.3389/fmicb.2019.02510.

Jackson, E., Ripoll, M. & Betancor, L. (2019). Efficient Glycerol Transformation by Resting Gluconobacter Cells. *Microbiology Open*; *8*; 1–10. doi: 10.1002/mbo3.926.

Jagtap, S. S., Bedekar, A. A., Liu, J. J., Jin, Y. S. & Rao, C. V. (2019). Production of Galactitol from Galactose by the Oleaginous Yeast *Rhodosporidium toruloides* IFO0880. *Biotechnology for Biofuels*; *2019*; 1–13. doi: 10.1186/s13068-019-1586-5.

Kasbawati, K., Mardawati, E., Samsir, R. & Suhartini, S. (2022). An Appropriate Unstructured Kinetic Model Describing the Batch Fermentation Growth of *Debaryomyces hansenii* for Xylitol Production Using Hydrolysis of Oil Palm Empty Fruit Bunch. *Biotechnology & Biotechnological Equipment*, *36*; 462–471. doi: 10.1080/13102818.2022.2098816.

Khan, A., Bhide, A. & Gadre, R. (2009). Mannitol Production from Glycerol by Resting Cells of *Candida magnoliae*. *Bioresource Technology*; *100*; 4911–4913. doi: 10.1016/j.biortech.2009.04.048.

Koko, M. Y. F., Sami, R., Muhoza, B., Khojah, E. & Mansour, A. M. A. (2021). Promising Pathway of Thermostable Mannitol Dehydrogenase (MtDH) from Caldicellulosiruptor Hydrothermalis 108 for D-mannitol Synthesis. *Separations*; *8*; 1-11. doi: 10.3390/separations8060076

Kosugi, A., Leong, K. H., Urata, E., Hayashi, Y., Kumada, S., Okada, K. & Onuki, Y. (2020). Effect of Different Direct Compaction Grades of Mannitol on the Storage Stability of Tablet Properties Investigated Using a Kohonen Self-Organizing Map and Elastic Net Regression Model. *Pharmaceutics*; *12*; 1–14. doi: 10.3390/pharmaceutics12090886.

Lai, Y. T., Cheng, K. C., Lai, C. N., & Lai, Y. J. (2019). Isolation and Identification of Aroma Producing Strain with Esterification Capacity from Yellow Water. *PLoS ONE*; *14*; 1–16. doi: 10.1371/journal.pone.0211356.

Lee, J. K., Koo, B. S., Kim, S. Y. & Hyun, H. H. (2003). Purification and Characterization of a Novel Mannitol Dehydrogenase from a Newly Isolated Strain of *Candida magnoliae*. *Applied and Environmental Microbiology*; *69*; 4438–4447. doi: 10.1128/AEM.69.8.4438-4447.2003.

Loman, A. A., Islam, S. M. ., & Ju, L. -K. (2018). Production of Arabitol from Enzymatic Hydrolysate of Soybean Flour *by Debaryomyces hansenii* Fermentation. *Biotechnological Products and Process Engineering*; *102*; 641–653. doi: 10.1007/s00253-017-8626-5.

López-Linares, J. C., Romero, I., Cara, C., Castro, E. & Mussatto, S. I. (2018). Xylitol Production by *Debaryomyces hansenii* and *Candida guilliermondii* from rapeseed Straw Hemicellulosic Hydrolysate. *Bioresource Technology*; *247*; 736–743. doi: 10.1016/j.biortech.2017.09.139.

Lu, F., Xu, W., Zhang, W., Guang, C. & Mu, W. (2019). Polyol Dehydrogenases: Intermediate Role in the Bioconversion of Rare Sugars and Alcohols. *Applied Microbiology and Biotechnology*; *103;* 6473–6481. doi: 10.1007/s00253-019-09980-z.

Mardawati, E., Andoyo, R., Muhaemin, M., Nurjanah, S., Natawigena, D., Herwanto, T. & Kramadibrata, A. M. (2019). Fermentation Process of Glycerol to Arabitol from Byproducts of *Reutalis trisperma* Biodiesel Using Yeast of *Debaryomyces hansenii*. *IOP Conference Series: Earth and Environmental Science*; *347*; 1-8. doi: 10.1088/1755-1315/347/1/012132

Martau, G. A., Coman, V. & Vodnar, D. C. (2020). Recent Advances in the Biotechnological Production of Erythritol and Mannitol. *Critical Reviews in Biotechnology*; *40*; 608–622. doi: 10.1080/07388551.2020.

Mérillon, J. -M. & Ramawat, K. G. (2018). Sweeteners. Pharmacology, Biotechnology and Applications. Berlin: Springer.

Msomi, N. Z., Erukainure, O. L. & Islam, M. S. (2021). Suitability of Sugar Alcohols as Antidiabetic Supplements: A Review. *Journal of Food and Drug Analysis*; *29*; 1–14. doi: 10.38212/2224-6614.3107.

Navarrete, C., Sánchez, B. J., Savickas, S. & Martínez, J. L. (2022). DebaryOmics: an Integrative –Omics Study to Understand the Halophilic Behaviour of *Debaryomyces hansenii*. *Microbial Biotechnology*; *15*; 1133–1151. doi: 10.1111/1751-7915.13954

Ng, W. (2020). Metabolism of Non-Growing Cells and Their Application in Biotransformation. Preprints; 2020; 1-6. doi: 10.20944/preprints202007.0402.v1.

Rice, T., Zannini, E., K. Arendt, E. & Coffey, A. (2020). A Review of Polyols–Biotechnological Production, Food Applications, Regulation, Labeling and Health Effects. *Critical Reviews in Food Science and Nutrition*; *60*; 2034–2051. doi: 10.1080/10408398.2019.1625859.

Shi, J., Tan, L., Ye, J. & Hu, L. (2020). Hypertonic Saline and Mannitol in Patients with Traumatic Brain Injury: A Systematic and Meta-Analysis. *Medicine*; *99*; 1-7. doi: 10.1097/MD.0000000000021655.

Suryadi, H., Katsuragi, T., Yoshida, N., Suzuki, S., & Tani, Y. (2000). Polyol Production by Culture of Methanol-Utilizing Yeast. *Journal of Bioscience and Bioengineering*; *89*; 236–240. doi: 10.1016/S1389-1723(00)88825-8.

Wang, X., Wang, X., Lu, X., Ma, C., Chen, K. & Ouyang, P. (2019). Methanol Fermentation Increases the Production of NAD(P)H-Dependent Chemicals in Synthetic Methylotrophic *Escherichia coli*. *Biotechnology for Biofuels*; *12*; 1–11. doi: 10.1186/s13068-019-1356-4.

Yang, H., Fu, Y., Zhang, Y., Zhou, J., Wang, D., Gao, Z. & Wang, X. (2021). Evaluation of Mannitol and Xylitol on the Quality of Wheat Flour and Extruded Flour Products. *Internation Journal of Food Science and Technology*; *56*; 4119–4128. doi: 10.1111/ijfs.15040.

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 279-289
DOI: 10.20473/jfiki.v9i32022.279-289
Available online at https://e-journal.unair.ac.id/JFIKI/

Jelly Candy Hydroxyapatite from Mackerel Fish Bone

Lia Anggresani[1,2], Santi Perawati[3]*, Ryan Afandi[3], Rahmadevi[4]
[1]Department of Midwifery, STIKes Syedza Saintika Padang, Padang, Indonesia
[2]Graduate School of Natural Science and Technology, Gifu University, Japan
[3]Department of Pharmacy, STIKes Harapan Ibu Jambi, Jambi, Indonesia
[4]Department of Pharmacy, Adiwangsa University, Jambi, Indonesia

*Corresponding author: santiperawati@gmail.com

Submitted: 7 May 2022
Accepted: 22 November 2022
Published: 9 December 2022

Abstract

Background*: Calcium is a mineral that is needed by bones and teeth. Calcium needs have not been achieved evenly among children, about 1000mg/day. Hydroxyapatite contains Ca and apatite which is good for the maintenance of bones and teeth. Hydroxyapatite is made from the bones of mackerel, where the bones of mackerel have a high source of calcium. For easy being consumed for children, the hydroxyapatite made in the form of jelly candy.* ***Objective****: The aim of this research was Hydroxyapatite Mackerel Fish Bone can be made as Jelly Candy.* ***Methods****: Hydroxyapatite from mackerel bones is made by the precipitation method. Then, hydroxyapatite was formulated into jelly candy with concentrations of hydroxyapatite was 18%, 19% and 20% respectively for Formula I, II and III. Jelly candy evaluation includes organoleptic test, gel strength, khamir and ALT ochre, weight uniformity, pH, hedonics, homogeneity and storage.* ***Results****: The evaluation showed that all jelly candy formulas are safe for consumption according to SNI 3547.2-2008.* ***Conclusion****: In conclusion, hydroxyapatite mackerel fish bone can be made as Jelly Candy with the best concentration in Formula 1.*

Keywords*: calcium, hydroxyapatite, jelly candy, mackerel fish bone, precipitation*

How to cite this article:
Anggresani, L., Perawati, S. Afandi, R. & Rahmadevi. (2022). Jelly Candy Hydroxyapatite from Mackerel Fish Bone. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 9(3), 279-289. http://doi.org/10.20473/jfiki.v9i32022.279-289

INTRODUCTION

Calcium is an important mineral in the human body, which can affect the development and growth of bones and teeth in humans, where 99% of calcium in the human body is found in bones (Shita & Sulistiyani, 2010). Age 9 to 18 years is the age that most need calcium with an average amount of calcium of 1000 - 1200 mg/day compared to other ages (Jauhari *et al*., 2019). Unfortunately, many children in Indonesia do not have enough calcium intake every day. Calcium sources are divided between animals and vegetables (Shita & Sulistiyani, 2010).

Hydroxyapatite (HAp) with molecular formula $Ca_5(PO_4)_3(OH)$ or better known as Formula $Ca_{10}(PO_4)_6(OH)_2$ is bio ceramic calcium apatite that can be found in human teeth and bones. Hydroxyapatite is the most similar to the mineral part of the bone, since the salt calcium phosphate (CaP) is the main mineral that composes bones and teeth (Mozartha, 2000). It is widely used as an adsorbent for removing heavy metals due to high-efficiency ion exchange between ion Ca and metal, purification of wastewater treatment and biomedical applications with excellent physical and mechanical properties. Many studies investigated the preparation and characterization of Hydroxyapatite from natural sources, such as cockle shells, cattle bones, and fish bones with various methods (Stötzel *et al.*, 2009).

One source of hydroxyapatite is mackerel bones. Fish bones have the most calcium content among other parts of the fish body because the main elements of fish bones are calcium, phosphorus and carbonate (Chadijah *et al*., 2018). Mackerel bones (Scomberomorus guttatus) can be processed into products that have a high economic value in the form of hydroxyapatite ($Ca_5(PO_4)_3(OH)$) (Anggresani *et al*., 2018). Aside from mackerel bones, hydroxyapatite can be found in egg shells (Noviyanti *et al*., 2017), blood shells (Ahmad, 2017), and tuna fish bones (Mutmainnah *et al*., 2017).

The development of science and technology in the field of pharmacy encourages pharmacists to make the right formulations to process hydroxyapatite into a form of preparation that is easily accepted by the community (Andasari, Zukhri and Nurjanah, no date). Pharmaceutical preparations made from hydroxyapatite active that has been made into a pharmaceutical product are the form of toothpaste (Anggresani *et al*., 2021; Hernawan *et al*., 2021; Wadu *et al.*, 2015), biomaterials (Hanura *et al*., 2017), and injectable bone substitutes (Budiatin *et al.*, 2016). There are also jelly preparations made from active calcium (Lesmana *et al*., 2008) and there are preparations of calcium jelly candy in the market. However, because hydroxyapatite has not been made in candy preparations, there needs to be efforts to develop hydroxyapatite preparations in a form that can be consumed more easily by the community, especially children. Children become unacceptable when directly contact with the tongue taste receptor, which has partially dissolved in it. Jelly candy is a kind of oral drug that many pharmaceuticals have been used nowadays for patients who lost their primary teeth (such as children), and patients who have Parkinson's, stroke, nausea, and thyroid disorder disease (Sunil *et al*., 2020). Jellies are translucent and transparent and can be used for internal and external application. Jellies offers efficacy, safety, low cost of treatment. In general, jelly candy reveals pleasant taste, magnificent appearance and convenient to handle.

Based on the description, researchers were interested in making hydroxyapatite jelly candy from mackerel fish bone due to the easy to be consumed. Jelly candy is made from water or juice of plants and gel-forming materials (Alridho *et al*., 2017).

MATERIALS AND METHODS

Materials

Reagents and chemicals used in this research were mackerel bone waste (*Scomberomorus guttatus*) were collected from Traditional Market in Jambi City, distilled water (Brataco® Indonesia), phosphoric acid (Merck® Germany), 85% 0.1 M, Ethanol (Merck® Germany) 50%, hydrochloric acid (Merck® Germany) 37% 1 M, diammonium hydrogen phosphate (Merck® Germany) 10 M, sodium hydroxide 0.1 %, and acetone (Merck® Germany), coco pandan syrup, gelatin, water, citric acid, benzoic acid, sodium benzoate, menthol and HAp.

Equipment

The equipment were used in this study were furnace (Sh scientiac® Canada), oven, balance analytics (Shimadzu® Japan), stirrer hot plate (Ika c-mag hs7® Germany), soaking container, sift mesh 80, porcelain cup, beaker glass, spatula, measuring pipette, burette, measuring glass, X-ray Analysis Fluorescence Spectrometer (XRF) UK, United Kingdom, X-Ray Diffraction (XRD) (Xpert pro analytical® United Kingdom, United Kingdom), Scanning Electron Microscopy (SEM) (Tabletop Microscope tm3000 United States).

Method

The powder formation of mackerel fish bone waste

Mackerel fish bones (*Scomberomorus guttatus*) 1 Kg was cleaned and boiled. Then washed it, immersed

P-ISSN: 2406-9388
E-ISSN: 2580-8303

in 10 L of 0.1% NaOH solution for 7 hours, drained then soaked in a container containing 50% acetone for 8 hours. Then, the bones are drained and dried for 7 days. Then crush it into powder. The powder was calcinated for 3 hours at 800°C. Then the powder is crushed then sieved with 80 mesh. Powder of mackerel fish bone (CaO) was formed and then analyzed using XRF (Anggresani *et al*., 2021).

The synthesis of hydroxyapatite

CaO powder 180 g was diluted with 1 L distilled water and then stirrer at speed 300 rpm for 30 minutes until the suspension $Ca(OH)_2$ was formed. Add diammonium hydrogen phosphate $(NH_4)_2HPO_4$ to the suspension $Ca(OH)_2$ using the variation of mol Ca/P was 1,67 and for 1 hour heated at 90ºC. Adjust pH using NaOH 1 M until it reaches 12. Aging the solution for 24 hours at room temperature. Then the formed deposition was filtered and dried using an oven at 120°C for 5 hours. The dry precipitated was calcinated at 900°C for 5 hours. Then analyzed with XRD and SEM (Anggresani *et al*., 2021).

The formulation of jelly candy hydroxyapatite from mackerel fish bone waste

Hydroxyapatite from Mackarel Fish Bone Waste. The formulation of jelly candy can be seen in Table 1.

The evaluation of jelly candy hydroxyapatite from mackerel fish bone waste

The evaluation of raw material

The raw material evaluation includes organoleptic and the solubility of hydroxyapatite, sodium benzoate, citric acid, gelatin, syrup, and menthol. All materials used in this formulation conform to the standard.

The evaluation of jelly candy

The evaluation of jelly candy includes a weight uniformity test, organoleptic test, pH test, and hedonic test with ethical clearance No. LB.02.06/2/007/2021, kapang khamir test and total plate number test, gel strength, homogeneity test and storage test (Badan Standarisasi Nasional, 2008) (from SNI Standard No. 3547-2-2008).

Weight uniformity test. Thirty pieces of jellies, each of them was weight and the average weight from each formula was calculated. *Organoleptic test*. Organoleptic tests included the observed of the color, smell, texture, and taste. *pH test*. This test used pH meter. Jelly candy hydroxyapatite mackerel fish bone was diluted with hot distilled water 10 mL. Then measure the pH using pH meter. *Hedonic test*. This test included color, taste and smell of the jelly candy hydroxyapatite mackerel fish bone made by the panelists. *Kapang khamir test*. This test was carried out to determine the shelf life of the product. Kapang khamir test was carried out by counting the colonies of kapang growth on jelly candy. According to the National Standardization Agency, the limit of kapang maximum was 1×10^2 colony/gram. *Total plate number test*. This test refers to National Standardization Agency (SNI) 3557.2-2008. *Gel strength*. Gel strength was measured using a texture analyzer with a load strength of 4500 grams and a cylindrical TA5 probe with a length of 40.25 mm. Gel strength was measured as the force required by the probe to compress a thick gel 5 mm at a speed 2.5 mm/second. *Homogeneity test*. This test was observed using a piece of glass, and then jelly candy was smeared in it. *Storage test*. The test was carried out by storing jelly candy at room temperature for 21 days. Then, jelly candy was observed on the 7th day, 14th day and 21st day.

RESULTS AND DISCUSSION

Calcium oxide (CaO) formation from mackerel fish bone

Mackerel fish bones was boiled for 45 min to cleanse the bones from the meat. After that, the bones were soaked with NaOH 0.1% and acetone 50% to remove the fat compound. Then crusher the bones and calcinated using a furnace at 800ºC for 3 hours to get CaO powder. The function of calcination is to remove calcium carbonate ($CaCO_3$) and becomes calcium oxide (CaO) (Mutmainnah *et al*., 2017).

Table 1. The formulation of jelly candy

Formula	Concentration (%)			
	Formula 0	**Formula 1**	**Formula 2**	**Formula 3**
Hydroxyapatite	-	18	19	20
Sodium Benzoate	0.15	0.15	0.15	0.15
Benzoic Acid	0.1	0.1	0.1	0.1
Citrate Acid	0.8	0.8	0.8	0.8
Gelatin	19	19	19	19
Syrup	35	35	35	35
Menthol	1	1	1	1
Distilled water	Ad 100	Ad 100	Ad 100	Ad 100

P-ISSN: 2406-9388
E-ISSN: 2580-8303

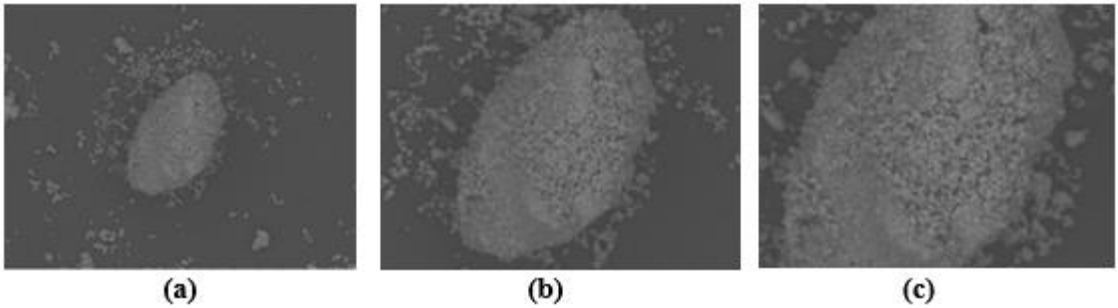


Figure 2. SEM analysis of hydroxyapatite with magnification (a) 500x, (b) 1000x, (c) 1500x

$$CaCO_3 \rightarrow CaO + CO_2$$

Then the powder analyzed with XRF (X-Ray Diffraction) to determined calcium oxide content. XRF analysis obtained CaO content of 49.846%. In Tuna Bones (Mutmainnah *et al*., 2017), the CaO content as much as 62.31%, cow bones the CaO content of 31.48% (Yuliana *et al*., 2017), and mackerel fish bones (Anggresani *et al*., 2018) as 50.814%. In this study has smaller CaO content from the previous studies. It is because when calcination occurs there is an organic material degradation process (Mutmainnah *et al*., 2017).

The synthesis of hydroxyapatite

Hydroxyapatite synthesis was made by calcium precursor and phosphate precursor in precipitation methods. In this study, the calcium precursor is CaO from mackerel fish bones and the phosphate precursor is $(NH_4)_2HPO_4$. Precipitation methods is an alkaline acid reaction that produces crystalline solids as well as water. This process is simpler, using cheap raw materials and homogeneity (Haris *et al*., 2016).

In synthesis, CaO from mackerel fish bones was dissolved in distilled water to get $Ca(OH)_2$ calcium hydroxide. Reactions that occur as follows:

$$CaO + H_2O \rightarrow Ca(OH)_2$$

Further, to synthesize hydroxyapatite, needed phosphate as a source of phosphate. In this study, we used diammonium hydrogen phosphate $(NH_4)_2HPO_4$ with a mole ratio of Ca/P 1.67. Hydroxyapatite with this mole ratio has the same crystalline arrangement as animal/human bones (Rana *et al.*, 2017). The reaction that occurs in the process of hydroxyapatite synthesis is:

$$10Ca(OH)_2 + 6(NH_4)_2HPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6H_2O + 12\ NH_4OH$$

The hydroxyapatite was analyzed using X-Ray Diffraction (XRD). High intensity was found in 2Ɵ: 32.58; 33.71; 33.14; 23.13; and 31.82 where the peak of this 2Ɵ was in accordance with ICSD standard of hydroxyapatite No. 96-900-3549 (Figure 1).

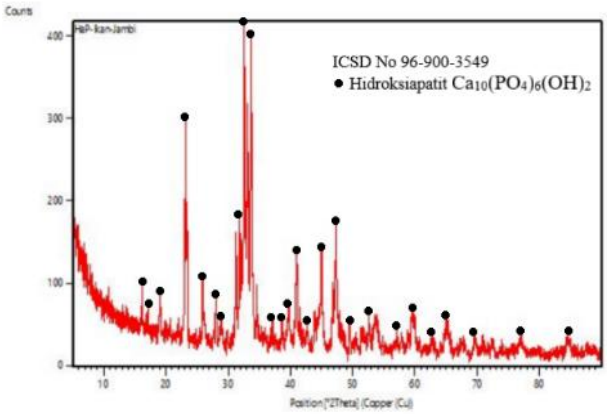


Figure 1. XRD analysis of hydroxyapatite

XRD analysis was conducted to see the composition of atoms in a crystalline material so that it can be known the structure, orientation and size of crystals (Munasir *et al.*, 2012). The crystal size of the XRD analysis can be known using the Scherrer method, the crystal size in this synthesis was 77.47 nm. Products that have a low FWHM will result in a larger crystal size (Warna *et al*., 2015).

SEM analysis was conducted to look at the morphology of particle surfaces of hydroxyapatite compounds. SEM analysis was done at magnification 500x, 1000x and 1500x (Figure 2).

This study with a mole ratio of Ca/P 1.67 particles formed granular like a sphere with an even particle distribution and the particle size is in the range of 0.1 to 0.3 µm. Particles shaped like spheres with a size of 20 – 30 µm will form at pH 10, while most hydroxyapatite synthesized at pH 8 are needle-like with a length of 0.25 µm (Wang *et al.*, 2010), but if there is the interaction between substances, it can affect the crystal morphology of a substance (Fadhila *et al*., 2020).

