BUKTI KORESPONDENSI

ARTIKEL JURNAL NASIONAL TERAKREDITASI

Judul Artikel	Physical Stability Test and Ethanol Cream Irritation Test OF Moringa oleifera L.
Jurnal	Jurnal Ilmiah Ibnu Sina (2021): 6(2) 186-195
Penulis	Sugihartini N.*, Zainab, Pamungkas A.

Tahap Submission

Submit pada tanggal 31 Maret 2021 dilakukan melalui OJS seperti tertera dalam lampiran 1.

Tahap Revisi

Permintaan revisi oleh editor melewati email meliputi jumlah kata dalam abstrak yang maksimal 250 kata, batas naskah dalam setiap halaman dan jumlah halaman maksimal 10 pada tanggal 10 September 2021 seperti tertera pada lampiran 2.

Perbaikan reviewer yang disampaikan melalui OJS meliputi penyesuaian template, jumlah kata dalam abstrak yang maksimal 250 kata, batas naskah dalam setiap halaman dan jumlah halaman maksimal 10 seperti tertera pada lampiran 3. Artikel hasil perbaikan disajikan pada lampiran 4.