Using calcination temperatures of 800°C and 1,000°C for 5 hours impacts the morphological changes of hydroxyapatite particles that bond between particle granules and form irregular rods (Setiawan & Basit, 2012). The particle size obtained is 0.19433 µm, which is in the range of 0.1 to 0.3 µm. This particle size is

P-ISSN: 2406-9388
E-ISSN: 2580-8303

almost the same as the previous research conducted by researchers who obtained particle sizes of 0.127 - 0.538 µm with an average of 0.396 µm (Siregar & Sulistyawati, 2019).

Jelly candy evaluation

a) Raw Material Inspection Results

Examination of raw materials carried out organoleptic and solubility in hydroxyapatite as an active substance with white powder and practically insoluble in aquadest and ethanol 95%. Sodium benzoate as a preservative, white in color, odorless and soluble in aquadest. Benzoic acid as a preservative, hablur powder, colorless, and odorless and soluble in aquadest. Citric acid as a taster of acid, white powder, odorless and very easily soluble in aquadest. Gelatin as a gel shaper and candy base, pale yellowish in color and soluble in hot water. Cocopandan syrup as a sweetener and dye, red liquid, and menthol as a flavor giver and mint aroma.

b) Jelly Candy Evaluation

Evaluation on Jelly Candy preparations was conducted to see which formula was best and meets the requirements of Jelly Candy in the National Agency Standard (SNI) No. 3547.2-2008 including weight uniformity test, organoleptic test, pH test, hedonic test, kapang khamir test, total plate number test, gel strength, homogeneity test and stability test.

A weight uniformity test was conducted to see the uniformity of the size of gummy candy preparations made, all formulas of hydroxyapatite jelly candy preparations 6 candies deviate from the condition of weight uniformity that should not be more than 5%. Factors that affect the uniformity of weights are shape, print and temperature (Firdaus *et al*., 2014). In addition, during the heating process causes the amount of water that evaporates in each formula is not the same and at the time of pouring printed or printing techniques. If the diameter and thickness are not uniform it will affect the number of doses of active substances (Syukri *et al*., 2018). Weight uniformity test in all formulation summarizes in Table 2.

Jelly candy without hydroxyapatite has a very striking red color, for jelly candy that uses hydroxyapatite has a pink color, this is due to the addition of hydroxyapatite substances affect the color quality of jelly candy (Figure 3). The addition of calcium carbonate to milk jelly candy affects the Brightness of milk jelly candy (Lesmana *et al*., 2008) which means the addition of calcium carbonate affects the Brightness of milk jelly candy which is getting murky due to insoluble calcium. The observation result of organoleptic test present in Table 3.

Table 3. Organoleptic test

Formulation	The Observation Result			
	Smell	**Color**	**Taste**	**Texture**
F0	Mint	Red	Sweet Mint	Chewy
F1	Mint	Pink	Sweet Mint	Chewy
F2	Mint	Pink	Sweet Mint	Chewy
F3	Mint	Pink	Sweet Mint	Chewy

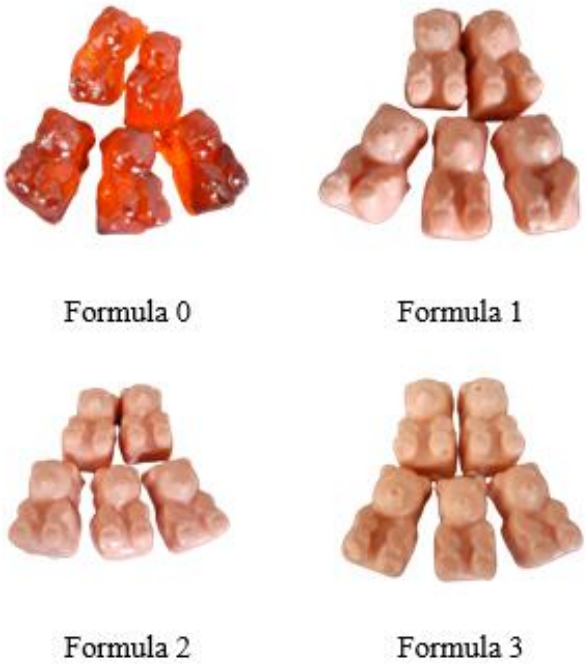


Figure 3. Jelly candy hydroxyapatite fish bone

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 2. Weight uniformity test in all formulation

No	F0	%D	F1	%D	F2	%D	F3	%D
1	1.6021	5.5309%*	1.9212	4.5745%	1.7285	4.7133%	1.9934	8.6084%*
2	1.6594	2.1522%	1.9242	4.4255%	1.7685	2.5082%	1.9647	7.0447%*
3	1.6675	1.6746%	1.9133	4.9669%	1.7866	1.5104%	1.8205	0.8118%
4	1.6369	3.4789%	1.9937	0.9735%	1.7743	2.1888%	1.8421	0.3650%
5	1.6325	3.7384%	1.9258	4.3460%	1.8216	0.4189%	1.8737	2.0867%
6	1.6028	5.4897%*	1.9649	2.4040%	1.8525	2.1223%	1.8174	0.9807%
7	1.6203	4.4878%	2.0653	2.5828%	1.7039	6.0694%*	1.8252	0.5557%
8	1.6228	4.3103%	2.0227	0.4668%	1.8645	2.7839%	1.8058	1.6127%
9	1.7554	3.5084%	2.0562	2.1308%	1.8223	0.4575%	1.8347	0.0381%
10	1.6664	1.7394%	2.0445	1.5496%	1.7851	1.5931%	1.8093	1.4220%
11	1.7098	0.8196%	2.0164	0.1539%	1.7627	2.8280%	1.8694	1.8524%
12	1.6817	0.8373%	2.0586	2.2500%	1.8951	4.4707%	1.8139	1.1714%
13	1.7474	3.0367%	2.0518	1.9122%	1.7317	4.5369%	1.8240	0.6211%
14	1.7140	1.0672%	2.0403	1.3410%	1.8611	2.5964%	1.8764	2.2338%
15	1.6892	0.3950%	2.0972	4.1672%	1.8436	1.6328%	1.8043	1.6944%
16	1.7081	0.7193%	2.0458	1.6142%	1.7583	3.0705%	1.8341	0.0708%
17	1.7414	2.6829%	2.0813	3.3775%	1.8087	0.2921%	1.8217	0.7464%
18	1.6485	2.7949%	2.0099	0.1688%	1.8503	2.0011%	1.8065	1.5745%
19	1.7941	5.7904%*	1.9156	4.8527%	1.8360	1.2127%	1.8173	0.9861%
20	1.7546	3.4612%	1.9824	1.5347%	1.8171	0.1708%	1.8273	0.4413%
21	1.6654	1.7984%	2.0436	1.5049%	1.8437	1.6372%	1.8176	0.9698%
22	1.7446	2.8716%	2.0538	2.0116%	1.8382	1.3340%	1.8007	1.8905%
23	1.7655	4.1040%	2.0111	0.1092%	1.8071	0.3803%	1.8325	0.1580%
24	1.7196	1.3974%	2.0788	3.2533%	1.8749	3.3572%	1.8089	1.4438%
25	1.7344	2.2701%	2.0470	1.6738%	1.8468	1.8081%	1.8263	0.4958%
26	1.7673	4.2101%	2.0081	0.2582%	1.8215	0.4134%	1.8223	0.7137%
27	1.7438	2.8244%	2.0354	1.0977%	1.8067	0.4024%	1.8150	1.1114%
28	1.7408	2.6475%	1.9817	1.5695%	1.8156	0.0882%	1.8139	1.1714%
29	1.6993	0.2004%	2.0933	3.9735%	1.8285	0.7993%	1.8173	0.9861%
30	1.6427	3.1369%	1.9138	4.9421%	1.8642	2.7673%	1.8260	0.5121%
Average	1.6959 ± 0.0544		2.0133 ± 0.0574		1.8140 ± 0.0462		1.8354 ± 0.0435	

Note : F0 : Formulation jelly candy without hydroxyapatite
F1 : Formulation jelly candy with hydroxyapatite 18%
F2 : Formulation jelly candy with hydroxyapatite 19%
F3 : Formulation jelly candy with hydroxyapatite 20%
%D : % deviation
* : Deviation > 5%

The test results of each hydroxyapatite jelly candy formula have a pH value range between 4.8 - 6.7 (Figure 4). The pH test is conducted to determine the pH value of the preparations that have been made. pH testing is important to know the acidity level of a product because acidity levels can affect consumer preference (Susanti *et al*., 2019).

pH testing in F1 obtained a more acidic pH compared to formulas that use hydroxyapatite, this is thought to be influenced by hydroxyapatite, because hydroxyapatite produced is made in a state of pH 12 which means more alkaline and can increase the pH value in hydroxyapatite jelly candy. The pH value of jelly candy is pH 4.3 to pH 6 (Lees, 1973), which means that hydroxyapatite jelly candy preparations enter the criteria.

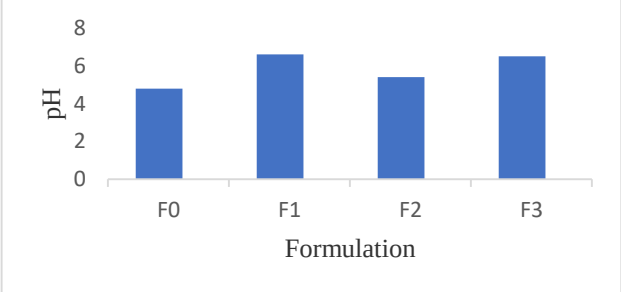


Figure 4. pH test of jelly candy hydroxyapatite mackerel fish bone

The hedonic test due to observe the respondent's preference in jelly candy hydroxyapatite mackerel fish bone. The hedonic test in smell indicated that F2 has the highest percentage in like jelly candy around 40%. On the other hand, F1 has the most significant percentage in

P-ISSN: 2406-9388
E-ISSN: 2580-8303

dislike jelly candy hydroxyapatite, about 30% (Figure 5).

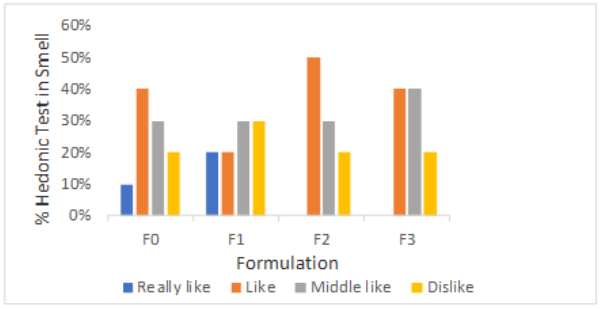


Figure 5. The result of the hedonic test in smell

Based on the result of hedonic test in color, the really like respondents were in F0 (formulation without hydroxyapatite) and the middle like respondents of the jelly candy were in F2 and F3 about 40%. The more hydroxyapatite added in the formulation, the jelly candy become soft pink and not eye-catching anymore like F0. However, the percentage of respondents dislike jelly candy hydroxyapatite were in F1 (Formulation 1) around 20% (Figure 6).

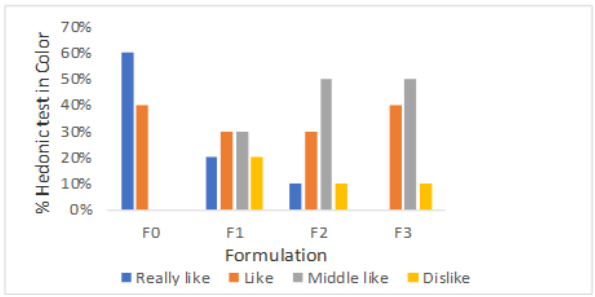


Figure 6. The result of hedonic test in color

Regarding this result of hedonic test in taste (Figure 7), the respondents like the taste of formulation 3 (F3) around 40%. However, the taste of jelly candy that the respondents disliked was in F1 and F2, about 40%. Meanwhile, the really like respondents in the texture of jelly candy (Figure 8) were in F0 by 40% and the dislike respondents was in F2 and F3 around 30%. Based on the result of hedonic test, F0 was the best formulation in color, taste, and texture.

The test of the kapang khamir test and total plate figures (Table 4) were obtained that all formulas of hydroxyapatite jelly candy are still below the standard limit of the time according to SNI 3547.2-2008 (Badan Standarisasi Nasional, 2008).

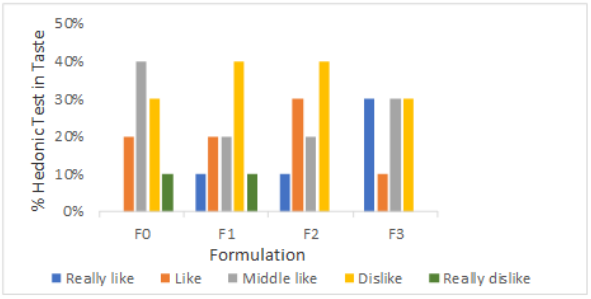


Figure 7. The result of hedonic test in taste

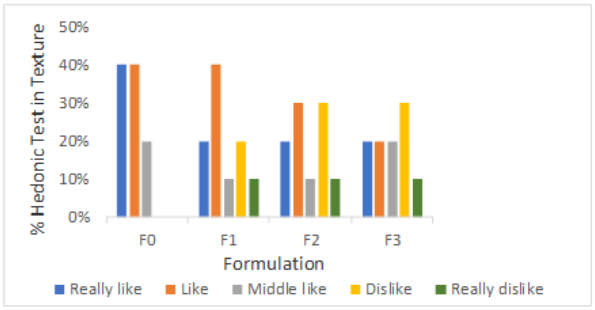


Figure 8. The result of hedonic test in texture

Regarding the results of the test of khamir time, it turns out that the sample produced the number of colonies that met the limit of khamir cup contamination on jelly candy preparations. Based on SNI, a jelly candy was safe if the total contamination of khamir time is not more than 1 x 10^2 colonies/g and the maximum ALT is 5 x 10^4 colonies/g, while in the test results of weak contamination and ALT is in the range of 1 x 10^1 to 2 x 10^1 and for ALT ranges from 2x10^1 to 8.5 x 10^2 then from the test results obtained shows that the preparation of hydroxyapatite jelly candy was not dangerous to be consumed because it does not exceed the standard limit of SNI 3547.2-2008 (Badan Standarisasi Nasional, 2008). Based on gel strength test results, the more hydroxyapatite added the higher the strength of the gel (Table 5).

Table 5. Gel strength test result

No	Formula	Gel Strength (N)
1.	F0	0.2173 N
2.	F1	1.8689 N
3.	F2	5.0647 N
4.	F3	6.3446 N

Table 4. Kapang Khamir Test and Total Plate Number Test Result

Formula	Kapang Khamir Result		Total Plate Number Test	
	Test Result (koloni/g)	Standard (koloni/g)	Test Result (koloni/g)	Standard (koloni/g)
F0	0	1 x 10^2	2 x 10^1	5 x 10^4
F1	1 x 10^1	1 x 10^2	10 x 10^1	5 x 10^4
F2	0	1 x 10^2	3 x 10^1	5 x 10^4
F3	2 x 10^1	1 x 10^2	85 x 10^1	5 x 10^4

P-ISSN: 2406-9388
E-ISSN: 2580-8303

The addition of different calcium concentrations may increase the strength value of the gel (Dewi *et al*., 2020). Increased moisture content can decrease hardness, where water will diffuse into the gel (Muhandri & Subarna, 2009). So that the gel formed becomes softer and causes the hardness to decrease.

A homogeneity test (in Figure 9) is done to find out whether the spread evenly or not hydroxyapatite in jelly candy. The test results were obtained that hydroxyapatite is distributed evenly in jelly candy even though jelly candy.

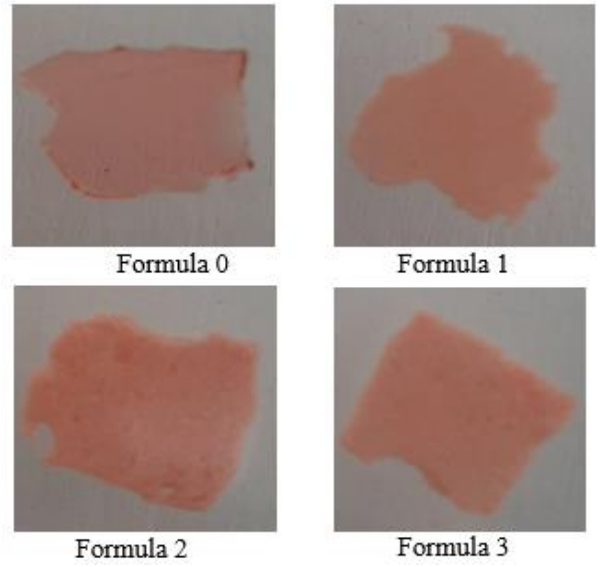


Figure 9. Homogeneity test

Jelly candy hydroxyapatite conducted observations stored at room temperature for three weeks, the testing of which included organoleptic examination of the taste, color, aroma, texture, and pH of jelly candies. Hydroxyapatite jelly candy changes every week. Storage of jelly candy changes on the 7th to 2nd day, where the change occurs in aroma, shape and taste while for the color in the jelly candy hydroxyapatite does not change from the 0th day to the 21st day. On the 14th day the aroma of mint begins to fade because the mint derived from menthol evaporates with increasing days. Formula 2 and formula 3 on the 7th and 21st days undergo changes in texture, this is because formulas 2 and 3 have more hydroxyapatite additions compared to formula 1. This is in line with the research that has been done that mentions the addition of calcium has an effect on the hardness of jelly candy (Lesmana *et al*., 2008).

The addition of calcium concentration impacts the gel's strength (Dewi et al., 2020), and the moisture content increases the gel's hardness (Muhandri & Subarna, 2009). In this study, formulas 2 and 3 have less moisture content than the formula 0 and 1, causing the jelly candy to harden on the 7th and 21st days. For the taste of hydroxyapatite jelly candy obtained changes on the 14th and 21st days in formulas 2 and 3; the change is the decrease in the taste of mint in preparations that cause the taste of sweet candy only; this is thought to be due to the addition of highly relevant hydroxyapatite resulting in the fading of mint on preparations on days 2 - 14 and 21. On the pH examination of hydroxyapatite, jelly candy obtained a stable pH value at pH 4 to 6.

The storage test of jelly candy hydroxyapatite was observed in the stability of the formulation, which stored at room temperature for 3 weeks and tested every week; where the observations consisted of several parameters are, including organoleptic test color, smell, texture and taste) and pH test (Figure 10). This is examined to guarantee that the jelly candy before and after the preparation was still in good parameters during the storage time. Based on Table 6, the changes occurred from the smell, texture and taste after the 7th day until the 21st day. However, there were no differences in the color from the 0 day until the 21st day. Formulations 2 and 3 were altered on the 7th day and 21st day, respectively, due to the more hydroxyapatite quantity in this formula. This research is in accordance with Lesmana *et al*. (2008), who stated that the addition of calcium affects the hardness of jelly candy.

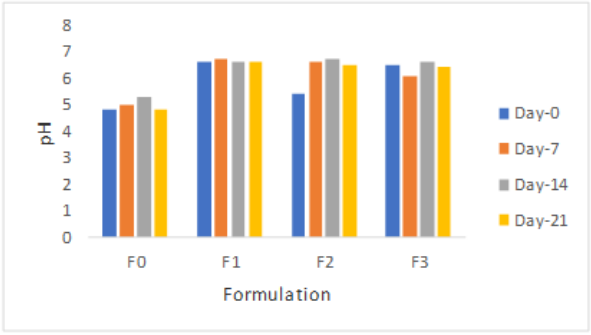


Figure 10. Storage test in pH

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 6. Storage test in organoleptic

Temperature	Observation	Formulation	Day			
			0	**7**	**14**	**21**
Room Temperature	Color	F0	Red	Red	Red	Red
		F1	Pink	Pink	Pink	Pink
		F2	Pink	Pink	Pink	Pink
		F3	Pink	Pink	Pink	Pink
	Smell	F0	Mint	Mint	Mint	Syrup
		F1	Mint	Mint	Mint	Syrup
		F2	Mint	Mint	Syrup	Syrup
		F3	Mint	Mint	Syrup	Syrup
	Texture	F0	Chewy	Chewy	Chewy	Chewy
		F1	Chewy	Chewy	Chewy	Chewy
		F2	Chewy	Chewy	Chewy	Chewy
		F3	Chewy	Chewy	Chewy	Chewy
	Taste	F0	Sweet Mint	Sweet Mint	Sweet Mint	Sweet Mint
		F1	Sweet Mint	Sweet Mint	Sweet Mint	Sweet Mint
		F2	Sweet Mint	Sweet Mint	Sweet	Sweet
		F3	Sweet Mint	Sweet Mint	Sweet	Sweet

CONCLUSION

Based on the results of this study, we can conclude that Hydroxyapatite Mackerel Fish Bone can be made as Jelly Candy, with the best concentration of Hydroxyapatite was 18%.

AUTHOR CONTRIBUTIONS

Conceptualization, L. A., S. P., R. A., R.; Methodology, L. A.; Validation, L. A., S. P., R.; Formal Analysis, L. A., S. P., R. A., R.; Investigation, L. A., S. P., R. A., R.; Resources, L. A., S. P., R. A., R.; Data Curation, L. A., S. P., R. A., R.; Writing - Original Draft, L. A., S. P., R. A., R.; Writing - Review & Editing, L. A., S. P., R. A., R.; Visualization, R.A.; Supervision, L. A., S. P.; Project Administration, L. A., S. P., R. A., R.; Funding acquisition, L. A., S. P., R. A., R.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Ahmad, I. (2017). Pemanfaatan Limbah Cangkang Kerang Darah (*Anadara granosa*) Sebagai Bahan Abrasif Dalam Pasta Gigi. *Jurnal Galung Tropika*; *6*; 49–59. doi: 10.31850/JGT.V6I1.210.

Alridho B, Ali, A. & Rossi, E.. (2017). Pembuatan Permen Jelly Ektrak Jahe Merah dengan Penambahan Karagenan. *Jom Faperta Ur*; *4*; 1–13.

Andasari, S. D., Zukhri, S. & Nurjanah, P. (2018). Formulasi Permen Jelly Bunga Turi (*Sesbania grandiflora* L.) dengan Variasi Kadar Gelatin dan Karagenan. *Cerata Jurnal Ilmu Farmasi*; 9; 26–38.

Anggresani, L., Perawati, S. & Rahayu, I. J. (2018). Limbah Tulang Ikan Tenggiri (*Scomberomorus guttatus*) sebagai Sumber Kalsium pada Pembuatan Hidroksiapatit. *Jurnal Katalisator*; *4*; 153–161.

Anggresani, L., Sari, Y. N. & Rahmadevi, R. (2021). Hydroxyapatite (HAp) from Tenggiri Fish Bones as Abrasive Material In Toothpaste Formula. *Jurnal Kimia Valensi*; *7*; 1–9. doi: 10.15408/jkv.v7i1.19165.

Noviyanti, A. R., Haryono & Rinal, P. D. R. E. (2017). Cangkang Telur Ayam sebagai Sumber Kalsium dalam Pembuatan Hidroksiapatit untuk Aplikasi Graft Tulang. *Chimica et Natura Acta*; *5*; 107–111.

Badan Standarisasi Nasional. (2008). Kembang gula – Bagian 2: Lunak. Jakarta: Badan Standarisasi Nasional.

Budiatin, A. S., Khotib, J., Hasmono, D. & Samirah. (2016). Injektabel Komposit Hydroksiapatit-Gelatin sebagai Sistem Penghantaran Alendronat. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*; *3*; 1-6.

Chadijah, S., Hardiyanti & Sappewali. (2018). Sintesis dan Karakterisasi Hidroksiapatit dari Tulang Ikan Tuna (*Thunnus albacores*) dengan XRF, FTIR, dan XRD. *Al-Kimia*; *6*; 178-184.

Dewi, I. K., Wijayanti, I. & Kurniasih, R. A. (2020). Pengaruh Nanokalsium terhadap Kekuatan Gel Kamaboko Ikan Mujair (*Oreochromis mossambicus*). *AgriTech*; *40*; 91–101.

Fadhila, M., Umar, S. & Zaini, E. (2020). Pembentukan Kokristal Asam Usnat – N-Methyl-DGlucamine dengan Metode Penguapan Pelarut dan Pengaruhnya terhadap Penurunan Interleukin-8 pada Tikus Inflamasi. *Jurnal Sains Farmasi & Klinis*; *7*; 23-30. doi: 10.25077/jsfk.7.1.23-30.2020.

Firdaus, F., Islamaya, W. & Fajriyanto, F. (2014) Formulasi Nutraseutikal Sediaan Gummy Candies Sari Buah Belimbing Manis (*Averrhoa carambola* L) dengan Variasi Kadar Manitol dan Corn Syrup sebagai Basis. *Teknoin*; *20*; 1–11. doi: 10.20885/teknoin.vol20.iss1.art5.

Hanura, A. B., Trilaksani, W. and Suptijah, P. (2017) Karakterisasi Nanohidroksiapatit Tulang Tuna Thunnus sp Sebagai Sediaan Biomaterial. *Jurnal Ilmu dan Teknologi Kelautan Tropis*; *53*; 619–629. doi: 10.1017/CBO9781107415324.004.

Haris, A., Fadli, A. and Yenti, S. R. (2016). Sintesis Hidroksiapatit dari Limbah Tulang Sapi menggunakan Metode Presipitasi dengan Variasi Rasio Ca/P dan Konsentrasi H3PO4. *Jom Fteknik; 3*; 1–11.

Hernawan, A. D., Anggresani, L. & Meirista, I. (2021). Formulasi Pasta Gigi Hidroksiapatit dari Limbah Tulang Ikan Tenggiri (*Scomberomorus guttatus*). *Chempublish Journal*; *6*; 34–45.

Jauhari, M., Thonthowi & Santoso, S. A. (2019). Asupan Protein dan Kalsium Serta Aktivitas Fisik pada Anak Usia Sekolah Dasar. *Ilmu Gizi Indonesia*; *2*; 79–88.

Lees, R. (1973) Sugar Confectionary and Chocolate Manufacture. New York: Springer.

Lesmana, S. N., Putut, T. I. & Kusumawati, N. (2008) Pengaruh Penambahan Kalsium Karbonat sebagai Fortifikan Kalsium terhadap Sifat Fisikokimia dan Organoleptik Permen Jeli Susu. *Jurnal Teknologi Pangan dan Gizi*; 7; 28–39. doi: 10.33508/JTPG.V7I1.148.

Mozartha, M. (2000). Hidroksiapatit dan Aplikasinya di Bidang Kedokteran Gigi. *Journal of Visual Languages & Computing*; *11*; 287–301.

Muhandri, T. & Subarna. (2009). Pengaruh Kadar Air NaCl dan Jumlah Passing terhadap Karakteristik Reologi Mi Jagung. Jurnal Teknologi dan Industri Pangan; *20*; 71-77.

Munasir, Triwikantoro, T., Zainuri & Darminto. (2012). Uji XRD dan XRF pada Bahan Meneral (Batuan dan Pasir) sebagai Sumber Material Cerdas ($CaCO_3$ dan SiO_2). *Jurnal Penelitian Fisika dan Aplikasinya (JPFA)*; *2*; 20-29. doi: 10.26740/jpfa.v2n1.p20-29.

Mutmainnah, M., Chadijah, S. & Rustiah, W. O. (2017) Hidroksiapatit dari Tulang Ikan Tuna Sirip Kuning (*Tunnus albacores*) dengan Metode Presipitasi. *Al-Kimia*; *5*; 119–126. doi: 10.24252/al-kimia.v5i2.3422.

Rana, M., Akhtar, N., Rahman, S., Jamil, H. M. & Asaduzzaman, S. M. (2017). Extraction of Hydroxyapatite from Bovine and Human Cortical Bone by Thermal Decomposition and Effect of Gamma Radiation: A Comparative Study. *International Journal of Complementary & Alternative Medicine*; *8*; 1-10. doi: 10.15406/ijcam.2017.08.00263.

Sunil, S., Sharma, U. K. & Arathy, S. A. (2020). Pharmaceutical Jellies: a Novel Way of Drug Delivery. *Journal of Pharmaceutical Sciences and Research*; *12*; 904–909.

Setiawan, D. & Basit F, M. (2012). Sintesis dan Karakterisasi Hidroksiapatit Untuk Aplikasi Sinovektomi Radiasi. *Jurnal Forum Nuklir*; *6*; 120-126. doi: 10.17146/jfn.2012.6.2.3338.

Shita, A. D. P. & Sulistiyani. (2010). Pengaruh Kalsium terhadap Tumbuh Kembang Gigi Geligi Anak. *Stomatognatic (J. K. G Unej)*; *7*; 40–44.

Siregar, R. F. and Sulistyawati, E. (2019). Karakteristik Hidroksiapatit Porous dari Prekursor Cangkang Keong Sawah dan Bahan Porogen Pati Sukun. *Eksergi*; *16*; 59-63. doi: 10.31315/e.v16i2.3082.

Stötzel, C., Müller, F. A., Reinert, F., Niederdraenk, F., Barralet, J. E. & Gbureck, U. (2009). Ion Adsorption Behaviour of Hydroxyapatite with Different Crystallinities. *Colloids and Surfaces B: Biointerfaces*; 74; 91–95. doi: 10.1016/j.colsurfb.2009.06.031.

Susanti, K. I. A., Tamrin & Asyik, N. (2019). Pengaruh Penambahan Sari Jahe Gajah (*Zingiber officinale*) terhadap Organoleptik, Sifat Fisik dan Kimia dalam Pembuatan Permen Jelly Daun Katuk (*Sauropus androgynus*). *Jurnal Sains dan Teknologi Pangan*; *4*; 2073–2085.

Syukri, Y., Wibowo, J. T. & Herlin, A. (2018). Pemilihan Bahan Pengisi untuk Formulasi Tablet Ekstrak Buah Mahkota Dewa (Phaleria macrocarpa Boerl). *JSFK (Jurnal Sains Farmasi & Klinis)*; *5*; 66–71. doi: 10.25077/JSFK.5.1.66-71.2018.

Wadu, I. *et al.* (2015). Pasta Gigi Pencegah Gigi Berlubang Berbahan Aktif Mikro Hidroksiapatit (Hap) dari Limbah Kerabang Telur Pasar Raya

Kota Salatiga. *Prosiding Seminar Nasional*; *2018*; 116–124.

Wang, P., Li, C., Gong, H., Jiang, X., Wang, H. & L, K. (2010). Effects of Synthesis Conditions on the Morphology of Hydroxyapatite Nanoparticles Produced by Wet Chemical Process. *Powder Technology*; *203*; 315–321. doi: 10.1016/j.powtec.2010.05.023.

Warna, N. S., Fadli, A. & Irdoni (2015). Sintesis Hidroksiapatit dari Cangkang Telur dengan Metode Presipitasi. *Jurnal Online Mahasiswa Fakulas Teknik*; *2*; 1–11.

Yuliana, R., Rahim, E. A. & Hardi, J. (2017). Sintesis Hidroksiapatit dari Tulang Sapi dengan Metode Basah pada Berbagai Waktu Pengadukan dan Suhu Sintering. *KOVALEN: Jurnal Riset Kimia*; *3*; 201–210.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 290-297
DOI: 10.20473/jfiki.v9i32022.290-297
Available online at https://e-journal.unair.ac.id/JFIKI/

Physicochemical Properties and Antioxidant Activity of Three Types of Monofloral Honey from Indonesia

Sulistyianingsih[1], Achmad Toto Poernomo[2], Riesta Primaharinastiti[2]*
[1]Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: r.nastiti@gmail.com

Submitted: 2 July 2022
Accepted: 21 November 2022
Published: 9 December 2022

Abstract

Background*: In addition to minerals, honey contains carbohydrates (glucose and fructose), protein, amino acids, water, enzymes, ash, vitamins, and other substances. Compounds of honey can affect the chemical properties of honey. Knowing the physicochemical properties of honey is very important because physicochemical properties affect the quality of honey. One of the biological activities of honey is an antioxidant. Antioxidants can interfere with oxidative processes, prevent disease, and play an important role in the body's defence system.* ***Objective****: to determine and compare physicochemical properties (color, viscosity, ash content, water content, reducing sugar (glucose), total phenolic compound, HMF) and antioxidant activities of monofloral honey samples from Indonesia.* ***Methods****: The color of honey are categorized using the Pfund scale. Viscosity measurement is carried out using a Brookfield viscometer. The water content is carried out using a refractometer. Phenolic content and antioxidant activities analysis were carried out by UV-VIS spectrophotometer.* ***Results****: The results show that rambutan honey from Malang has the highest physicochemical properties and antioxidant activity, which had an amber color, water content of 21.7% b/b, acidity 20.7 mL NaOH/Kg, viscosity of 33.08 poise, ash content of 0.17% b/b, reducing sugar 69.38%b/b, total phenolics content 533.7 mg GAE/Kg sample and IC_{50} 0.111 µg/mL.* ***Conclusion****: The quality of honey varies from region to region. The best honey (according to SNI) is rambutan honey from Malang.*

Keywords*: honey, physicochemical properties, antioxidant activity, total phenolics*

How to cite this article:
Sulistyianingsih, Poernomo, A. T. & Primaharinastiti, R. (2022). Physicochemical Properties and Antioxidant Activity of Three Types of Monoflora Honey from Indonesia. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 9(3), 290-297. http://doi.org/10.20473/jfiki.v9i32022.290-297

INTRODUCTION

Honey is a natural liquid, that is generally sweet tasting and produced by bees (*Apis* sp.) from floranectars or other parts of the plant (SNI, 2013). Honey is widely circulated and produced in Indonesia, and can be divided into monofloral and multifloral. Monofloral honey is obtained from *Apis cerana* or *Apis mellifera* bees with feed derived from one type of nectar source. This honey is commonly named based on the source of nectar, such as kelengkeng honey, rambutan honey, randu honey, and other. Monofloral honey has a specific aroma, flavor and color based on the source of honey (Suranto, 2007). Results from several studies showed that monofloral honey, which is randu honey, kelengkeng honey, and rambutan honey had different physicochemical properties (Chayati, 2008).

Honey contains not only minerals but also carbohydrates, protein, water, ash, small of vitamins, enzymes, amino acids, and other substances (Buba et al., 2013). The mineral contents in honey are Cr, Na, Al, Ni, Ca, K, Mg, Zn, Co, Cu, and Fe (Conti et al., 2014. The compounds in honey will affect physicochemical properties of honey. Knowing the physicochemical properties of honey is very important because physicochemical properties affect the quality of honey. Several areas in Indonesia are known to produce monofloral honey are Kediri, Malang and Bogor. *Ceiba pentandra* (randu) honey, *Dimocarpus longan* (kelengkeng) honey, and *Nephelium lappaceum* (rambutan) honey are types of honey that are produced continuously in Indonesia.

Empirically, honey has long been used as a component in traditional medicine in various countries. One of the biological activities of honey is an antioxidant. Natural antioxidants protect the body from the attack of free radicals, and can delay the onset of chronic diseases (Wahdaningsih *et al.*, 2011). Based on RISKESDAS (2007), the prevalence of degenerative diseases such as heart disease, hypertension, stroke, tumors, and diabetes were 16.1; 53.7; 20.2; 8.8; and 3.7%. Several studies have been done to determine the antioxidant activity of honey against chronic diseases. Research by Saputra & Wulan (2015) proves that honey with antioxidant activity can reduce the risk of chronic obstructive pulmonary disease and lung cancer. The antioxidant potential of manuka honey, a type of honey that has been registered as a wound care product, contain methyl syringate (a type of phenolic compound), which is considered to be quite potential to interfere with the process of amplification of inflammation by ROS (Molan, 2011). Manuka honey is also commonly used for cosmetic treatments (Suranto, 2004). Vallianoul *et al.*, (2014), stated that antioxidants in manuka honey play a role in cleaning the skin, eliminating skin discoloration, and increasing skin elasticity. Research (Chayati & Isnatin, 2015) shows differences in the antioxidant activity of monofloral honey originating from Central Java, which is rambutan, kelengkeng, coffee, randu, and calliandra honey, respectively, 11.9; 8.73; 5.56; 13.1 and 48.0%.

Given the many benefits of honey to human health, the purpose of this study is to identify and compare physicochemical parameters (viscosity, color, ash content, water content, reducing sugar (glucose), acidity, total phenolic content, Hydroxymethylfurfural (HMF), and antioxidant activities of monofloral honey from Indonesia.

MATERIALS AND METHODS

Materials

Randu honey from Kediri, kelengkeng honey from the cultivation of National Beekeeping Center (Pusbahnas) Bogor, and rambutan honey from Malang, DPPH (2,2-difenil-1-pikrilhidrazil) p.a (Sigma-Aldrich.), aquadest, gallic acid p.a (Sigma. Co.), Folin-Ciocalteau (E. Merck), ethanol p.a (E. Merck), hydrochloric acid 37% (E. Merck), dan $AlCl_3$ 10% (E. Merck).

Instruments

Spektrofotometer UV-Vis Shimadzu UV 260 and Brookfield DV3TLV digital viscometer.

Method

Color analysis

One hundred milliliter of honey was placed in a clear glass jar with a bright enough light and compared with the standard. The colors of honey are categorized with the Pfund scale.The color scale are divided into seven levels, which are dark amber, amber, light amber, extra light amber, white, extra white, and water white (White, 1984).

Viscosity

Viscosity measurements were carried out using the Brookfield DV3TLV Digital Viscometer. The honey sample was put into a special container on the viscometer. The rotor is dipped in the honey sample.

Ash content

The cup that has been heated in the oven at 105°C for 24 hours is cooled in a desiccator and weighed. Two grams of honey were weighed and placed into the cup and weighed, then burned on a hot plate at 400°C until smokeless and turn to ashes. Then put into the furnace at a temperature of 550°C for 6 hours, then removed and

P-ISSN: 2406-9388
E-ISSN: 2580-8303

cooled in a desiccator. The cup was weighed, and the weight was recorded. Ash content analysis was replicated twice (Sudarmadji, 2007).

Water content

The water content measurement was carried out by a refractometer to read the index of refraction of a honey sample at a temperature of 20°C. The water content analysis was replicated twice. The water content of honey is determined by comparing the refractive index values of honey and water (SNI, 2018).

Reducing sugar (glucose)

Anhydrous Na_2CO_3 was weighed and dissolved in ± 300 mL of distilled water to make Luff's solution. Fifty grams of citric acid was added in 50 mL of distilled water while stirring. Twenty-five grams of $CuSO_4.5H_2O$ was added and dissolved in 100 mL of distilled water. The solution was transferred to a 1000 mL volumetric flask, and squeezed to the mark with distilled water, stored for 24 hours. Reducing sugar is determined by weighing 1.5 grams of honey and then putting it into a 500 mL erlenmeyer. 100 mL of 3% HCl was added and heated for 3 hours in an upright cooler. Then cooled and neutralized using 30% NaOH solution and a little 3% CH_3COOH. The solution was transferred to 500 mL volumetric flask to volume and filtered. 10 mL of the filter results in a 500 mL Erlenmeyer, then added 25 mL of luff solution and 15 mL of distilled water. Heated for 3 minutes, then cooled. 15 mL of 20% KI solution and 25 mL of 25% H_2SO_4 were added. Titrated with 0.1 N sodium thiosulfate solution and added a small amount of 0.5% starch solution. The same treatment was carried out for the blanks. Glucose level analysis was replicated twice. (SNI, 1992).

HMF Content

The honey sample was weighed as much as 5 g and put into a 50 mL volumetric flask, then dissolved with distilled water until the volume of the solution was 50 mL. The Carrez I solution and the Carrez II solution were added as much as 0.50 mL, shaken and diluted with aquadest to the line mark. Added a drop of alcohol to remove the foam on the solution's surface. The solution was filtered and the first 10 mL of the filter was discarded. The filter results were pipetted as much as 5 mL and put into 18 mL x 150 mL test tube. 5 mL of aquadest was pipetted and put into a tube for the sample solution and as a comparison, solution 5 mL of 0.20% sodium bisulfate was added, then homogenized until completely mixed, and the absorbance of the sample against the comparison was determined at a wavelength of 284 nm and 336 nm. HMF level analysis was replicated twice (SNI, 2018). The results were expressed in mg HMF / 100 g honey using the following formula:

$$\text{HMF} = \left(\frac{mg}{100}\ g\ madu\right) = \frac{A284 - A336\ x\ 14{,}97\ x\ 5}{bobot\ sampel\ (g)}$$

Acidity

Acidity analysis was carried out by weighing 10 grams of honey, then dissolving with 75 mL of CO2-free water in a 250 mL beaker, stirring with a stirring rod and inserting a pH meter to record the pH, titrated with 0.05 M NaOH at a rate of 5.0 mL/min. The titration was stopped when the pH reached 8.50. 0.05 M NaOH was taken with a 10 mL pipette and immediately titrated with 0.05 M HCl to pH 8.30. In the blank test, 75 mL of CO_2-free water was titrated with NaOH to pH 8.5. Acidity analysis was replicated twice (SNI, 2018).

Total phenolics content

Procedure for preparing a calibration curve: A standard solution of gallic acid (H_2O solvent) was prepared with a concentration of 5 to 25 ppm. One mL of each concentration was pipetted into a test tube. 0.5 mL of Folin-Ciocalteu was added and left for 5 minutes, and then 2 mL of 10% sodium carbonate solution was added.

Sample preparation: 0.5 g honey was weighed into a 25 mL volumetric flask and added water to the measuring line. 1.0 mL aliquot was pipetted into the vial and 0.5 mL Folin-Ciocalteu was added. The mixture was incubated for 5 minutes followed by addition of 2 mL 10% sodium carbonate. The mixture was further incubated for 10 minutes followed by absorbance measurement of 770 nm. The total content of phenolic compounds was expressed as gallic acid equivalents (mg GAE/Kg honey) using the standard curve for gallic acid. Total phenolics content analysis was replicated twice (Alfian & Susanti, 2012).

Antioxidant activity

One milliliter of the aqueous honey solution was mixed with 1 mL DPPH ethanol. The mixture was left in the dark for 30 minutes, and the absorbance was spectrophotometrically read at 519 nm. Ascorbic acid was used for calibration, and the results were expressed as mmol of ascorbic acid per Kg of honey. Antioxidant activity analysis was replicated twice. The antioxidant activity of honey can be read from the IC_{50} value. The IC_{50} value is the sample concentration value to measure the ability of the sample to consider its antioxidant activity to be 50% free radical. Antioxidant activity analysis was replicated twice (Chayati & Isnatin, 2015).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

RESULTS AND DISCUSSION

Honey color

Using the Pfund scale, honey can be categorized into dark amber, amber, light amber, extra light amber, white, extra white, and water white (Pontis *et al.*, 2014). Analysis of the test results for honey color parameters in Table 1 and Figure 1 shows that monofloral honey (randu, kelengkeng and rambutan) has a color from amber to extra light amber. The color of honey consists of water-soluble and fat-soluble fractions. Brightly colored honey has less water soluble than fat-soluble honey. The existence of an oxidation process causes a color change in honey (Adriani, 2011). The dye's persistence is caused by a mixture of several amino acids with iron from packaging or processing equipment (Sihombing, 2005). In this research, rambutan honey was darker than randu and kelengkeng honey. Indonesia has a very diverse vegetation that blooms regularly. This enables beekeepers to gather various single or multi-flowered honey in different colors. Previous studies have shown that transition metals react with organic compounds in honey to form colorful complexes. The darker honey color indicates higher total phenolic content and antioxidant activity (Harris, 2014).

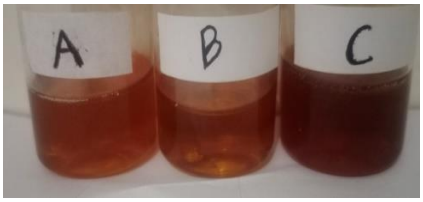


Figure 1. Color of (A) randu; (B) kelengkeng and (C) rambutan honey

Viscosity

The viscosity requirement based on SNI (Indonesian National Standard) is minimum 10 poise. The value of test results for viscosity parameter (Table 1) randu honey, kelengkeng and rambutan was 4.85, 75, and 3.31 poise. The viscosity of rambutan and kelengkeng honey the label has a viscosity value according to SNI standards. The viscosity of honey can be affected by temperature and water content. The higher the water content, the higher the liquid content of honey, and the lower the water content, the higher the density of honey. The viscosity of randu honey from Kediri is lower than the SNI standard. . This is because the water content in randu honey from Kediri is higher than kelengkeng and rambutan honey. Honey with high water content is very susceptible to fermentation because water can stimulate the growth and development of yeast cells. The condition of honey that has a higher water content or is more dilute (lower viscosity) can also cause a faster fermentation process which can change the taste of honey to become sour (Apriani, 2013).

Ash Content

Ash content is a mixture of inorganic or mineral components in a food ingredient. If the ash content is high, the mineral content is also high. Testing the ash content in honey needs to be done to determine the total mineral content of honey because each honey has a different mineral content. It depends on the source of soil and nectar around the bees (Sihombing, 2005). The honey quality requirement for ash content based on SNI 8664:2018 is a maximum 0.5% w/w. The higher the ash content of the sample, the higher the mineral content of the honey (Qadar *et al.*, 2015).

As a result of ash analysis, Randu honey from Kediri, kelengkeng honey from the cultivation of the National Beekeeping Center (Pusbahnas) Bogor, and rambutan honey from Malang, have the appropriate ash content with the standard set by SNI 8664:2018 which are 0.17; 0.10; 0.17 %w/w. This matter indicates that the mineral content in those areas is still quite good because still in accordance with the standards that have been set.

Water content

The results of the water content analysis show that each type of honey from different regions has different water content. The results show that kelengkeng honey from Bogor has moisture of 19% w/w. The reason is that the environmental temperature in the Bogor area is higher by 31.9°C (Badan Pusat Statistik, 2021), so honey in that area has low hygroscopic properties. Honey in this area is following with the water content standard in SNI 8664:2018. Therefore, honey in the Bogor area has good quality. Good quality honey is honey that contains water, about 17 - 21% (Sihombing, 2005).

The water content of randu honey from Kediri is 27.30%. These results indicate that the water content in this area is slightly higher than the honey quality requirements in SNI 8664:2018. This can be influenced by the environmental temperature in Kediri which is around 22.5°C (Badan Pusat Statistik, 2020). The water content of rambutan honey from Malang has a water content of 21.7%. The results of the water content in this area are in accordance with the water content standard in SNI 8664:2018. This is because Malang has an altitude of 498.48 above sea level and an environmental temperature of around 22.38°C (Badan Pusat Statistik, 2021). Low temperatures cause honey to absorb more water (Evahelda *et al.*, 2017). Therefore, the lower the

P-ISSN: 2406-9388
E-ISSN: 2580-8303

ambient temperature, the higher the moisture content in honey.

In addition, the level of maturity of honey that has not been perfect also affects the water content (Savitri, *et al.,* 2017). This is in accordance with the taking of honey in Kediri, which is not in accordance with the harvest time so that the maturity is not perfect. Generally, the honey harvest time that has been determined is at 11-12 days marked by a nest covered with beeswax (Fatma, *et al.,* 2017).

Reducing sugar (glucose)

Reducing sugar is an important parameter to determine the quality of honey. Honey has two important components, namely sugar and water. However, two types of sugar are more dominant, glucose and fructose as much as 70 - 80% and water 10 - 20% (Evahelda *et al*., 2017). The results for the analyzed level of reducing sugar (glucose) honey can be seen in Table 1. Requirements for reducing sugar content based on SNI are at least 65% w/w. The reducing sugar content produced in this study from randu honey, kelengkeng, and rambutan were 63.74; 72.36 and 69.53%b/w. These results stated that kelengkeng and rambutan honey complied with the requirements for glucose-reducing sugar levels. Randu honey with a value of 63.74% w/w does not meet the requirements for reducing sugar content because it is less than 65%/w. Alcohols reacting with oxygen can form reactions with acetic acid. The formation of acetic acid can cause an increase in acidity in honey (Kuntadi, 2013). The different types of plants that are a food source for bees to produce honey will affect the characteristics of honey, such as taste, aroma, color, quality, and sugar content in honey (Mulu *et al.,* 2004).

Acidity

Based on SNI 8664:2018 the maximum acidity value is 50 mL NaOH/Kg. The results of the acidity test on some honey samples Table 1 showed that kelengkeng and rambutan honey have acidity levels of 11.63 and 20.67; mL NaOH/Kg that had the acidity value according to SNI standards. Kelengkeng and rambutan honey have good quality because it indicates that microbes will not grow in the honey. This can be seen in the slightly thick honey texture (Savitri, *et al.,* 2017). Meanwhile, randu honey, has high acidity values, 70.0 mL NaOH/Kg. These results are not following SNI 8664:2018. High acidity values can be affected by the water content of honey. Honey is acidic and has a high water content which will increase fermentation. Increasing the fermentation process can produce an increasingly sour taste of honey and decrease the value of reducing sugar (glucose) (Prica & Balos, 2014). Storage at high humidity also affects the acidity of honey (Savitri, *et al.,* 2017). In addition, high acidity in honey can also be caused by unhygienic post-harvest processing, which can lead to honey being easily contaminated. It is very important to pay attention to the acidity level of honey to keep honey hygienic and safe for consumption (Karnia *et al.,* 2019).

HMF Content

HMF content analysis has been performed as a measure of honey quality and has served as a reference for some studies to determine the authenticity of honey. The HMF value is an indicator for measuring the freshness of honey, the heating process, and the shelf life. If the honey is stored for too long, HMF levels will increase (Suranto, 2004). This is because the C atoms of glucose, fructose, and monosaccharides decompose when heated and undergo levulinic oxidation to formic acid (Anjana, 2014). The permissible HMF level for honey is 40 mg/Kg according to Indonesian National Standard (SNI 01-8664-2018). Based on the presented results Table 1 HMF content of kelengkeng honey (27.86 mg/Kg) and rambutan honey (36.59 mg/Kg) were in accordance with the mentioned standards. Higher levels of HMF (76.75 mg/Kg) have been found in randu honey, and this significant increase can be attributed to inferior products due to overheating, improper storage, or the addition of inverted sugar syrup.

Excessive heating can cause HMF levels to increase (Minarti *et al.,* 2016). High levels of HMF in honey will reduce honey quality because the HMF content is related to several other chemical characteristics of honey, such as water content, pH, free acid content, reducing sugar content, and enzymatic activity in honey (Kowalski *et al.,* 2013). HMF levels increased during the heating process, with a decrease in water content, reducing sugar, diastase enzyme activity, and increased free acid levels.

Total phenolics content

Total phenol content was determined using the Folin-Ciocalteu method and standard gallic acid by UV-Vis spectrophotometry. Gallic acid plays an important role, so we use it as a comparative substance because it has a heteropolymer with three hydroxy phenol groups. Phenolic hydroxy groups will be oxidized by the Folin-Ciocalteu reagent under alkaline conditions. Folin-Ciocalteu reagent will oxidize gallic acid in its phenolic hydroxy group to form a molybdenum-tungsten complex with a blue color (Alfian & Susanti, 2012).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 1. Analysis results of physicochemical properties and antioxidant activity

Honey Samples	Physicochemical properties								Antioxidant Activity
	Color	Viscosity	Ash content	Water	Glucose	Acidity	HMF	Phenolic content	
Randu	Light amber	4.85	0.17 ± 5.6	27.3 ± 2.1	63.74 ± 4.5	70.0 ± 4.8	76.75 ± 1.6	465.9 ± 7.3	0.096 ± 2.1
Kelengkeng	Light amber	72.2	0.10 ± 0.14	19.0 ± 2.1	72.35 ± 3.7	11.6 ± 0.2	27.86 ± 3.8	272.4 ± 45.7	0.251 ± 8.8
Rambutan	Amber	33.08	0.17 ± 1.0	21.7 ± 0.9	69.38 ± 1.2	20.7 ± 5.4	36.59 ± 0.2	533.7 ± 14.0	0.111 ± 2.7

Note: The data represent the mean ± RPD (Relative Percent Different) data of the two replications

Total phenolic content is influenced by environmental factors such as light, rainfall, soil nutrients, altitude, and humidity. Besides that, total phenolic content is also affected by the cultivation process, such as fertilization, irrigation, and post-harvest treatment (Malinikova *et al.,* 2013). The results in this study showed that total phenolic compounds in rambutan honey (533.7 mg GAE/Kg honey) were higher than in randu honey and kelengkeng honey were 465.9 and 272.4 mg GAE/Kg honey (Table 1). According to Ferreira *et al.* (2009), dark honey was richer in phenolic compounds, and this was also confirmed in our study. Phenolic compounds are associated with antioxidant activity. Plants that have high phenolic compounds also have high antioxidant activity. Phenolic compounds protect antioxidants because phenolic compounds can scavenge the action of free radicals and react with reactive oxygen species (ROS) so that they no longer damage cells in the human body.

Antioxidant Activity

Antioxidant activity was determined by the DPPH method with a UV-Vis spectrophotometer. DPPH is a molecule containing unstable nitrogenous radicals that can bind with hydrogen ions, so it was used to test antioxidant activity. The presence of antioxidant compounds in the sample caused a color change of the methanol DPPH solution, which was initially dark purple to pale yellow. This color change occurs because DPPH is reduced, leading to electrons becoming paired (Zuraida *et al*., 2010).

In testing the antioxidant activity Table 1, IC_{50} values of randu, kelengkeng and rambutan were 0.095; 0.240 and 0.109 ppm. Kelengkeng honey from Bogor showed the highest DPPH radical scavenger activity compared with randu honey from Kediri and rambutan honey from Malang. Pointis *et al,* (2014) demonstrated a positive relationship between phenol concentration, antioxidant capacity and color of monoflora honey. The higher the concentration of phenolic compounds, the greater the antioxidant activity (Shahwar *et al.,* 2010). Capacity of phenolic antioxidants is affected by several factors, one of which is the functional group associated with the main structure. The study by Mohsen & Ammar (2009), showed that the radical scavenging activity tested on Phenolics is related to the number and position of hydroxyl group bonds (OH) in the molecule. The more hydroxyl groups are substituted in the molecule, the stronger the antioxidant capacity becomes because more hydrogen atoms can be generated (Yu Lin et al., 2009).

CONCLUSION

The quality of honey varies from region to region. The best honey (according to SNI) is rambutan honey from Malang has the highest physicochemical properties and antioxidant activity and has an amber color, water content of 21.7% b/b, acidity 20.7 mL NaOH/Kg, viscosity 33.08 poise, ash content 0.17 %b/b, reducing sugar 69.38 % b/b, total phenolics content 533.7 (mg/Kg GAE) and IC_{50} 0.111 ppm.

ACKNOWLEDGMENT

The authors would like to deliver to gratitude to Indonesia Endowment Found for Education (LPDP) Scholarship from the Ministry of Finance of the Republic of Indonesia. for the financial support.

AUTHOR CONTRIBUTIONS

Conceptualization, R. P.; Methodology, S., R. P., A. T. P.; Sotfware, A. T. P.; Validation, S.; Formal Analysis, S.; Investigation, S.; Resources, S.; Data Curation, S., R. P., A. T. P.; Writing - Original Draft, S.; Writing - Review & Editing, R. P., A. T. P.; Visualization, R. P.; Supervision, R. P.; Project Administration, R. P.; Funding acquisition, R. P.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

REFERENCES

Alfian, R. & Susanti, H. (2012). Penetapan Kadar Fenolik Total Ekstrak Metanol Kelopak Bunga Rosella Merah *(Hibiscus sabdariffa* Linn*)* dengan Variasi Tempat Tumbuh Secara Spektrofotometri. *Jurnal Ilmiah Kefarmasian*; *2*; 73-80.

Apriani. (2013). Studi Tentang Nilai Viskositas Madu Hutan dari Beberapa Daerah di Sumatera Barat untuk Mengetahui Kualitas Madu. *Pillar Of Physics Journal*; *2*; 91-98.

Badan Standardisasi Nasional (SNI). (2013). SNI 3545:2013. Madu. Jakarta: Badan Standardisasi Nasional.

Badan Standardisasi Nasional (SNI). (2018). SNI 8664:2018. Madu. Jakarta: Badan Standardisasi Nasional.

Buba F, Gidado A & Shugaba A. (2013). Analysis of Biochemical Composition of Honey Samples from North-East Nigeria. *Biochemistry and Analytical Biochemistry*; 2; 1-7. doi: 10.4172/2161-1009.1000139.

Chayati, I. (2008). Sifat Fisikokimia Madu Monoflora dari Daerah Istimewa Yogyakarta dan Jawa Tengah. *Agritech*; *28*; 9-14.

Chayati, I. & Isnatin, M. (2015). Kajian Kadar Flavonoid, Aktivitas Antioksidan, dan Kapasitas Antioksidan Madu Monoflora. *Mgmi*; *6*; 11-24

Conti, M. E., Maria, G. F., Luc, F., Giustino, M., Francesco, B. & Ivo, L. (2014). Characterization of Argentine Honeys on The Basis of Their Mineral Content and Some Typical Quality Parameters. *Chemistry Central Journa*l; *8*; 1-10.

Evahelda, E., Filli, P., Nura, M. & Budi, S. (2017). Sifat Fisik dan Kimia Madu dari Nektar Pohon Karet di Kabupaten Bangka Tengah, Indonesia. *Agritech*; *37*; 363-368. doi: 10.22146/agritech.16424.

Fatma, S. Haryanti & Agung, S. W. (2017) Uji Kualitas Madu pada Beberapa Wilayah Budidaya Lebah Madu di Kabupaten Pati. *Jurnal Biologi*; *6*; 58-65.

Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M. & Estevinho, L. M. (2009). Antioxidant Activity Of Portuguese Honey Samples: Different Contributions of The Entire Honey and Phenolic Extract. *Food Chemistry*; *114*; 1438-1443. doi: 10.1016/j.foodchem.2008.11.028.

Karnia, I., Hamidah, S. & Thamrin, G. A. R. (2019). Pengaruh Masa Simpan Madu Kelulut (*Trigona sp*) terhadap Kadar Gula Pereduksi dan Keasaman. *Jurnal Sylva Scienteae*; *2*; 1094-1099.

Kowalski, S., Łukasiewicz, M. & Berski W. (2013). Applicability of Physicochemical Parameters of Honey for Identification of The Botanical Origin. *Acta Scientiarum Polonorum Technologia Alimentaria*; *12*; 51-59.

Kuntadi. (2013). Pengaruh Umur Larva terhadap Potensi Kualitas Ratu yang Dihasilkan pada Penangkaran Lebah Ratu Apis cerana L. Hymenoptera: Apidae dengan Teknik Grafting. *Jurnal Entomologi Indonesia*; *10*; 1-6.

Malinikova, E., Kukla, J., Kuklová, M. & Balazova, M. (2013). Altitudinal Variation of Plant Traits: Morphological Characteristics in Rosaceae (*Fragaria vesca* L.). *Annals of Forest Research*; 56; 79–89. doi: 10.1108/09533239510093215.

Minarti, S. (2010). Ketersediaan Tepungsari dalam Menopang Perkembangan Anakan Lebah Madu *Apis mellifera* di Areal Randu (*Ceiba pentandra*) dan Karet (*Hevea brasilliensis*). *Jurnal Ternak Tropika*; *11*; 54-60.

Mohsen, S. M. & Ammar, A. S. M. (2009). Total Phenolic Contents and Antioxidant Activity of Corntassel extracts. *Journal of Food Chemistry*; *112*; 595–598.

Molan, P. C. (2011). The Evidence and the Rationale for the Use of Honey as Wound Dressing. *Wound Practice and Research*; *19*; 204-220.

Mulu, A., Tessema, B. & Derbie, F. (2004). In Vitro Assesment of the Antimicrobial Potential of Honey on Common Human Pathogens. *The Ethiopian Journal of Health Development*; *18*; 107-112.

Pontis, J.A., Costa, L.A.M.A.D., Silva, S.J.R.D. & Flach, A. (2014). Color Phenolic and Flavour Content and Antioxidant Activity of Honey from Roraima Brazil. *Journal of Food Science and Technology*; *34*; 69-73.

Prica, N. & Balos, M. Z. (2014). Moisture ad Acidity as Indicator's of The Quality of Honey Originating from Vojvodina Region. *Arhiv Veterinarske Medicine*; *7*; 99-109.

Qadar, S., Noora, A. & Maminga. (2015). Karakteristik Fisika Kimia madu Hutan Desa Terasa. *Jurnal Techno*; *4*; 37-41.

RISKESDAS. (2007). Laporan Hasil Riset Kesehatan Dasar 2007. Jakarta: Badan Penelitian dan Pengembangan Kesehatan, Riset Kesehatan Dasar, Departemen Kesehatan Republik Indonesia.

Saputra, T. & Wulan, A. (2015). Madu ssebagai Pencegah Penyakit Paru Obstruksi Kronik. *Majority*; *5*; 37-42.

Savitri, N. P. T., Hastuti, E. D. & Suedy, S. W. A. (2017). Kualitas Madu Lokal dari Beberapa Wilayah di Kabupaten Temanggung. *Buletin Anatomi dan Fisiologi*; *2*; 58-66.

Shahwar, D., Ahmad, N., Ullah, S. & Raza, M. A. (2010). Antioxidant Activities of the Selected Plants from the Family Euphorbiaceae, Lauraceae, Malvaceae and Balsaminaceae. *Journal of Biotechnology*; 9; 1086–1096. doi: 10.5897/AJB09.1622.

Sihombing, D. T. H. (2005). Ilmu Ternak Lebah Madu. Yogyakarta: Gajah Mada University Press.

Sudarmadji, S. (2007). Analisa Bahan Makanan dan Pertanian. Yogyakarta: Liberty.

Suranto, A. (2004). *Khasiat dan Manfaat Madu Herbal.* Jakarta: Agromedia Pustaka.

Suranto, Adji. (2007). Terapi Madu. Jakarta: Penebar Swadaya.

Vallianoul, N. G., Gounaril, P., Skourtis, A., Penagos, J. & Kazazis, C. (2014). Honey and Its AntiInflammatory, Antibacterial and Antioxidant Properties. *General Medicine*; *2*; 1 -5.

Wahdaningsih, S., Setyowati, E. P. & Wahyuono, S. (2011). Aktivitas Penangkap Radikal Bebas dari Batang Pakis (*Alsophila glauca J . Sm*). *Majalah Obat Tradisional*; *16*; 156–160.

White, J. W. (1984). Instrumental Color Classification of Honey: Collaborative study. *Journal of the Association of Official Analytical Chemists*; *67*; 1129–1131.

Yu, L. H., Kuo, Y. H., Lin., Y. L. & Chiang, W. (2009). Antioxidative Effect and Active Components from Leaves of Lotus (*Nelumbo nucifera*). *Journal of Agricultural and Food Chemistry*; *57*; 6623–6629. doi: 10.1021/jf900950z.

Zuraida., S., Sajuthi, D. & Irma H. S. (2017). Fenol, Flavonoid, dan Aktivitas Antioksidan pada Ekstrak Kulit Batang Pulai *(Alstonia scholaris* R. Br*). Penelitian Hasil Hutan*; *35*; 211-219.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 298-304
DOI: 10.20473/jfiki.v9i32022.298-304
Available online at https://e-journal.unair.ac.id/JFIKI/

Assessing the Neurotoxicological Effect of the Acute Paraquat Aerosols Exposure in Causing Parkinsonism on Mouse through Behavioral Assays

Sherlo Adha Maulana[1]*, Santi Nurul Kamilah[1], Choirul Muslim[1], Aceng Ruyani[2], R. R. Sri Astuti[1]
[1]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Bengkulu, Bengkulu, Indonesia
[2]Graduate School of Science Education, Faculty of Teacher Training and Education, University of Bengkulu, Bengkulu, Indonesia

*Corresponding author: sherlo.a.maulana@gmail.com

Submitted: 2 July 2022
Accepted: 21 November 2022
Published: 9 December 2022

Abstract

***Background**: In the scientific community, there is no consensus that paraquat, a widely used herbicide, has a strong relationship with the occurrence of Parkinson's disease. A reliable epidemiological explanation of how paraquat can induce parkinsonism is urgently needed because it relates to the agriculture community's potential public health problem. **Objective**: In this study, mice exposed to aerosols of paraquat solution were assessed by behavioral assays designed to observe whether mice exposed to paraquat aerosols develop cardinal symptoms of Parkinson's disease, such as tremor-at-rest, bradykinesia, rigidity, and postural instability. **Methods**: To obtain the intended information, we carried out the observation on distal extremities, catalepsy test, wire suspension test, and swimming test consisting of the head position sub-test, the involvement of limbs sub-test, and the swimming direction test, respectively, to both the group of mice exposed to paraquat aerosols and the one which is not. **Results**: According to the result of the independent-samples t-test calculation on the data obtained from behavioral assays, a significant difference is shown only by the wire suspension test used to assess the development of forelimb rigidity and not the others. **Conclusion**: Therefore, this study showed that daily exposure for a week to paraquat aerosols insignificantly causes tremor-at-rest, bradykinesia, and postural instability in studied mice but dramatically affects their forelimb performance in the form of rigidity.*

***Keywords**: paraquat, Parkinson's disease, parkinsonism, aerosol, behavioral assay*

How to cite this article:
Maulana, S. A., Kamilah, S. N., Muslim, C., Ruyani, A. & Astuti, A. R. R. (2022). Assessing the Neurotoxicological Effect of the Acute Paraquat Aerosols Exposure in Causing Parkinsonism on Mouse through Behavioral Assays. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 9(3), 298-304. http://doi.org/10.20473/jfiki.v9i32022.298-304

INTRODUCTION

To eradicate undesired weeds and grasses from the plantation field during the planting period, farmers usually apply herbicides by spraying them in the field for a period (Konthonbut *et al*., 2020). However, in most developing countries where deficient working conditions, such as the type of equipment being used are poor, improper maintenance of equipment by farmers, climatic conditions in which farmers usually stand upwind while spraying, and illiteracy make controlled and safe use of herbicide is complex (Watts, 2011). Among the widely used herbicides in agriculture is paraquat (1,1-dimethyl-4,4-bipyridinium dichloride). As one of the highly toxic nonselective contact ammonium herbicides, paraquat is associated with the occurrence of Parkinson's disease (Tanner *et al.*, 2011: Gao *et al*., 2020), a neurological movement disorder characterized by the loss of the nigrostriatal dopaminergic pathway (Tieu, 2011).

Even though there are several pathways that paraquat can exist in the body and give rise to health problems, those are ingestion of the residue in water or food, dermal penetration, and inhalation of the aerosols, a recent finding by Anderson *et al.* (2021) showed that paraquat could be found and translocated to various brain regions of mice exposed to paraquat aerosols. It is known that paraquat can trigger oxidative stress that leads to neuronal damage (Guo *et al*., 2018). However, it is still unclear how paraquat can specifically attack dopaminergic neurons in substantia nigra pars compacta (Tieu, 2011) to induce parkinsonism.

In our laboratory, we exposed aerosols of paraquat solution onto the face of mice and isolated them for 10 minutes in an isolation cage. After daily paraquat exposure for a week, we carried out behavioral assays on studied mice to know whether acute paraquat aerosols exposure can induce the manifestation of movement disorders associated with Parkinson's disease (parkinsonism), such as tremor-at-rest, bradykinesia (slowness of a performed movement), rigidity, and postural instability (inability to lance due to loss of postural reflexes) (Berardelli *et al*., 2001; Jankovic, 2008). In contrast to many studies on the effect of paraquat on the occurrence of Parkinson's disease, which were conducted via intraperitoneal injection, we attempted to demonstrate whether the result would be consistent if one of the actual conditions in which most farmers are exposed to paraquat in their plantation field, namely exposure to paraquat aerosols inhaled when applying that herbicide by spraying, was mimicked.

MATERIALS AND METHODS

Animals

This study is based only on BALB/c male mice 8-12 weeks in age bred at Andalas University. Mice were caged individually and provided standard rodent chow and water *ad libitum*. After being acclimatized (12 hours day and 12 hours night) for a week at Animal Anatomy and Physiology Laboratory, University of Bengkulu, mice were randomly divided into two groups containing eight mice each. The first group was the control group (P0), which was the group that is only being maintained in their cage during the study, whereas the other group was the mice exposed to aerosols of paraquat solution (P1). All mice used in this study were treated ethically with procedures approved by the Committee of Ethics at the Faculty of Medicine and Health Sciences, University of Bengkulu.

Paraquat exposure

The source of paraquat used in this study is Gramoxone containing 276 g/L paraquat dichloride (equal to 200 g/L paraquat ions) produced by Syngenta Indonesia. Before being used in the study, 5 mL of Gramoxone was mixed with aqua dest until the volume of the solution reached 500 mL. The paraquat solution was then poured into a spray bottle.

Paraquat exposure was conducted by spraying the aerosols of paraquat solution onto the face of each P1 mouse three times (±2.62 mL of paraquat solution received by each mouse) in the isolation cage. After 10 minutes of isolation, each mouse will be placed back into its maintenance cage. This procedure was done daily for a week for P1 mice.

Behavioral assays

Behavioral assays were done in a quiet, dimly lit room in the evening time three days after the last exposure of paraquat aerosols applied to P1 mice. Behavioral assays that were carried out in this study consist of the catalepsy test, inclined plane test, wire suspension test, and swimming test, which comprises a head position sub-test, limb movement sub-test, and swim direction sub-test. The detail of how each behavioral assay was done is as follows:

The catalepsy test was carried out based on Grabow & Dougherty (2001), with the scoring method used in this study as follows: 0 if the mouse went ahead toward the gap when it was placed at the edge of the table; 1 if the mouse kept staying in its position at the edge of the table more than 10 seconds; and 2. if the mouse turned backward in less than 10 seconds to avoid the gap.

The wire suspension test was performed by allowing the mouse grasps a horizontal wire and then

P-ISSN: 2406-9388
E-ISSN: 2580-8303

evaluating its ability to pull its hanged body. It got 0 if it could not hold the wire provided to it and pull its hanged body up by flexing its forelimbs, and one if vas versa.

The inclined plane test was conducted based on Grabow & Dougherty (2001), with the scoring method as follows: 0 if the mouse slipped down from the inclined plane; 1 if the mouse could keep its body in the inclined plane; and 2, if the mouse was not only able to keep its body in the inclined plane but also performed negative geotaxis movement.

The swimming test was conducted by placing the mouse in a water-filled aquarium and evaluating the response shown by the mouse while in the water. This test observed three aspects: head position, swimming direction, and whether the mouse uses its limbs to swim. For evaluating *the head position*, the mouse would get 0 as its score if the entire head of the mouse sank in the water; 1 if the nose of the mouse was above the surface of the water; 2 if the nose and upper head of the mouse were above the surface of the water; 3 if nose and eyes, as well as ¾ of mouse's ears, were above the surface of the water; and 4, if nose, eyes and entire ears of the mouse were above the surface of the water. For evaluating *the swimming direction*, the mouse would get 0 as its score when the mouse sank, 1 when the mouse floated in the water, 2 when the mouse swam circularly, and 3 when the mouse straightly swam toward its desired direction. For evaluating *the involvement of limbs*, the mouse would get 0 if the limbs were not involved in swimming; 1 if only hindlimbs were involved; and 2 if both fore- and hindlimbs were involved.

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics version 28.0.1.1 (IBM SPSS, Inc,) for Windows. Statistically significant differences between two normally distributed groups were analyzed by performing the Independent-Samples T-Test, in which statistical significance was set at $p < 0.05$. All data were presented as mean + standard deviation.

RESULTS

A slight, insignificant decline in the mean score of the catalepsy test for the group of mice exposed to paraquat

The catalepsy test is a behavioral assay designed to assess whether mice lose spontaneous mobility (Simon *et al*., 1970). The loss of spontaneous movement indicates that the mice have difficulty initiating a movement and/or slowly performing it (Jankovic, 2008). Therefore, this test could show whether mice exposed to paraquat aerosols in this research developed bradykinesia.

As illustrated by Figure 1a, paraquat aerosol exposure could slightly decrease the score for the catalepsy test of the mice exposed to paraquat compared to the control group. However, the result of the independent-sample t-test ($p = 0.149$) shows that there is no significant difference between the mean score of paraquat-exposed mice and the control group ($p > 0.05$).

A significant decrease in the mean score of the wire suspension test for paraquat-exposed mice

Rigidity in Parkinson's disease manifests in flexor muscle inhibition and extensor muscle facilitation (Andrews *et al*., 1972). In this study, the wire suspension test score represents the ability of a mouse to flex its forelimbs when it pulls up its body which hung with its forelimbs grasp a string of wire. Figure 1b shows that the mean score of the wire suspension test in paraquat-exposed mice highly decreases compared to the control group's mean score. Further independent-sample t-test on this data ($p = 0.004$) confirmed that the difference between the mean score of both the control and paraquat-exposed group is significant ($p < 0.05$).

A statistically insignificant decline was observed in the mean score of the inclined plane test for mice exposed to paraquat

To maintain the balance of the body in an inclined plane, mice need to have good postural reflexes, and to assess whether paraquat-exposed mice lose their postural reflexes, this research observed the response of both the group of mice exposed to paraquat aerosols and the control group when they were placed in an inclined plane. Figure 1c presents the bar graph of the mean score of both groups, showing that paraquat exposure could decline the score of the inclined plane test result in several mice exposed to paraquat aerosols, even though if it was compared to the control group, the difference between both groups is statistically insignificant ($p = 0.060$) based on the independent-samples t test calculation ($p > 0.05$).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

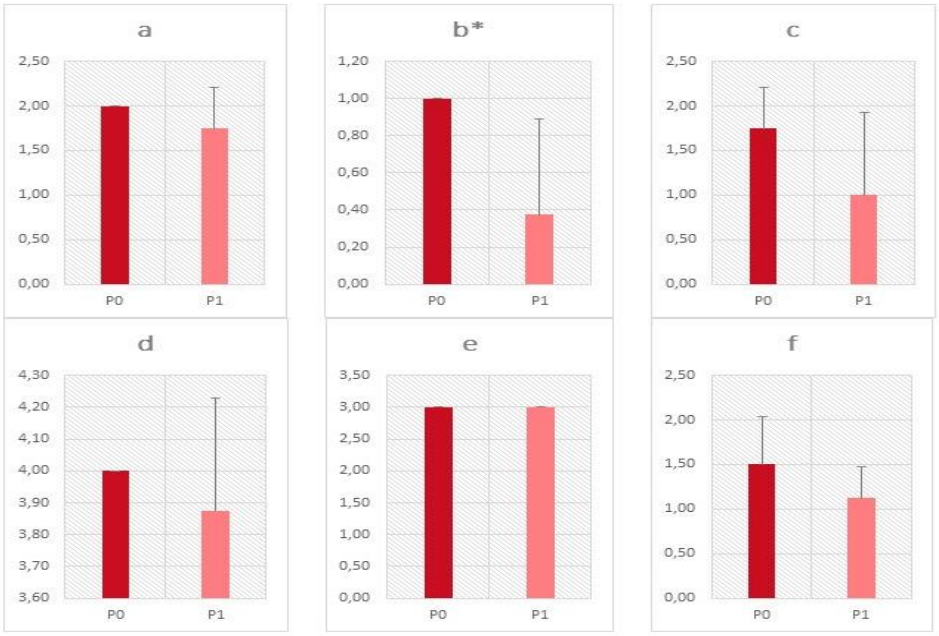


Figure 1. The result of behavioral assays of mice exposed to paraquat aerosols (P1, pink) and the control group (P0, red). Each bar represents the mean score of the assessed behavioral assays (see Materials and Methods). Behavioral assays tested for both groups comprises the catalepsy test (a), wire suspension test (b), inclined plane test (c), and swimming test. The swimming test assessed for both groups consists of the head position sub-test (d), swimming direction sub-test (e), and limbs-using sub-test (f). Error bars on the graphs show the standard deviation. Asterix sign means the test result shows a significant difference statistically

An insignificant difference in the result of the swimming test between the group of mice exposed to paraquat aerosols and the control group

Swimming is a complex movement that includes the ability to coordinate each limb to keep the body's balance and move the body to the desired direction while in water. In this study, the ability to maintain body stability in water was assessed by scoring the ability of a mouse to keep its head out of the water, whereas the ability to perform a movement at a required speed to avoid drowning was assessed by scoring the ability to swim toward a certain direction. Furthermore, to check whether the mouse can easily use its limbs for swimming properly, we observe the movement of limbs during swimming activity.

Each behavioral assay score represents the mouse's ability to conduct normal movements required to swim when the mouse does not develop parkinsonism. When a mouse swims and can maintain its head out of the water, it is more likely to have no problem balancing its posture in the water and vice versa. Moreover, if the mouse has no problem swimming properly toward any direction it wants, it means the mouse does not develop bradykinesia because if it does, the mouse will be drowning. The ability of the mouse to move its limbs when it swims will inform that its flexor and extensor limb muscles can function properly, indicating that there is no rigidity.

As depicted by Figure 1d, the mean score for the observation of mouse head position is slightly decreased in mice exposed to paraquat aerosols compared to the control group, which was confirmed insignificant (p > 0.05) by the independent-samples t-test calculation (p = 0,334). In the ability to swim properly in any direction, both the control group and mice exposed to paraquat show the exact same mean score (Figure 1e). For the result of the use of limbs sub-test (Figure 1f), the statistical calculation (p = 0.120) proved that the difference between the control group and the group of mice exposed to paraquat aerosols is insignificant (p > 0.05).

There is no observed tremor-at-rest in the group of mice exposed to paraquat aerosols

Tremor-at-rest, or rest tremor, is the most common and easily recognized symptom of Parkinson's disease and almost always be prominent in the distal part of extremities. Observation on the distal part of mice's extremities was done during this research, and there is no observed tremor-at-rest in both mice exposed to paraquat aerosols or the control group.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

DISCUSSION

Many studies showed that cardinal symptoms of Parkinson's disease only emerge when the dysfunction of the basal ganglia circuit involved in motor control, including locomotor movement and posture, ensued from the degeneration of 50-80% of substantia nigra dopaminergic neurons, which are part of the input level of the circuit (Grillner *et al*., 2013; DeMaagd & Philip, 2015; Yttri and Dudman, 2018). Tremor-at-rest, for example, is a condition caused by the failure of the circuit's direct pathway output level (which includes the GABAergic substantia nigra pars reticulata and the globus pallidus internal) as a result of the dysfunctional input level, with the effect on the indirect pathway output level (which includes the subthalamic nucleus) causing bradykinesia (Wichmann & DeLong, 1996; Tai *et al.*, 2012; Grillner *et al*., 2013; Yttri & Dudman, 2018). Moreover, because of the reciprocal connection between the basal ganglia and the mesencephalic locomotor region cholinergic neurons involved in the control of locomotion, posture, and balance, the dysfunction of the basal ganglia circuit also gives rise to postural instability in Parkinson's disease patients (Grabli *et al.*, 2012; Pahapill & Lozano, 2000; Caggiano *at al*., 2018).

In the previous study by Fahim *et al.* (2013), rats which were administered by intraperitoneal injection of paraquat for three weeks in a row showed a significant reduction in motor activity and difficulty in movement following the degeneration of dopaminergic neurons in substantia nigra pars compacta. However, our study on mice exposed to paraquat aerosols for a week showed a different result. As shown in Figure 1 (except Figure 1b), the mean score for behavioral assays designed to assess mouse motor performance in this study showed a statistically insignificant reduction or none in the group of mice acutely exposed to paraquat aerosols compared to the control group. This result may imply that the typical degeneration of dopaminergic neurons in substantia nigra that led to a significant reduction in motor activity and difficulty in movement is absent in mice exposed to paraquat aerosols in our study. Furthermore, it also suggests that the degree of paraquat neurotoxicity may depend on the route paraquat comes into the body, how a body of different species deals with it, and the duration of exposure, since those three aspects are what differs our study from Fahim *et al.* (2013).

However, the result of the wire suspension test for both the control group and the group of mice acutely exposed to paraquat aerosols in this study (Figure 1b) is prominent evidence that paraquat can induce neuronal damage because it can cause rigidity to emerge in the forelimb of paraquat aerosols exposed mice. One of the possibilities underlying rigidity in Parkinson's disease is brainstem degeneration (Bologna & Paparella, 2020).

A study by Esposito *et al.* (2014) found that the brainstem nucleus medullary reticular formation ventral part, which its high synaptic density was found contacts to the biceps, extensor carpi radialis, and extensor digiti quarti motor neurons, is a key brainstem area specifically connecting to a subset of forelimb-innervating spinal motor neuron. Degeneration of those brainstem nuclei might affect forelimb motor performance, such as causing rigidity. Therefore, the result of our study in which forelimb rigidity is observed also supports the current view that was established by the study by Braak *et al.* (2003), that the early stage of Parkinson's disease is started in the brainstem and will only show its full clinical manifestation when neurodegeneration has reached substantia nigra.

However, as far as our knowledge, there is no research focused on the effect of paraquat on brainstem degeneration either anatomically or immunohistochemically, so we suggest that future research may focus on this area to fully understand how paraquat can gradually induce neuronal damage in the brain leading to Parkinson's disease. Moreover, since farmers who worked in plantation fields are regularly exposed to herbicide throughout their lifetime, to fully understand the effect of paraquat aerosols in inducing the occurrence of Parkinson's disease, unlike this study which focused only on the impact of acute exposure, we need to know whether the chronic exposure of paraquat aerosols may, too, lead to parkinsonism.

CONCLUSION

In summary, our study shows that daily exposure for a week to paraquat aerosols insignificantly causes tremor-at-rest, bradykinesia, and postural instability in studied mice but dramatically affects their forelimb performance in the form of rigidity.

ACKNOWLEDGMENT

The authors acknowledge Deni Parlindungan for his assistance in purchasing the mice used in this research.

AUTHOR CONTRIBUTIONS

Conceptualization, S. A. M.; Methodology, C. M.; Validation, S. N. K., R. R. S. A.; Formal Analysis, S. A. M.; Investigation, S. A. M.; Data Curation, S. A. M.; Writing - Original Draft, S. A. M.; Writing - Review &

Editing, S. N. K.; Visualization, S. A. M.; Supervision, C. M., A. R.; Project Administration, C. M., S. N. K.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Anderson, T., Merrill, A. K., Eckard, M. L., Marvin, E., Conrad, K., Welle, K., Oberdörster, G., Sobolewski, M., Cory-Slechta, D. A. (2021). Paraquat Inhalation, a Translationally Relevant Route of Exposure: Disposition to the Brain and Male-Specific Olfactory Impairment in Mice. *Toxicological Sciences*; *180*; 175-185. doi: 10.1093/toxsci/kfaa183.

Andrews, C. J., Burke, D. & Lance, J. W. (1972). The Response to Muscle Stretch and Shortening in Parkinsonian Rigidity. *Brain*; *95*; 795-812. doi: 10.1093/brain/95.4.795.

Berardelli, A., Rothwell, J. C., Thompson, P. D. & Hallett, M. (2001). Pathophysiology of Bradykinesia in Parkinson's Disease. *Brain*; *124*; 2131-2146. doi: 10.1093/brain/124.11.2131.

Bologna, M. & Paparella, G. (2020). Pathophysiology of rigidity in Parkinson's disease: Another Step Forward. *Clinical Neurophysiology*; *131*; 1971-1972. doi: 10.1016/j.clinph.2020.05.013.

Braak, H., Del Tredici, K., Rüb, U., de Vos, R. A., Jansen, S. E. N. & Braak, E. (2003). Staging of Brain Pathology Related to Sporadic Parkinson's Disease. *Neurobiol Aging*; *24*; 197-211. doi: 10.1016/s0197-4580(02)00065-9.

Caggiano, V., Leiras, R., Goñi-Erro, H., Masini, D., Bellardita, C., Bouvier, J., Caldeira, V., Fisone, G. & Kiehn, O. (2018). Midbrain Circuits that set Locomotor Speed and Gait Selection. *Nature*; *553*; 455-460. doi: 10.1038/nature25448.

DeMaagd, G. & Philip, A. (2015). Parkinson's Disease, and Its Management (Part 1): Disease Entity, Risk Factors, Pathophysiology. *Clinical Presentation, and Diagnosis*; *40*; 504-532.

Esposito, M. S., Capelli, P. & Arber, S. (2014). Brainstem Nucleus MdV Mediates Skilled Forelimb Motor Tasks. *Nature*; *508*; 351-356. doi: 10.1038/nature13023.

Fahim, M. A., Shehab, S., Nemmar, A., Adem, A., Dhanasekaran, S. & Hasan, M. Y. (2013). Daily Subacute Paraquat Exposure Decreases Muscle Function and Substantia Nigra Dopamine Level. *Physiological Research*; *62*; 313-321. doi: 10.33549/physiolres.932386.

Gao, L., Yuan, H. & Xu, E. (2020). Toxicology of Paraquat and Pharmacology of the Protective Effect of 5-Hydroxy-1-Methylhydantoin on Lung Injury Caused by Paraquat Based on Metabolomics. *Scientific Reports*; *10*; 1-16.

Grabli, D., Karachi, C., Welter, M. L., Lau, B., Hirsch, E. C., Vidailhet, M. & François, C. (2012). Normal and Pathological Gait: What We Learn from Parkinson's Disease. *Journal of Neurology, Neurosurgery, and Psychiatry*; *83*; 979-985. doi: 10.1136/jnnp-2012-302263.

Grabow, T. S. & Dougherty, P. M. (2001). Cervicomedullary Intrathecal Injection of Morphine Produces Antinociception in the Orofacial Formalin Test in the Rat. *Anesthesiology*; *95*; 1427-1434. doi: 10.1097/00000542-200112000-00023.

Grillner, S., Robertson, B. & Stephenson-Jones, M. (2013). The Evolutionary Origin of the Vertebrate Basal Ganglia and Its Role in Action Selection. *The Journal of Physiology*; *591*; 5425-5431. doi: 10.1113/jphysiol.2012.246660.

Guo, J. D., Zhao, X., Li, Y., Li, G. R. & Liu, X. L. (2018). Damage to Dopaminergic Neurons By Oxidative Stress in Parkinson's Disease (Review). *International Journal of Molecular Medicine*; *41*; 1817-1825. doi: 10.3892/ijmm.2018.3406.

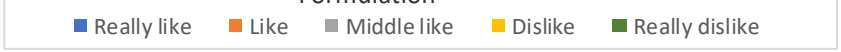
Jankovic, J. (2008). Parkinson's Disease: Clinical Features and Diagnosis. *Journal of Neurology, Neurosurgery, and Psychiatry*; *79*; 368-376. doi: 10.1136/jnnp.2007.131045.

Konthonbut, P., Kongtip, P., Nankongnab, N., Tipayamongkholgul, M., Yoosook, W., & Woskie, S. (2020). Paraquat Exposure of Backpack Sprayers in Agricultural Area in Thailand. *Human and Ecological Risk Assessment: an International Journal*; *26*; 2798-2811. doi: 10.1080/10807039.2019.1684187.

Pahapill, P. A. & Lozano, A. M. (2000). The Pedunculopontine Nucleus and Parkinson's Disease. *Brain*; *123*; 1767-1783. doi: 10.1093/brain/123.9.1767.

Simon, P., Malatray, J. & Boissier, J. R. (1970). Antagonism by Amantadine of Prochlorpemazine-Induced Catalepsy. *Journal of Pharmacy and Pharmacology*; *22*; 546-547. doi: 10.1111/j.2042-7158.1970.tb10567.x.

Tai, C. H., Pan, M. K., Lin, J. J., Huang, C. S., Yang, Y. C. & Kuo, C. C. (2012). Subthalamic Discharges as a Causal Determinant of Parkinsonian Motor



Deficits. *Annals of Neurology*; *72*; 464-476. doi: 10.1002/ana.23618.

Tanner, C. M., Kamel, F., Ross, G. W., Hoppin, J. A., Goldman, S. M., Korell, M., Marras, C., Bhudhikanok, G. S., Kasten, M., Chade, A. R., Comyns, K., Richards, M. B., Meng, C., Priestley, B., Fernandez, H. H., Cambi, F., Umbach, D. M., Blair, A., Sandler, D. P. & Langston, J. W. (2011). Rotenone, Paraquat, and Parkinson's Disease. *Environmental Health Perspectives*; *119*; 866-872. doi: 10.1289/ehp.1002839.

Tieu, K. (2011). A Guide to Neurotoxic Animal Models of Parkinson's Disease. *Cold Spring Harbor Perspectives in Medicine*; *1*; 1-20. doi: 10.1101/cshperspect.a009316.

Watts, M. (2011). Pesticide action network Asia and the Pacific by Watts. http://wssroc.agron.ntu.edu.tw/note/Paraquat.pdf. Accessed: 7 January 2022.

Wichmann, T. & DeLong, M. R. (1996). Functional and Pathophysiological Models of the Basal Ganglia. *Current Opinion in Neurobiology*; *6*; 751-758. doi: 10.1016/s0959-4388(96)80024-9.

Yttri, E. A. & Dudman, J. T. (2018). A Proposed Circuit Computation in Basal Ganglia: History-Dependent Gain. *Movement Disorders Journal*; *33*; 704-716. doi: 10.1002/mds.27321.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 305-313
DOI: 10.20473/jfiki.v9i32022.305-313
Available online at https://e-journal.unair.ac.id/JFIKI/

Viability and Antibacterial Activity of *Bifidobacterium bifidum* in Fermented Robusta Coffee for Diarrhea Treatment

Miksusanti[1], Elsa Fitria Apriani[2]*, Dimas Nur Hidayat[2]
[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Padang, Indonesia
[2]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Padang, Indonesia

*Corresponding author: elsafitria@mipa.unsri.ac.id

Submitted: 2 June 2022
Accepted: 21 November 2022
Published: 9 December 2022

Abstract

Background*: Diarrhea can be treated with probiotic bacteria such as Bifidobacterium bifidum, which decreases the intestinal environment's pH to become acidic so that pathogenic bacteria cannot thrive. Objective: To make fermented coffee that can increase the number of probiotic bacteria Bifidobacterium bifidum and has antidiarrheal activity against pathogenic bacteria Escherichia coli.* ***Methods****: Robusta coffee (20.25% and 19.75%) was fermented using Saccharomyces cerevisiae, and then the probiotic bacteria Bifidobacterium bifidum was added. Unfermented coffee was compared with the same concentration (20.25% and 19.75%) to obtain four formulas. Organoleptic panelists tested all formulas to determine the best formula for fermented and non-fermented coffee. The number of Bifidobacterium bifidum and antibacterial activity was calculated on the optimum formulation using the Total Plate Count and Disc Diffusion Method.* ***Result****: The optimum formula obtained at fermented and unfermented coffee concentration was 20,25%. The number of probiotic bacteria Bifidobacterium bifidum growing in fermented and non-fermented coffee was 7.3 x 108 ± 32.4 and 3.1 x 108 ± 30.7 ($p < 0.05$). The diameter of the inhibition zone of the best fermented and non-fermented coffee was 11.5 ± 0.5 mm and 8.5 ± 0.5 mm, respectively ($p < 0.05$).* ***Conclusion****: Fermented coffee can increase the growth of the probiotic bacteria Bifidobacterium bifidum and has strong antibacterial activity against Escherichia coli bacteria.*

Keywords*: Bifidobacterium bifidum, disc diffusion, Escherichia coli, fermented coffee, total plate count*

How to cite this article:
Miksusanti, Apriani, E. F. & Hidayat, D. N. (2022). Viability and Antibacterial Activity of *Bifidobacterium bifidum* in Fermented Robusta Coffee for Diarrhea Treatment. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(3), 305-313. http://doi.org/10.20473/jfiki.v9i32022.305-313

INTRODUCTION

Diarrhea is a disease associated with environmental hygiene. Diarrhea is mainly caused by food contamination of pathogenic bacteria such as *Escherichia coli*, *Salmonella*, and *Shigella* (Wang *et al*., 2015). According to research by Zhou *et al*. (2018), *E. coli* is the most common bacteria that causes diarrhea. Diarrheal disease management can be treated with antibiotics such as metronidazole, vancomycin, ciprofloxacin, tetracycline, and doxycycline (Giannelli, 2017). In addition, there are other antidiarrheal agents, such as probiotics (Guarino *et al*., 2015).

Probiotics are live microorganisms that provide the host health benefits when administered appropriately. Microorganisms that can act as probiotics are non-pathogenic microorganisms tolerant of acids and bile, modulate the immune system, and can produce lactic acid (Riddle *et al*., 2016). One of the most widely used microorganisms as probiotics is *Bifidobacterium bifidum* (*B. bifidum*) (Pandey *et al*., 2015). Probiotics can prevent the attachment of pathogenic bacteria to the intestinal mucosa, increase the immune response, and increase the microflora in the intestine (Plaza-Diaz *et al*., 2019). The growth of probiotic bacteria can be assisted with a prebiotic agent.

Prebiotics are a nutrient source rich in simple carbohydrates (Arslanoglu *et al*., 2008). The fermentation process can obtain simple carbohydrates (Pokuesava *et al*., 2011). In this study, robusta coffee was used as a source of carbohydrates. Based on the research of Mindarti *et al*. (2020), Robusta coffee contains carbohydrates up to 62.78% *w/w*. The main carbohydrate in robusta coffee is sucrose (Wulandari *et al*., 2021). Complex carbohydrates in robusta coffee will be fermented using *Saccharomyces cerevisiae*, which can break down complex carbohydrates into simpler components to be used as a source of nutrition for *B. bifidum* (Rizal *et al*., 2020). *S. cerevisiae* can break down sucrose into glucose and fructose (Marques *et al*., 2016).

The fermented coffee will be added with the probiotic bacteria *B. bifidum* to become a symbiotic product. In fermented products, the probiotic bacteria *B. bifidum* will degrade carbohydrates into organic acids such as lactic acid, succinic acid, and acetic acid (Chichlowski *et al*., 2011; Stiverson *et al*., 2014; Wang *et al*., 2021). These organic acids have a strong antimicrobial effect against bacterial pathogens (Makras *et al*., 2006). *Bifidobacterium* can kill pathogenic bacteria such as *Clostridioides difficile* (Yang & Yang, 2019), *Salmonella enterica* (Symonds *et al*., 2012), and *E. coli* (Abdelhamid et al., 2018).

Based on the description above, researchers are interested in making a fermented robusta coffee as a symbiotic product containing the probiotic bacteria *B. bifidum* and testing the growth of the probiotic bacteria using the total plate count method. Then, the antibacterial activity was tested against pathogenic bacteria *E. coli* using the Disc Diffusion Method. Non-fermented robusta coffee was used as a comparison.

MATERIALS AND METHODS

Materials

The materials used consisted of robusta coffee (South Sumatra, Indonesia), *S. cerevisiae* (buy at Sriwijaya University, Indonesia), distilled water (Bratachem, Indonesia), Man Rogosa and Sharpe media (Merck, Indonesia), *B. bifidum* BRL-130 (buy at Gadjah Mada University, Indonesia), *E. coli* ATCC-25922 (buy at Sriwijaya University, Indonesia), ciprofloxacin, sodium alginate (Merck, United States), barium chloride (Sigma-Aldrich, United States), sodium chloride (Sigma-Aldrich, United States), hydrochloric acid (Bratachem, Indonesia), and concentrated sulfuric acid (Bratachem, Indonesia).

Equipment

The equipment used consisted of Freeze Dryer (Nuaire® NU9483GC), glassware (Pyrex® and Iwaki®), magnetic stirrer (IKA® C-MAG HS 4), micro pipette (DragonLab®), analytical balance (Ohaus®), autoclave (Lequitron®), incubator (Biosan®), Oven (Memmert®), Furnace (Thermolyne®), butyrometer.

Methods

Production of *Bifidobacterium bifidum* probiotic powder

The suspension of the probiotic bacteria *B. bifidum* in de Man, Rogosa & Sharpe (MRS) broth media was dried using the freeze-drying method. The probiotic powder was made by mixing *Bifidobacterium* suspension into 10% skim milk solution and 4% sodium alginate solution, then incubated at 37ºC for 8 hours. The mixed suspension was then dried at -23ºC for 24 hours (Holkem *et al*., 2016).

Production of fermented robusta coffee

Robusta coffee was fermented using 3% *S. cerevisiae* yeast for 5 hours. After that, it is washed thoroughly and dried in the sun. After drying, the coffee beans are cleaned and then roasted at a temperature of 120ºC. Then the coffee beans are ground to obtain fermented robusta coffee powder (Pereira *et al*., 2014).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 1. The formula of probiotic coffee

Samples	Formula Concentration (%)			
	F1	**F2**	**F3**	**F4**
Fermented coffee	20.25	-	19.75	-
Non-fermented coffee	-	20.25	-	19.75
Probiotic powder	1.00	1.00	1.00	1.00
Glucose	3.75	3.75	3.75	3.75

Production of robusta coffee symbiotic

Robusta coffee symbiotic products are made from fermented and non-fermented coffee with added probiotic powder containing the *B. bifidum*. Fermented coffee powder or non-fermented coffee is added with probiotic powder. Then add glucose and mix until smooth. The formula for probiotic coffee can be seen in Table 1.

Organoleptic test

Organoleptic tests were conducted to determine the best formula with assessments including color, smell, taste, and texture from samples shown by 30 untrained panelists with a target age of 15 to 50 years. Samples are placed in containers and coded according to the formula. Panelists were asked to rate each sample on the questionnaire sheet. The scale used in this study consisted of five numerical scales, namely strongly dislike (1), dislike (2), neutral (3), like (4), and like very much (5).

Proximate test of robusta coffee beans and fermented coffee

The proximate test refers to the rules of SNI 01-2891-1992, including water, ash, protein, fat, and carbohydrate content.

Water content test

Moisture content was measured using the Thermogravimetric Method. The best probiotic coffee preparations were dried in an oven at 105°C for 5 hours and then weighed until a constant weight was obtained.

Total ash content test

Total ash content was measured by calculating the constant weight of the sample, which had been heated at 600°C for 2 hours using a furnace.

Protein content test

A number of samples were mixed with 1.9 g K_2SO_4, 40 mg HgO, and 2 mL concentrated H_2SO_4, then boiled until the solution became clear. The solution was then distilled with 10 mL of 60% NaOH, 5 mL of boric acid, and 5 mL of MB:MM indicator for 15 minutes. The solution was then titrated with 0.02 N HCl.

Fat content test

A total of 10 mL of H_2SO_4 and a number of samples were put into the butyrometer. Add amyl alcohol and stir until homogeneous. The mixture was heated at a temperature of 65 - 70°C for 5 minutes and then mixed. Put the mixture upside down and reheat for 2 - 3 minutes. Calculate the percent fat on the butyrometer line.

Carbohydrate content test

The carbohydrate content of the sample was calculated by subtracting 100% of the nutritional content of the sample from the moisture content, total ash content, protein content, and fat content.

Probiotic bacteria growth test

The growth test of the probiotic bacteria *B. bifidum* was carried out using the Total Plate Count Method. In this test, a suspension of *B. bifidum* was used as a positive control, and distilled water as a negative control. Selected probiotic coffee formula from fermented and non-fermented coffee was brewed with warm water. Then the dilution was performed from 10^{-1} to 10^{-8} using 0.9% NaCl solution. Then the solution was poured into MRS Agar media and incubated at 37°C for 48 hours (Rosburg *et al*., 2010). The amount of growth of probiotic bacteria was calculated using equation 1.

$$Number\ of\ Colony\ (N) = \frac{\sum C}{(1xn1)+(0.1xn2)+(0.01xn3)xdf} \quad [1]$$

Information:

N = Number of product colonies (cfu/mL or cfu/g)

ΣC = Number of colonies in all counted plates

n1 = Number of cups in the first dilution is calculated

n2 = Number of cups in the second dilution is calculated

n3 = Number of cups in the third dilution is calculated

Fp = First dilution calculated

Antibacterial activity test against *E. coli*

The antibacterial activity test was carried out using the Disc Diffusion Method (Mohammed *et al*., 2020). The antibiotic ciprofloxacin was used as a positive control, and distilled water was used as a negative control. Filter paper with a diameter of 6 mm was dipped in each sample (positive control, negative control, and coffee samples) for 5 minutes and then planted on MRS Agar media which already contained the pathogenic bacterium *E. coli*. The Petri dish was then incubated at 37°C for 24 hours. Antibacterial activity was measured from the diameter of the resulting inhibition zone.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Data analysis

Data analysis was carried out statistically using SPSS version 23. The normality test was carried out using the Shapiro-Wilk method. If the data were normally distributed ($p > 0.05$), then it was continued with the one-way ANOVA test to see the differences between the test groups.

RESULTS AND DISCUSSION

Probiotic powder containing *B. bifidum* bacteria has a smooth and dry texture. Drying was carried out using the freeze-drying method with the help of coating materials such as sodium alginate and skim milk. According to research by Chandramouli *et al*. (2004), sodium alginate coating has several advantages, such as being non-toxic, easy to form a gel matrix around bacterial cells, and easy release of active substances when contact with the intestine fluid. In addition, skim milk, which contains high protein, can also prevent damage to bacterial cell membranes (Amine *et al*., 2014).

The growth and metabolic activity of the probiotic bacteria *B. bifidum* can be selectively increased by the presence of carbohydrates in the environment. Robusta coffee is used as a carbohydrate source for the growth of the probiotic bacteria *B. bifidum*. Robusta coffee is known to contain mucilage consisting of pectin and carbohydrates (Haile & Kang, 2019). Based on the research of Mindarti *et al*. (2020), Robusta coffee contains carbohydrates up to 62.78% *w/w*. In this study, the robusta coffee beans used had a water content of 6.63 ± 0.14%, ash content of 3.68 ± 0.18%, protein content of 7.84 ± 0.11%, fat content of 0.86 ± 0.03%, and carbohydrate content of 75.98 ± 0.07% (Table 2). In addition, S. cerevisiae also contains β-Galactosidases which can convert lactose into galactooligosaccharides (GOS) (Macfarlane *et al*., 2008; Osman *et al*., 2012). Carbohydrates that can be used as nutrients for probiotic bacteria B. bifidum are simple carbohydrates such as inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and lactulose (Gibson *et al*., 2010). In this study, robusta coffee will be fermented using the yeast *Saccharomyces cerevisiae* to help the degradation process of complex carbohydrates into prebiotic compounds. Fermentation is a chemical process that can convert complex compounds into simpler compounds. S. cerevisiae is known to have the enzyme Fructosyl-transferase (FTase), which is an enzyme capable of converting fructose into inulin and fructooligosaccharides (FOS) (Louis *et al*., 2016; Mohkam *et al*., 2016). S. cerevisiae can also hydrolyze sucrose into glucose and fructose (Marques *et al*., 2016).

Organoleptic is an essential parameter in functional food products to increase consumer attractiveness. Fermented and non-fermented robusta coffee was carried out by organoleptic tests using 30 panelists to determine the best formula. Based on this test, fermented coffee formula one and non-fermented formula two were chosen as the best formula according to Figure 1. The results of statistical analysis showed that there were no significant differences in taste and odor parameters ($p > 0.05$) while color and texture had significant differences ($p < 0.05$). Formula 1 generally has the best results from all parameters. It indicates that the fermentation process in coffee can improve the quality of the coffee produced. Robusta coffee fermented with *S. cerevisiae* has been proven to make coffee that has good taste qualities such as the presence of a caramel aroma in the coffee, a sweet taste at the beginning and bitter at the end, and a fresh aroma (Bressani *et al*., 2020; Evangelista *et al*., 2014; Silva *et al*., 2013). This change is because the coffee fermentation process will produce new byproducts such as acetic acid, citric acid, malic acid, lactic acid, and succinic acid (Da Mota *et al*., 2020).

Figure 1. Organoleptic results of symbiotic coffee (n = 30, assessed using questionnaire)

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 2. Test results of coffee beans and fermented coffee the best formula (n = 5)

Parameter (%)	Robusta coffee beans	Fermented coffee	SNI 6685, 2009
Water content	6.63 ± 0.14	8.76 ± 0.13	Max 7
Ash content	3.68 ± 0.18	4.48 ± 0.21	Max 1
Protein content	7.84 ± 0.11	38.11 ± 0.49	Min 1
Fat content	0.86 ± 0.03	1.77 ± 0.02	Min 0.6
Carbohydrate content	75.98 ± 0.07	46.87 ± 0.61	-

Table 4. Inhibition zone diameter in samples (n = 5)

Treatment Group	Inhibition zone diameter (mm)	Category
Positive control	26.5 ± 0.5	Very strong
Negative control	0.0 ± 0.0	None
F1- Fermented coffee	11.5 ± 0.5	Strong
F2- Non-fermented coffee	8.5 ± 0.5	Moderate

The proximate results of the best-fermented robusta coffee have met the standards of SNI 6685, 2009, namely water content of 8.76 ± 0.13%, ash content of 4.48 ± 0.21%, the protein content of 38.11 ± 0.49%, fat content was 1.77 ± 0.02%, and carbohydrate content was 46.87 ± 0.61 (Table 2).

Formula 1 and 2 were continued by testing the viability of probiotic bacteria. The results of the viability test of the probiotic bacteria *B. bifidum* can be seen in Table 3.

Table 3. Viability of the probiotic bacteria *B. bifidum* in samples (n = 5)

Treatment Group	Number of colonies (cfu/mL)
Positive control	7.4×10^8
Negative control	1×10^8
F1-Fermented coffee	7.3×10^8
F2-Non-fermented coffee	3.1×10^8

Based on the results of statistical analysis for the viability of probiotic bacteria, there was no significant difference between the positive control and the fermented coffee formula 1 ($p > 0.05$). In contrast, the non-fermented coffee formula 2 differed significantly from the positive control and formula 1 ($p < 0.05$). It proves that the fermentation process increases the growth of the probiotic bacteria *B. bifidum*. According to the description above, *S. cerevisiae* can convert complex carbohydrates such as sucrose and lactose into FOS and GOS. FOS and GOS have been shown to increase the growth of the bacterium *B. bifidum* (Saulnier *et al*., 2008).

Furthermore, Formula 1 and 2 were continued with antibacterial activity tests against *E. coli* pathogenic bacteria. The results of the antibacterial activity test can be seen in Table 4.

Statistical analysis showed significant differences between groups ($p < 0.05$). It indicates that fermented coffee and non-fermented coffee produce different antibacterial activities. Fermented coffee makes a larger diameter of inhibition zone than non-fermented coffee and is included in the category of antibacterial solid compounds, namely 11.5 ± 0.5 mm. It proves that the fermentation process can positively affect antibacterial activity. As described above, the robusta coffee fermentation process will produce prebiotic compounds such as FOS and GOS. FOS and GOS will then be further fermented by the bacterium *B. bifidum*. FOS will be fermented into organic acids such as lactic acid and succinic acid, while GOS will be fermented into acetic acid, which can inhibit the growth of *E. coli* bacteria (Bondue & Delcenserie, 2015; Stiveson *et al*., 2014). Organic acids will make the environment acidic so *E. coli* bacteria cannot grow. In addition, the undissociated form of organic acids will enter the *E. coli* bacterial cell and dissociate in the cytoplasm, which causes the bacterial cell to lysis (Bermudez-Brito *et al*., 2012). *B. bifidum* probiotics can also produce bacteriocins in the form of Bifidocin A. Bifidocin A enters the cells of *E. coli* bacteria to form pores that cause leakage of intracellular compounds such as proteins, nucleic acids, and ions (Liu *et al*., 2015). *B. bifidum* can stimulate mucus secretion, strengthening intestinal epithelial defenses (Denkova *et al*., 2017). Pathogenic bacteria attach to the intestinal epithelium due to the interaction between Microbe-Associated Molecular Pattern (MAMP) and Pattern Recognition Receptor (PRR) (Madsen, 2012). *B. bifidum* can recognize these receptors to replace pathogens attached to the intestinal epithelium (Sarkar & Mandal, 2016).

In addition to the probiotic effect, the antibacterial activity of fermented and non-fermented robusta coffee can also be caused by the content of robusta coffee.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Robusta coffee contains flavonoid compounds, alkaloids, tannins, and terpenoids (Less & Kamengon, 2021). Flavonoids can act as antibacterial by inhibiting nucleic acid synthesis, inhibiting porin formation, disrupting membrane structure, and changing membrane permeability (Xie *et al*., 2015). Alkaloids can act antibacterial by inhibiting ATP-dependent transport of compounds across the cell membrane (Mabhiza *et al*., 2016). Tannins work as antibacterial by interfering with the metabolism of bacterial cells (Kaczmarek, 2020). Meanwhile, terpenoids act as antibacterial by disrupting the integrity of cell membranes (Guimarães *et al*., 2019). However, the antibacterial activity of fermented and non-fermented coffee was not as strong as the antibiotic ciprofloxacin. But for fermented coffee, the antibacterial activity is in a strong category.

CONCLUSION

The fermented robusta coffee produced in this study meet with SNI requirements in characteristics parameter namely water content, ash content, the protein content, the fat content, and carbohydrate content. Fermented robusta coffee also has a strong antibacterial activity against *E. coli* while the non-fermented coffee has moderate antibacterial activity. Fermented robusta coffee can also increase the growth of *B. bifidum* bacteria due to the presence of prebiotic compounds such as FOS and GOS. So, fermented robusta coffee can be used as an alternative symbiotic product for diarrhea management.

AUTHOR CONTRIBUTIONS

Conceptualization, M., E. F. A.; Methodology, M., E. F. A.; Sotfware, M., E. F. A.; Validation, M., E. F. A., D. N. A.; Formal Analysis, M., E. F. A., D. N. A.; Resources, M., E. F. A., D. N. A.; Investigation, M., E. F. A., D. N. A.; Data Curation, M., E. F. A., D. N. A.; Writing - Original Draft, M., E. F. A.; Writing - Review & Editing, M., E. F. A.; Visualization, M., E. F. A; Supervision, M., E. F. A.; Project Administration, M., E. F. A.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Abdelhamid, A. G., Esaam, A. & Hazaa, M. M. (2018). Cell Free Preparations of Probiotics Exerted Antibacterial and antibiofilm Activities Against Multidrug Resistant *E. coli*. *Saudi Pharmaceutical Journal; 26*; 603–607. doi: 10.1016/j.jsps.2018.03.004.

Amine, K. M., Champagne, C. P., Salmieri, S., Britten, M., St-Gelais, D., Fustier, P. & Lacroix, M. (2014). Effect of Palmitoylated Alginate Microencapsulation on Viability of *Bifidobacterium longum* during Freeze-drying. *Food Science and Technology*; *56*; 111-117. doi: 10.1016/j.lwt.2013.11.003.

Arslanoglu, S., Moro, G. E., Schmitt, J., Tandoi, L., Rizzardi, S. & Boehm, G. (2008). Early Dietary Intervention with a Mixture of Prebiotic Oligosaccharides Reduces the Incidence of Allergic Manifestations and Infections during the First Two Years of Life. *Journal of Nutrition*; *138*; 1091–1095. doi: 10.1093/jn/138.6.1091.

Bermudez-Brito, M., Plaza-Diaz, J., Muñoz-Quezada, S., Gómez-Llorente, C. & Gil, A. (2012). Probiotic Mechanisms of Action. *Annals of Nutrition and Metabolism*; *61*; 160–174.

Bondue, P. & Delcenserie, V. (2015). Genome of Bifidobacteria and Carbohydrate Metabolism. *Korean Journal for Food Sciences of Animal Resources*; *35*; 1-9. doi: 10.5851/kosfa.2015.35.1.1.

Bressani, A. P. P., Martinez, S. J., Sarmento, A. B. I., Borém, F. M. & Schwan, R. F. (2020). Organic Acids Produced during Fermentation and Sensory Perception in Specialty Coffee using Yeast Starter Culture. *Food Research International*; *128*; 108773. doi: 10.1016/j.foodres.2019.108773.

Chandramouli, Y., Gandhimati, R., Yasmeen, B. R., Vikram, A., Mahitha, B. & Imroz, S.M. (2004). Review On Cocrystal as An Approach with Newer Implication in Pharmaceutical Field. *International Journal of Medicinal Chemistry and Analysis*; *2*; 91-100.

Chichlowski, M., German, J. B., Lebrilla, C. B. & Mills, D. A. (2011). The Influence of Milk Oligosaccharides on Microbiota of Infants: Opportunities for Formulas. *Annual Review of Food Science and Technology*; *2*; 331-351. doi: 10.1146/annurev-food-022510-133743.

Da Mota, M. C. B., Batista, N. N., Rabelo, M. H. S., Ribeiro, D. E., Borém, F. M. & Schwan, R. F. (2020). Influence of Fermentation Conditions on the Sensorial Quality of Coffee Inoculated with Yeast. *Food Research International*; *136*; 109482. doi: 10.1016/j.foodres.2020.109482.

Denkova, R., Goranov, B., Teneva, D., Denkova, Z. & Kostov, G. (2017). Antimicrobial Activity of

Probiotic Microorganisms: Mechanisms of Interaction and Methods of Examination. *Antimicrobial Research: Novel Bioknowledge and Educational Programs*; *34*; 201-212.

Evangelista, S. R., Miguel, M. G., de Souza Cordeiro, C., Silva, C. F., Pinheiro, A. C. & Schwan, R. F. (2014). Inoculation of Starter Cultures in a Semi-Dry Coffee (*Coffea arabica*) Fermentation Process. *Food Microbiology*; *44*; 87–95.

Giannelli, F. R. (2017). PA-C Antibiotic-associated Diarrhea. *Journal of the American Academy of Physician Assistants*; *30*; 46-47. doi: 10.1097/01.JAA.0000524721.01579.c9.

Gibson, G. R., Scott, K. P., Rastall, R. A., Tuohy, K. M., Hotchkiss, A., Dubert-Ferrandon, A., Gareau, M., Murphy, E. F., Saulnier, D. & Loh, G. (2010). Dietary Prebiotics: Current Status and New Definition. *Food Science & Technology Bulletin Functional Foods*; *7*; 1–19.

Guarino, A., Guandalini, S. & Vecchio, A. (2015). Probiotics for Prevention and Treatment of Diarrhea. *Journal of Clinical Gastroenterology*; *49*; 37-45. doi: 10.1097/MCG.0000000000000349.

Guimarães, A. C., Meireles, L. M., Lemos, M. F., Guimarães, M., Endringer, D. C., Fronza, M. & Scherer, R. (2019). Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. *Molecules; 24*; 2471. doi: 10.3390/molecules24132471.

Haile, M. & Kang, W. H. (2019). The Role of Microbes in Coffee Fermentation and Their Impact on Coffee Quality. *Journal of Food Quality*; *2019*; 1-16. doi: 10.1155/2019/4836709.

Holkem, A., Raddatz, G., Nunes, G., Cichoski, A., Jacob-Lopes, E., Grosso, C. & Menezes, C. (2016). Development and Characterization of Alginate Microcapsules Containing Bifidobacterium BB-12 Produced by Emulsification/Internal Gelation Followed by Freeze Drying. *Food Science and Technology*; *71*; 302-308. doi: 10.1016/j.lwt.2016.04.012.

Kaczmarek, B. (2020). Tannic Acid with Antiviral and Antibacterial Activity as A Promising Component of Biomaterials-A Minireview. *Materials; 13*; 3224.

Liu, G., Song, Z., Yang, X., Gao, Y., Wang, C. & Sun, B. (2015). Antibacterial Mechanism of Bifidocin A, a Novel Broad-Spectrum Bacteriocin Produced by *Bifidobacterium animalis* BB04. *Food Control*; *62*; 309-316. doi: 10.1016/j.foodcont.2015.10.033.

Louis, P., Flint, H. J. & Michel, C. (2016). How to Manipulate the Microbiota: Prebiotics. *Advances in Experimental Medicine and Biology*; *902*; 119-142. doi: 10.1007/978-3-319-31248-4_9.

Macfarlane, G., Steed, H. & Macfarlane, S. (2008). Bacterial Metabolism and Health-Related Effects of Galacto-Oligosaccharides and Other Prebiotics. *Journal of Applied Microbiology*; *104*; 305–344.

Mabhiza, D., Chitemerere, T. & Mukanganyama, S. (2016). Antibacterial Properties of Alkaloid Extracts from *Callistemon citrinus* and *Vernonia adoensis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *International Journal of Medicinal Chemistry*; *2016*; 1-7. doi: 10.1155/2016/6304163.

Madsen, K. L. (2012). Enhancement of Epithelial Barrier Function by Probiotics. *Journal of Epithelial Biology & Pharmacology*; *5*; 55–59.

Makras, L., Triantafyllou, V., Fayol-Messaoudi, D., Adriany, T., Zoumpopoulou, G., Tsakalidou, E., Servin, A. & DeVuyst, L. (2006). Kinetic Analysis of the Antibacterial Activity of Probiotic Lactobacilli Towards *Salmonella enterica* Serovar *typhimurium* Reveals a Role for Lactic Acid and Other Inhibitory Compounds. *Research in Microbiology*; *157*; 241–247. doi: 10.1016/j.resmic.2005.09.002.

Marques, W., Raghavendran, V., Stambuk, B. U. & Gombert, A. K. (2016). Sucrose and *Saccharomyces cerevisiae*: A Relationship Most Sweet. *Federation of European Microbiological Societies Yeast Research*; *16*; 1-16. doi: 10.1093/femsyr/fov107.

Mindarti, S., Zalizar, L., Damat, Saati, E. A. & Fajriani, S. (2020). Characterization of Fiber Fraction, Physical and Chemical Properties of Coffee Flour (*Coffea* sp.) as Functional Foodstuff for Diabetes Mellitus Patient. *IOP Conferences Series: Earth and Environmental Science*; *462*; 1-10.

Mohammed, M., Raman, N., Alhoot, M. & Alwan, R. (2020). Antibacterial Activities of *Allium sativum* (Garlic) Extracts Against *Staphylococcus aureus* and *Escherichia coli*. *European Journal of Molecular & Clinical Medicine*; *7*; 526-534.

Mohkam, M., Nezafat, N., Berenjian, A., Negahdaripour, M., Behfar, A. & Ghasemi, Y. (2016). Role of *Bacillus genus* in the Production of Value-Added Compounds. Basel: Springer.

Osman, A., Tzortzis, G., Rastall, R. A. & Charalampopoulos, D. (2012). BbgIV is an Important *Bifidobacterium* β-galactosidase for the Synthesis of Prebiotic Galactooligosaccharides at High Temperatures. *Journal of Agriculture and Food Chemistry*; *60*; 740–748.

Pandey, K. R., Naik, S. R. & Vakil, B. V. (2015). Probiotics, Prebiotics and Symbiotics - a Review. *Journal of Food Science and Technology*; *52*; 7577–7587.

Pereira, G., Thomaz-Soccol, V., Pandey, A., Madeiros, A., Lara, J., Gollo, A. & Soccol, C. (2014). Isolation, Selection and Evaluation of Yeasts for Use in Fermentation of Coffee Beans by the Wet Process. *International Journal of Food Microbiology*; *188*; 60-66. doi: 10.1016/j.ijfoodmicro.2014.07.008.

Plaza-Diaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M. & Gil, A. (2019). Mechanisms of Action of Probiotics. *Advances in nutrition*; *10*; 49–66. doi: 10.1093/advances/nmy063.

Pokusaeva, K., Fitzgerald, G. F. & van Sinderen, D. (2011). Carbohydrate metabolism in Bifidobacteria. *Genes & Nutrition*; *6*; 285–306. doi: 10.1007/s12263-010-0206-6.

Riddle, M. S., DuPont, H. L. & Connor, B. A. (2016). ACG Clinical Guideline: Diagnosis, Treatment, and Prevention of Acute Diarrheal Infections in Adults. *American Journal of Gastroenterology*; *111*; 602-622. doi: 10.1038/ajg.2016.126.

Rizal, S., Murhadi, Kustyawati, M. A. & Hasanudin, U. (2020). Growth Optimization of *Saccharomyces cerevisiae* and *Rhizopus oligosporus* during Fermentation to Produce Tempeh with High β-glucan Content. *Biodiversitas*; *21*; 2667-2673. doi: 10.13057/biodiv/d210639.

Rosburg, V., Boylston, T. & White, P. (2010). Viability of Bifidobacteria Strains in Yogurt with Added Oat Beta-Glucan and Corn Starch During Cold Storage. *Journal of Food Science*; *75*; 439–444. doi: 10.1111/j.1750-3841.2010.01620.x.

Sarkar, A. & Mandal, S. (2016). Bifidobacteria - Insight into Clinical Outcomes and Mechanisms of Its Probiotic Action. *Microbiological Research*; *192*; 159-171. doi: 10.1016/j.micres.2016.07.001.

Saulnier, D. M., Gibson, G. R. & Kolida, S. (2008). In Vitro Effects of Selected Symbiotics on the Human Faecal Microbiota Composition. *Federation of European Microbiological Societies Microbiology & Ecology*; *66*; 516–527.

Silva, C. F., Vilela, D. M., de SouzaCordeiro, C., Duarte, W. F., Dias, D. R. & Schwan, R. F. (2013). Evaluation of a Potential Starter Culture for Enhances Quality of Coffee Fermentation. *World Journal of Microbiology & Biotechnology*; *29*; 235–247. doi: 10.1007/s11274-012-1175-2.

Stiverson, J., Williams, T., Chen, J., Adams, S., Hustead, D., Price, P., Guerrieri, J., Deacon, J. & Yu Z. (2014). A Comparative Evaluation of Prebiotic Oligosaccharides using In Vitro Cultures of Infant Fecal Microbiome. *Applied and Environmental Microbiology*; *80*; 7388-7397. doi: 10.1128/AEM.02200-14.

Symonds, E. L., O'Mahony, C., Lapthorne, S., O'Mahony, D., Sharry, J. M., O'Mahony, L. & Shanahan, F. (2012). *Bifidobacterium infantis* 35624 Protects Against Salmonella-Induced Reductions in Digestive Enzyme Activity in Mice by Attenuation of the Host Inflammatory Response. *Clinical and Translational Gastroenterology*; *3*; 15.

Wang, X., Wang, J., Sun, H., Xia, S., Duan, R. & Liang, J. (2015). Etiology of Childhood Infectious Diarrhea in a Developed Region of China: Compared to Childhood Diarrhea in a Developing Region and Adult Diarrhea in a Developed Region. *PLoS ONE*; *10*; 1-14. doi: 10.1371/journal.pone.0142136.

Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y. & Geng, W. (2021). Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. *Frontiers in Bioengineering and Biotechnology*; *9*; 1-19. doi: 10.3389/fbioe.2021.612285.

Wulandari, S., Ainuri, M. & Sukartiko, A. C. (2021). Biochemical Content of Robusta Coffees Under Fully-Wash, Honey, and Natural Processing Methods. *IOP Conferences Series: Earth and Environmental Science*; *819*; 1-9.

Xie, Y., Yang, W., Tang, F., Chen, X. & Ren, L. (2015). Antibacterial Activities of Flavonoids: Structure-Activity Relationship and Mechanism. *Current Medicinal Chemistry*; *22*; 132–149. doi: 10.2174/0929867321666140916113443.

Yang, J. & Yang, H. (2019). Antibacterial Activity of *Bifidobacterium breve* Against *Clostridioides difficile*. *Frontiers in Cellular and Infection Microbiology*; *9*; 1-12. doi: 10.3389/fcimb.2019.00288.

Zhou, Y., Zhu, X., Hou, H. et al. (2018). Characteristics of Diarrheagenic *Escherichia coli* among Children Under 5 Years of Age with Acute Diarrhea: A Hospital Based Study. *BMC Infectious Disease*; *18*; 1-10. doi: 10.1186/s12879-017-2936-1.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 314-322
DOI: 10.20473/jfiki.v9i32022.314-322
Available online at https://e-journal.unair.ac.id/JFIKI/

Knowledge and Perception on Overclaim against the Behaviors of Implementing the COVID-19 Prevention Protocol Communities in Indonesia

Elsa Christina[1], Didik Setiawan[1,2]*, Dina Ratna Juwita[1], Lianawati[2]
[1]Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto, Purwokerto, Indonesia
[2]Center for Health Economic Studies, Universitas Muhammadiyah Purwokerto, Purwokerto, Indonesia

*Corresponding author: d.didiksetiawan@gmail.com

Submitted: 31 May 2022
Accepted: 30 November 2022
Published: 9 December 2022

Abstract

Background*: COVID-19 occurs in various countries and has been declared a pandemic by WHO. Multiple efforts have been made to reduce the number of cases of COVID-19. However, the incidence of COVID–19 continues to increase, along with control efforts carried out by various parties, causing overclaims for the prevention or treatment of COVID–19.* ***Objective*** *This study aimed to determine the relationship between knowledge and public perception of the behavior of implementing the COVID-19 prevention protocol in Central Java Province.* ***Methods****: This cross-sectional study used primary data collected online via WhatsApp, Telegram, Instagram, and Facebook in December 2020. A total of 1,098 of 1,115 respondents passed the inclusion and exclusion criteria. Backward Elimination is used to determine factors related to behavior in the multivariable model stage using multiple logistic regression.* ***Results****: The knowledge, perception, and behaviors prevalence of implementing COVID-19 prevention protocols were good & enough 79.1% (95% CI 76.63 – 81.45), 96.6% (95% CI 95.38 – 97.55), and 92.3% (95% CI 90.62 – 93.78) respectively. The result revealed that Knowledge (adjOR = 2.034, 95% CI 1.253 - 3.302, P = 0.004) and Perception (adjOR = 4.064, 95% CI 1.859 - 8.882, P = < 0.001) were possibly associated with behaviors of implementing COVID-19 prevention protocols among communities in Central Java Province.* ***Conclusion*** *This study found a slight prevalence of good & enough knowledge, perception, and behaviors of implementing COVID-19 prevention protocols in a representative sample among Communities in Central Java Province. Knowledge and perceptions were statistically significant with behaviors.*

Keywords*: knowledge, perception, behaviors, COVID–19*

How to cite this article:
Christina, E., Setiawan, D., Juwita, D. R. & Lianawati. (2022). Knowledge and Perception on Overclaim against the Behaviors of Implementing the COVID-19 Prevention Protocol Communities in Indonesia. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 9(3), 314-322. http://doi.org/10.20473/jfiki.v9i32022.314-322

INTRODUCTION

COVID-19 (Corona Virus Disease 2019) is a deadly disease, and the number of patients continues to increase rapidly throughout the world (Manchia *et al*., 2022; WHO, 2020). It was originally discovered in Hubei Province, China, and quickly spread to 215 other countries around the world. Furthermore, on March 20, 2020, WHO declared COVID-19 a pandemic. The number of cases of COVID-19 continues to increase. As of 4 October 2020, there were 34 million cases and more than 1 million deaths worldwide. In Indonesia, on November 2022, there were 6,627,538 confirmed cases of COVID-19 with 159,524 deaths, reported to WHO. In Central Java Province, 23,521 confirmed cases and 2,018 confirmed deaths. The positive number for COVID-19 in Indonesia is 17.51%, this figure is the highest in Southeast Asia (Johns Hopkins University and Medicine, 2022; WHO, 2022).

Control of COVID-19 in Indonesia has been pursued by issuing the 5th revision of the Corona Virus Disease (COVID-19) Prevention and Control Guidelines by the Ministry of Health of the Republic of Indonesia. One of the keys to breaking the chain of transmission of COVID-19 is not to create a source of transmission by implementing new habits or by implementing health protocols in every activity (MoH Indonesia, 2020). However, data showed that the incidence and mortality due to COVID-19 continue to increase. The implementation of protocols and screening programs has not provided optimal results. This is demonstrated by the number of COVID-19 cases that have occurred since September 8, 2020, which is consistently greater than 3,000 cases per day (Islam *et al*., 2020; Susanna, 2020; WHO, 2020).

Ineffective COVID-19 control has led to many control measures that have resulted in overclaims that can mislead and harm the public (Cheng *et al*., 2021; Stewart *et al*., 2022; Vijaykumar et al. 2021). This is evidenced by claims of prevention and treatment that do not yet have a solid scientific basis, including; The Corona Herbavid-19, a herbal medicine by the DPR-RI COVID-19 Resistance Task Force claimed to have succeeded in curing positive COVID-19 patients (Garnesia, 2020). Various Indonesian spices such as ginger and turmeric are believed to increase the body's immunity to ward off the coronavirus transmission. The Ministry of Agriculture claims that eucalyptus necklaces or eucalyptus plants can ward off the coronavirus (Wibowo, 2020). Eucalyptus is still in the research-based stage of computational analysis (in silico). Hydroxychloroquine (an antimalaria drug) is claimed to be effective as a COVID-19 drug but has not shown significant resultsUniversitas Airlangga's COVID-19 combination drug, which was declared capable of curing COVID-19 patients, has not yet gone through the clinical trial stage (Mawalia, 2020; Wibowo, 2020). The news about "The COVID-19 vaccine test in Indonesia was successful" shows that the vaccine test has not been completed (Li, 2021; PT Bio Farma, 2020).

To put it another way, claiming to be able to prevent and cure COVID-19 can lead to various interpretations in society. These exaggerated claims can potentially influence risk perception patterns and public behavior. Studies on the relationship between risk perception patterns and responsive behaviors that emerged during the pandemic yield inconsistent and insignificant results, which influence people's behavior in response to COVID-19 (Xu & Peng, 2015). Previous research claimed that inaccurate information could mislead the public. Besides, people can lose their sense of crisis, so they do not want to prevent, treat, or participate in vaccinations, and even lose trust in health workers and the government (Cha *et al*., 2021; Fanelli 2009). This could make the COVID-19 pandemic worse and more difficult to control (Islam *et al*., 2020; Tangcharoensathien *et al*., 2020). Since it was declared a pandemic, COVID-19 has become the biggest threat to society and has been exacerbated by the overclaims made by various parties. This can make the community feel safe and neglect the COVID-19 prevention protocol so that the Overclaim can affect people's perception and behaviors in implementing the COVID-19 transmission prevention protocol.

MATERIALS AND METHODS

Tools

This study will determine the relationship between knowledge and public perception of the behavior of implementing the COVID-19 prevention protocol in Central Java Province. After reviewing the literature, the researcher designed this research questionnaire. Communities in other provinces outside Central Java province carried out validation. Evaluation of the reliability of the questionnaire was carried out using a pilot study on 40 respondents (Riwidikdo & Setiawan 2006). Test the validity and internal consistency of the questionnaire using Cronbach Alpha and set the minimum acceptable validity value $\alpha = 0.32$ (Budiman & Riyanto 2013). The validity test results on the knowledge, perception and behavior questionnaire were 0.539 - 0.773; 0.525 - 0.644; 0.592 - 0.808. The results

P-ISSN: 2406-9388
E-ISSN: 2580-8303

of the reliability test on the knowledge, perception and behavior questionnaire were 0.912, 0.786, and 0.919.

The final questionnaire consisted of four sections. The first part of the questionnaire contains demographic information of the respondents, including gender, age, education level, and profession. The second part contains 15 statements to evaluate respondents' knowledge about the overclaiming of COVID-19 prevention and treatment. The third part consists of 6 statements assessing respondents' perceptions of overclaiming COVID-19 prevention and treatment. The last section contains ten statements about the respondent's behavior regarding the behavior of implementation COVID-19 health prevention. All questions were closed except for the demographic questionnaire.

Respondents were asked to choose the option "True" or "False" for the statements of knowledge and perception. The correct answer (yes) is given a score of one (1), while the wrong answer (no) is given a score of zero. A three-point Likert scale was used for behavioral statements (constantly = 3, sometimes = 2, never = 1). Therefore, the minimum and maximum scores for knowledge, perception, and behavior are 0 to 15, 0 to 6, and 10 to 30, respectively. The questionnaire was distributed online via WhatsApp, Telegram, Instagram and Facebook in December 2020. The behavior, knowledge, and perception were calculated from the total score as a continuous data and then transformed by x-tile (3q) into a categorical data (ordinal) good, enough, and poor. The variable category was regenerated to good & enough and poor (dichotomous).

Method

Study design and sampling

A cross-sectional study design was applied in this study. Data collection in this study was conducted online from communities in Central Java Province in December 2020. About 1,098 of 1,115 respondents passed the inclusion and exclusion criteria selection. The sampling technique used is non-probability sampling with an accidental sampling method. The sample size was calculated using the Rao software online sample size calculator with a 95% CI and 5% margin of error. A total of 1,098 respondents participated in the survey. Analysis was performed on 385 usable forms.

Inclusion criteria in this study were people who live in the Central Java Province are over the age of 18 years, are willing to be a respondent, and have read or heard the news about the prevention or treatment of COVID-19, which is claimed to be excessive (Overclaim), smartphone users, can read and fill out questionnaires, and have a telephone number that can be contacted. The exclusion criteria were health workers, or the respondent's occupation is related to health sciences.

Overclaimed is that various forms of misinformation during a pandemic, as well as science communication strategies that confuse the public, fake news and misinformation or exaggerated information about the outbreak, can thrive on social media with potentially dangerous consequences.

All questionnaires were labelled, including the respondent's date, time, and location. Informed consent was taken before distributing the questionnaire to each respondent, and the confidentiality of the respondent's information was maintained. No incentives are given to any respondent. Informed consent was done through online media before filling in the data. Respondents were explained and filled out a consent form before being able to access the questionnaire.

Statistical analysis

This study used an observational analytic study with a cross-sectional design. The descriptive stage was used to determine the characteristics of this study, and comparative statistics were used to define whether differences between both (good and poor behavior) groups existed. Furthermore, the inferential stage is used to determine the relationship between knowledge and public perception of the behavior of implementing the COVID-19 prevention protocol in Central Java Province. In the bivariate stage, we used chi-square analysis to evaluate the association of knowledge and perception on behavior. The backward elimination method was used to determine factors related to behavior in the multivariable model stage using multiple logistic regression using the SPSS version 23. The results are presented as an adjusted odds ratio (adjOR) and 95% confidence interval (CI).

RESULTS AND DISCUSSION

Demographic characteristic

Among a total of 1,098 respondents who passed our inclusion and exclusion criteria, most of them were female (65.3%), aged 18 - 29 years old (92.5%), finishing their secondary school (53.5%), and their current status as a student (73.4%) (Table 1). Generally, there are no statistical differences in characteristics between people with excellent and poor behavior (*p-value* of > 0.05).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 1. The Respondent characteristics between good and poor behaviors groups implementing COVID-19 Prevention protocols in Central Java

Variable	Behaviors				Total (%) (n = 1134)		P-value
	Good (%) (n = 1,014)		Poor (%) (n = 84)				
Gender							
Male	348	34.32	33	39.29	381	34.70	0.358
Female	666	65.68	51	60.71	717	65.30	
Age (years)							
18 – 29	938	92.50	78	92.86	1016	92.53	0.956
30 – 59	75	7.40	6	7.14	81	7.38	
≥ 60	1	0.10	0	-	1	0.09	
Education level							
Primary education	10	0.99	2	2.38	12	1.09	0.377
Secondary education	541	53.35	46	54.76	587	53.46	
University education	76	7.50	3	3.57	79	7.19	
Post graduation	387	38.17	33	39.29	420	38.25	
Occupation							
Student	750	73.96	56	66.67	806	73.41	0.671
Govt. Employee	50	4.93	5	5.95	55	5.01	
Private employee	181	17.85	20	23.81	201	18.31	
Military	1	0.10	0	-	1	0.09	
Housewife	32	3.16	3	3.57	35	3.19	

Table 2. Factor associated with behaviors of implementing COVID-19 prevention protocols in Central Java Province

Variable	Behaviors (%)				OR	P-value
	Good (n = 1,014)		Poor (n = 84)			
Knowledge						
Good & enough	815	80.37	54	64.29	2.275	< 0.001
Poor	199	19.63	30	35.71		
Perception						
Good & enough	987	97.34	74	88.10	4.940	< 0.001
Poor	27	2.66	10	11.90		

Table 3. Factor associated with behaviors of implementing COVID-19 prevention protocols among communities in Central Java Province by using multiple logistic

Variable	Model	
	1	2
	Pseudo R2 = 0.0392	Pseudo R2 = 0.0353
Perception	4.30 (1.95 – 9.50) ***	4.06 (1.86 – 8.88) ***
Knowledge	2.05 (1.26 – 3.33) **	2.03 (1.25 – 3.30) **
Gender	0.78 (0.49 – 1.24)	
Age	0.83 (0.34 – 2.03)	
Education level	0.62 (0.23 – 1.65)	
Occupation	1.58 (0.60 – 4.18)	

Factor associated with behaviors

Our study showed that although more people have better knowledge (80.37% vs. 64.29%) and perception (97.34% vs. 88.10%) among both good and poor behavior groups, those minor differences in knowledge (OR of 2.275, p-value 0.000) and perception (OR of 4.940, p-value 0.000) resulting significant increase on how much people will have good behavior on implementing COVID-19 prevention protocols on their daily life (Table 2).

Multiple logistic regression was used for multivariable analysis to define the relationship between respondent characteristics, knowledge, perception, and behavior in implementing the COVID-19 prevention protocol. The initial result showed that respondent characteristics do not significantly influence their behavior (Table3). Furthermore, the final result revealed that Knowledge (adjOR = 2.034, 95% CI 1.253 - 3.302, P = 0.004) and Perception (adjOR = 4.064, 95% CI 1.859 - 8.882, P = < 0.001) were possibly associated

P-ISSN: 2406-9388
E-ISSN: 2580-8303

with behaviors of implementing COVID-19 prevention protocols among communities in Central Java Province.

Discussion

The knowledge, perception, and behaviors prevalence of implementing COVID-19 prevention protocols was Good & Enough 79.1% (95% CI 76.63 – 81.45), 96.6% (95% CI 95.38 – 97.55), and 92.3% (95% CI 90.62 – 93.78) respectively. It was different from the previous study in Cameron that showed the result of the prevalence of high overall knowledge score, perception, and behaviors/practice towards COVID-19 were 84.19%, 69%, and 60.8%, respectively (Ngwewondo *et al*., 2020). A study in Ethiopia found that 62.3% of respondents had good knowledge, 56.6% had positive attitudes/perceptions of COVID-19, and 47.5% had good behavior/practices towards COVID-19 (Adhena & Hidru 2020). Cross-Sectional Study from Nigeria found that 88.59% of respondents had good knowledge about COVID-19, and most of the health workers had bad attitudes (n = 101, 25.06%) or were indifferent toward work (n = 233, 57.82). %) in the COVID-19 era, and 81.39% have a high level of practice to prevent COVID-19 infection. (Pauline *et al*., 2020) Previous research from Uganda showed that 91% of research respondents had good knowledge, 74% had positive attitudes/perceptions towards COVID-19, and 57% had good practices/behaviors towards COVID-19 (Olum *et al*., 2020) It may be different from this research because the research from Cameron, Ethiopia, Nigeria, and Uganda has a different region and culture from Indonesia. Indonesia is an archipelagic country with hundreds of cultures in it. Information is often biased due to the acceptance of understanding between each tribe. The prevalence of Knowledge, Perception, and Behavior has comparable numbers compared to previous studies. This is important to consider for handling pandemic in Indonesia.

The importance of knowledge impacting attitudes and behavior in the application of health protocols during the COVID-19 pandemic (Zegarra-Valdivia *et al*., 2020). Previous studies have shown evidence of a high prevalence of COVID-19-related knowledge among all participants included in this study. Similar to this study, there are still significant gaps in perception and behavior towards COVID-19 (Nwagbara *et al*., 2021). Research from North Sulawesi Indonesia revealed that most respondents had good knowledge, positive attitudes, and good practices toward COVID-19 prevention. However, knowledge of specific topics is still insufficient (Simanjorang *et al*., 2021).

This present study revealed that Knowledge (adjOR = 2.034, 95%CI 1.253 - 3.302, P = 0.004) and Perception (adjOR = 4.064, 95%CI 1.859 - 8.882, P = <0 .001) were possibly associated with behaviors of implementing COVID-19 prevention protocols among communities in Central Java Province. This study is similar to previous study, which stated that most students had inadequate knowledge about COVID-19 (good knowledge about COVID-19 = 23.5%, 95% CI 19.5% to 28.1%) and were less involved in COVID-19 prevention behavior (Handebo *et al*., 2021). Risk perception and knowledge related to COVID-19 can influence protective behavior (Rattay *et al*., 2021). Perception of cognitive and particularly effective risk is a further significant predictor of behavior in the face of COVID-19 (Betsch *et al*., 2021). Previous studies in China highlighted the usefulness of cognitive assessment (i.e., perceived severity, perceived controllability, and knowledge of COVID-19), as a core process in dealing with stress, in explaining public emotions and behaviors in the face of public health issues (Li *et al*., 2020). A qualitative study in Ghana found that health knowledge has increased due to COVID-19 regarding access to health information and increased understanding of health issues. Increased knowledge and access to information reduce the risk of being misinformed or claimed to be redundant from pandemic protocols (Saah *et al*., 2021).

Sufficient knowledge provides an understanding of certain situations, including dealing with the COVID-19 pandemic. Understanding the situation provides self-confidence so that it is not easily provoked or manipulated by invalid or overclaimed information (Mao *et al*., 2021) Behavioral changes require information about potential threats to the health of oneself or others. Valid information and increased knowledge affect handling the COVID-19 pandemic (Azlan *et al*., 2020; Chesser *et al*., 2020; Šuriņa *et al*., 2021).

This knowledge investigation of a new infectious disease (COVID-19) is needed to identify knowledge gaps and sources of misinformation that can assist public health efforts in designing and implementing more focused intervention measures (Sallam *et al*., 2020). Sufficient knowledge, and accurate understanding, can minimize the occurrence of misinformation and overclaims and speed up the completion of the pandemic (Guan *et al*., 2020; Rothan & Byrareddy, 2020; Sallam *et al*., 2020).

A previous study among university students in the UK found that perception ($\beta = 0.13$, $p = 0.016$) was statistically significant for the unique variance in hand

P-ISSN: 2406-9388
E-ISSN: 2580-8303

hygiene behavior during the pandemic (Barrett & Cheung 2021). Previous study with 633 participants revealed that better understanding shapes perceptions that affect behavior (Lim *et al*., 2021). Another previous study revealed that the perception of COVID-19 can positively predict behavior indirectly (Mahmoud *et al*., 2021).

Public perception of COVID-19 affects health behavior. Previous qualitative studies conducted among communities in Kathmandu, Kanchanpur, Bajura and Jhapa districts in Nepal revealed a very good general understanding among respondents about COVID-19, personal precautions and population level strategies. Respondents recognized the important role of the media in increasing awareness, knowledge, and straightening perceptions. Participants also expressed concern over the misleading (overclaimed) news that was spread by several media (Bhatt *et al*., 2020).

Planning an effective educational intervention for handling the COVID-19 pandemic requires knowledge, perception, and preventive behavior as awareness of the health risks posed by this disease (Albaqawi *et al*., 2020). This is important to increase the general public's knowledge in preventing the spread of COVID-19. Knowledge can make people aware of the seriousness of this pandemic situation (Gohel *et al.*, 2021). Human behavior plays an important role as the world grapples with public health threats such as the COVID-19 pandemic. Appropriate behavior can be an important factor in reducing cases of the COVID-19 pandemic (Jalloh *et al*., 2021).

CONCLUSION

This study found a slight prevalence of good & enough knowledge, perception, and behaviors of implementing COVID-19 prevention protocols in a representative sample among Communities in Central Java Province. Knowledge and perceptions were statistically significant with behaviors.

ACKNOWLEDGMENT

The authors would like to acknowledge all the respondents from Central Java province willing to respond to this research.

AUTHOR CONTRIBUTIONS

Conceptualization, E. C., D. S.; Methodology, E. C., D. S.; Sotfware, E. C., D. S.; Validation, D. S., D. R. J.; Formal Analysis, E. C., D. R. J.; Investigation, D. S., D. R. J.; Resources, E. C., D. R. J., L.; Data Curation, E. C..; Writing - Original Draft, E. C.; Writing - Review & Editing, D. S., D. R. J., L.; Visualization, D. S., D. R. J., L.; Supervision, D. S., D. R. J.; Project Administration, D. S., L.; Funding Acquisition, D. S.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Adhena, G., & Hidru, H. D. (2020). Knowledge, Attitude, and Practice of High-Risk Age Groups to Coronavirus Disease-19 Prevention and Control in Korem District, Tigray, Ethiopia: Cross-Sectional Study. *Infection and Drug Resistance 13*; 3801–3809. doi: 10.2147/IDR.S275168.

Albaqawi, Mohammad, H., Alquwez, N., Balay-odao, E., Bajet, J. B., Alabdulaziz, H., Alsolami, F., Tumala, R. B., Alsharari, A. F., Hanan, M. M. T., Felemban, E. M. & Cruz, J. P. (2020). Nursing Students' Perceptions, Knowledge, and Preventive Behaviors Toward COVID-19: A Multi-University Study. *Frontiers in Public Health*; *8*; 1–9. doi: 10.3389/fpubh.2020.573390.

Azlan, A. A., Hamzah, M. R., Sern, T. J., Ayub, S. H. & Mohamad, E. (2020). Public Knowledge, Attitudes and Practices towards COVID-19: A Cross-Sectional Study in Malaysia. *PLoS ONE*; *15*; 1–15. doi: 10.1371/journal.pone.0233668.

Barrett, C. & Cheung, K. L. (2021). Knowledge, Socio-Cognitive Perceptions and the Practice of Hand Hygiene and Social Distancing during the COVID-19 Pandemic: A Cross-Sectional Study of UK University Students. *BMC Public Health*; *21*; 1–18. doi: 10.1186/s12889-021-10461-0.

Betsch, C., Korn, L., Burgard, T., Gaissmaier, W., Felgendreff, L., Eitze, S., Sprengholz, P., Böhm, R., Stollorz, V., Ramharter, M., Promies, N., De Bock, F., Schmid, P., Renner, B., Wieler, L. H. & Bosnjak, M. (2021). The Four Weeks before Lockdown during the COVID-19 Pandemic in Germany: A Weekly Serial Cross-Sectional Survey on Risk Perceptions, Knowledge, Public Trust and Behaviour, 3 to 25 March 2020. *Euro Surveillance: Bulletin Europeen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin*; *26*; 1–13. doi: 10.2807/1560-7917.ES.2021.26.42.2001900.

Bhatt, N., Bhatt, B., Gurung, S., Dahal, S., Jaishi, A. R., Neupane, B. & Budhathoki, S. S. (2020). Perceptions and Experiences of the Public Regarding the COVID-19 Pandemic in Nepal: A

Qualitative Study Using Phenomenological Analysis. *BMJ Open*; *10*; 1–11. doi: 10.1136/bmjopen-2020-043312.

Budiman & Agus Riyanto. (2013). Kapita Selekta Kuesioner Pengetahuan dan Sikap Dalam Penelitian Kesehatan. Jakarta: Salemba Medika.

Cha, M., Cha, C., Singh, K., Lima, G., Ahn, Y. Y., Kulshrestha, J. & Varol, O. (2021). Prevalence of Misinformation and Factchecks on the COVID-19 Pandemic in 35 Countries: Observational Infodemiology Study. *JMIR Human Factors*; *8*; 1–6. doi: 10.2196/23279.

Cheng, M., Yin, C., Nazarian, S. & Bogdan, P. (2021). Deciphering the Laws of Social Network-Transcendent COVID-19 Misinformation Dynamics and Implications for Combating Misinformation Phenomena. *Scientific Reports*; *11*; 1–14. doi: 10.1038/s41598-021-89202-7.

Chesser, A., Ham, A. D. & Woods, N. K. (2020). Assessment of COVID-19 Knowledge Among University Students: Implications for Future Risk Communication Strategies. *Health Education and Behavior*; *47*; 540–43. doi: 10.1177/1090198120931420.

Fanelli, D. (2009). How Many Scientists Fabricate and Falsify Research? A Systematic Review and Meta-Analysis of Survey Data. *PLOS ONE*; *4*; 1-11. doi: 10.1371/journal.pone.0005738.

Garnesia, I. (2020). Menguji Klaim Herbavid-19 Sebagai Obat COVID-19. Jakarta: Tirto Id.

Gohel, K. H., Patel, P. B., Shah, P. M., Patel, J. R., Pandit, N. & Raut, A. (2021). Knowledge and Perceptions about COVID-19 among the Medical and Allied Health Science Students in India: An Online Cross-Sectional Survey. *Clinical Epidemiology and Global Health*; *9*; 104–109. doi: 10.1016/j.cegh.2020.07.008.

Guan, W., Ni, Z., Hu, Y., Liang, W., Ou, C., He, J., Liu, L., Shan, H., Lei, H., Hui, D. S. C., Du, B., Li, L., Zeng, G., Yuen, K., Chen, R., Tang, C., Wang, T., Chen, P., Xiang, J., Li, S., Wang, J., Liang, Z., Peng, Y., Wei, L., Liu, Y., Hu, Y., Peng, P., Wang, J., Liu, J., Chen, Z., Li, G., Zheng, G., Qiu, S., Luo, J., Ye, C., Zhu, S. & Zhong, N. (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. *New England Journal of Medicine*; *382*; 1708–1720. doi: 10.1056/nejmoa2002032.

Handebo, S., Adugna, A., Kassie, A. & Shitu, K. (2021). Determinants of COVID-19-Related Knowledge and Preventive Behaviours among Students in Reopened Secondary Schools: Cross-Sectional Study. *BMJ Open*; *11*; 1–10. doi: 10.1136/bmjopen-2021-050189.

Islam, M. S., Sarkar, T., Khan, S. H., Kamal, A. H. M., Hasan, S. M. M., Kabir, A., Yeasmin, D., Islam, M. A., Chowdhury, K. I. A., Anwar, K. S., Chughtai, A. A. & Seale, H. (2020). COVID-19-Related Infodemic and Its Impact on Public Health: a Global Social Media Analysis. *American Journal of Tropical Medicine and Hygiene*; *103*; 1621–1629. doi: 10.4269/ajtmh.20-0812.

Jalloh, M. F., Nur, A. A., Nur, S. A., Winters, M., Bedson, J., Pedi, D., Prybylski, D., Namageyo-Funa, A., Hageman, K. M., Baker, B. J., Jalloh, M. B., Eng, E., Nordenstedt, H. & Hakim, A. J. (2021). Behaviour Adoption Approaches during Public Health Emergencies: Implications for the COVID-19 Pandemic and Beyond. *BMJ Global Health*; *6*; 1–9. doi: 10.1136/bmjgh-2020-004450.

Johns Hopkins University and Medicine. (2022). Johns Hopkins Coronavirus Resource Center. Baltimore: The Johns Hopkins University School of Medicine.

Li, J. B., Yang, A., Dou, K., Wang, L. X., Zhang, M. C. & Lin, X. Q. (2020). Chinese Public's Knowledge, Perceived Severity, and Perceived Controllability of COVID-19 and Their Associations with Emotional and Behavioural Reactions, Social Participation, and Precautionary Behaviour: A National Survey. *BMC Public Health*; *20*; 1–14. doi: 10.1186/s12889-020-09695-1.

Li, Q. (2021). The Efficacy, Safety and Immunogenicity Study of Inactivated SARS-CoV-2 Vaccine for Preventing Against COVID-19. https://clinicaltrials.gov/ct2/show/NCT04659239. Accessed: 19 March 2021.

Lim, V. W., Lim, R. L., Tan, Y. R., Soh, A. S. E., Tan, M. X., Othman, N. B., Dickens, S. B., Thein, T. L., Lwin, M. O., Ong, R. T. H., Leo, Y. S., Lee, V. J. & Chen, M. I. C. (2021). Government Trust, Perceptions of Covid-19 and Behaviour Change: Cohort Surveys, Singapore. *Bulletin of the World Health Organization*; *99*; 92–101. doi: 10.2471/BLT.20.269142.

Mahmoud, A. B., Polay, D. H., Reisel, W. D., Fuxman, L., Grigoriou, N., Mohr, I. & Aizouk, R. (2021). Who's More Vulnerable? a Generational Investigation of COVID-19 Perceptions' Effect on Organisational Citizenship Behaviours in the MENA Region: Job Insecurity, Burnout and Job

Satisfaction as Mediators. *BMC Public Health*; *21*; 1–17. doi: 10.1186/s12889-021-11976-2.

Manchia, M., Gathier, A. W., Yapici-Eser, H., Schmidt, M. V., de Quervain, D., Amelsvoort, T. V., Jonathan, B., Cryan, J. F., Howes, O. D., Pinto, L., van der Wee, N. J., Domschke, K., Branchi, I. & Vinkers, C. H. (2022). The Impact of the Prolonged COVID-19 Pandemic on Stress Resilience and Mental Health: A Critical Review across Waves. *Annals of Oncology*; 55; 22–83.

Mao, Y., Chen, H., Wang, Y., Chen, S., Gao, J., Dai, J., Jia, Y., Xiao, Q., Zheng, P. & Fu, H. (2021). How Can the Uptake of Preventive Behaviour during the COVID-19 Outbreak Be Improved? An Online Survey of 4827 Chinese Residents. *BMJ Open*; *11*; 1–11. doi: 10.1136/bmjopen-2020-042954.

Mawalia, K. A. (2020). Peneliti UNAIR Temukan Lima Kombinasi Obat Efektif Lawan Corona. https://news.unair.ac.id/2020/06/12/peneliti-unair-temukan-lima-kombinasi-obat-yang-efektif-lawan-corona-2/?lang=id. Accessed: 10 March 2021.

MoH Indonesia. (2020). Pedoman Kesiapsiagaan Menghadapi Coronavirus Disease (Covid-19). Jakarta: Kementerian Kesehatan Republik Indonesia.

Ngwewondo, A., Nkengazong, L., Ambe, L. A., Ebogo, J. T., Mba, F. M., Goni, H. O., Nyunaï, N., Ngonde, M. C. & Oyono, J. L. E. (2020). Knowledge, Attitudes, Practices of/towards COVID 19 Preventive Measures and Symptoms: A Cross-Sectional Study during the Exponential Rise of the Outbreak in Cameroon. *PLoS Neglected Tropical Diseases*; *14*; 1–15. doi: 10.1371/journal.pntd.0008700.

Nwagbara, U. I., Osual, E. C., Chireshe, R., Bolarinwa, O. A., Saeed, B. Q., Khuzwayo, N. & Hlongwana, K. W. (2021). Knowledge, Attitude, Perception, and Preventative Practices towards COVID-19 in Sub-Saharan Africa: A Scoping Review. *PLoS ONE*; *16*; 1–20. doi: 10.1371/journal.pone.0249853.

Olum, R., Kajjimu, J., Kanyike, A. M., Chekwech, G., Wekha, G., Nassozi, D. R., Kemigisa, J., Mulyamboga, P., Muhoozi, O. K., Nsenga, L., Lyavala, M., Asiimwe, A. & Bongomin, F. (2020). Perspective of Medical Students on the COVID-19 Pandemic: Survey of Nine Medical Schools in Uganda. *JMIR Public Health and Surveillance*; *6*; 1–12. doi: 10.2196/19847.

Pauline, M., Ngozichukwu, C., Azubuike, C. M. C., Mbachu, I. I., Ndukwu, C. I., Ezeuko, A. Y. A., Udigwe, I. B., Nnamani, C. P., Umeh, U. M., Ezeagwuna, D. A., Onah, S. K., Eze, H. O., Okereke, U. C. & Orji-Ifeanyi, E. N. (2020). COVID-19 Infection: Knowledge, Attitude, Practices, and Impact among Healthcare Workers in a South-Eastern Nigerian State. *Journal of Infection in Developing Countries*; *14*; 943–52. doi: 10.3855/JIDC.13248.

PT Bio Farma. (2020). Efficacy, Safety and Immunogenicity Study of SARS-CoV-2 Inactivated Vaccine (COVID-19). Rockville: U.S. National Library of Medicine.

Rattay, P., Michalski, N., Domanska, O. M., Kaltwasser, A., de Bock, F., Wieler, L. H. & Jordan, S. (2021). Differences in Risk Perception, Knowledge and Protective Behaviour Regarding COVID-19 by Education Level among Women and Men in Germany. Results from the COVID-19 Snapshot Monitoring (COSMO) Study. *PLoS ONE*; *16*; 1–26. doi: 10.1371/journal.pone.0251694.

Riwidikdo, H. & Setiawan, A. (2006). Statistik Kesehatan: Belajar Mudah Teknik Analisis Data Dalam Penelitian Kesehatan (plus Aplikasi SPSS). Yogyakarta: Mitra Cendekia Press.

Rothan, H. A. & Byrareddy, S. N. (2020). The Epidemiology and Pathogenesis of Coronavirus Disease (COVID-19) Outbreak. *Journal of Autoimmunity Journal*; *109*; 1-4.

Saah, F. I., Amu, H., Seidu, A. A. & Bain, L. E. (2021). Health Knowledge and Care Seeking Behaviour in Resource-Limited Settings amidst the COVID-19 Pandemic: A Qualitative Study in Ghana. *PLoS ONE*; *16*; 1–15. doi: 10.1371/journal.pone.0250940.

Sallam, M., Dababseh, D., Yaseen, A., Al-Haidar, A., Taim, D., Eid, H., Ababneh, N. A., Bakri, F. G. & Mahafzah, A. (2020). COVID-19 Misinformation: Mere Harmless Delusions or Much More? A Knowledge and Attitude Cross-Sectional Study among the General Public Residing in Jordan. *PLoS ONE*; *15*; 1–18. doi: 10.1371/journal.pone.0243264.

Simanjorang, C., Tooy, G., Wuaten, G. & Pangandaheng, N. (2021). Knowledge, Attitudes, and Practices Towards COVID-19 among North Sulawesi Indonesia Residents. *Journal of Health Education*; *6*; 57–64. doi: 10.1080/10556699.1994.10603001.

Stewart, R., Madonsela, A., Tshabalala, N., Etale, L. & Theunissen, N. (2022). The Importance of Social Media Users' Responses in Tackling Digital COVID-19 Misinformation in Africa. *Digital Health*; 8; 1-15. doi: 10.1177/20552076221085070.

Šuriņa, S., Martinsone, K., Perepjolkina, V., Kolesnikova, J., Vainik, U., Ruža, A., Vrublevska, J., Smirnova, D., Fountoulakis, K. N. & Rancans, E. 2021. "Factors Related to COVID-19 Preventive Behaviors: A Structural Equation Model. *Frontiers in Psychology*; *12*; 1–15. doi: 10.3389/fpsyg.2021.676521.

Susanna, D. (2020). When Will the COVID-19 Pandemic in Indonesia End?. *Kesmas*; *15*; 160–162. doi: 10.21109/KESMAS.V15I4.4361.

Tangcharoensathien, V., Calleja, N., Nguyen, T., Purnat, T., D'Agostino, M., Garcia-Saiso, S., Landry, M., Rashidian, A., Hamilton, C., AbdAllah, A., Ghiga, I., Hill, A., Hougendobler, D., van Andel, J., Nunn, M., Brooks, I., Sacco, P. L., de Domenico, M., Mai, P., Gruzd, A., Alaphilippe, A. & Briand, S. (2020). Framework for Managing the COVID-19 Infodemic: Methods and Results of an Online, Crowdsourced Who Technical Consultation. *Journal of Medical Internet Research*; *22*; 1–8. doi: 10.2196/19659.

Vijaykumar, S., Rogerson, D. T., Jin, Y. & Costa, M. S. O. (2021). Dynamics of Social Corrections to Peers Sharing COVID-19 Misinformation on WhatsApp in Brazil. *Journal of the American Medical Informatics Association: JAMIA*; *29*; 33–42. doi: 10.1093/jamia/ocab219.

WHO. (2020). WHO Director-General's Opening Remarks at the Media Briefing on COVID-19: WHO Director General's Speeches. https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020. Accessed: 11 March 2021.

WHO. (2022). COVID-19 Weekly Epidemiological Update. *World Health Organization*; *58*; 1–23.

Wibowo, Y. C. (2020). 4 Alasan Kita Perlu Tolak Klaim Keampuhan Obat Herbal Di Tengah Pandemi COVID-19: The Conversation. https://theconversation.com/4-alasan-kita-perlu-tolak-klaim-keampuhan-obat-herbal-di-tengah-pandemi-covid-19-140642. Accessed: 11 March 2021.

Xu, J. & Peng, Z. (2015). People at Risk of Influenza Pandemics: The Evolution of Perception and Behavior. *PLoS ONE*; *10*; 1–14. doi: 10.1371/journal.pone.0144868.

Zegarra-Valdivia, Vilca, C. & Guerrero, A. (2020). Knowledge, Perception and Attitudes in Regard to COVID-19 Pandemic in Peruvian Population. *Pediatria Integral*; *24*; 69–70.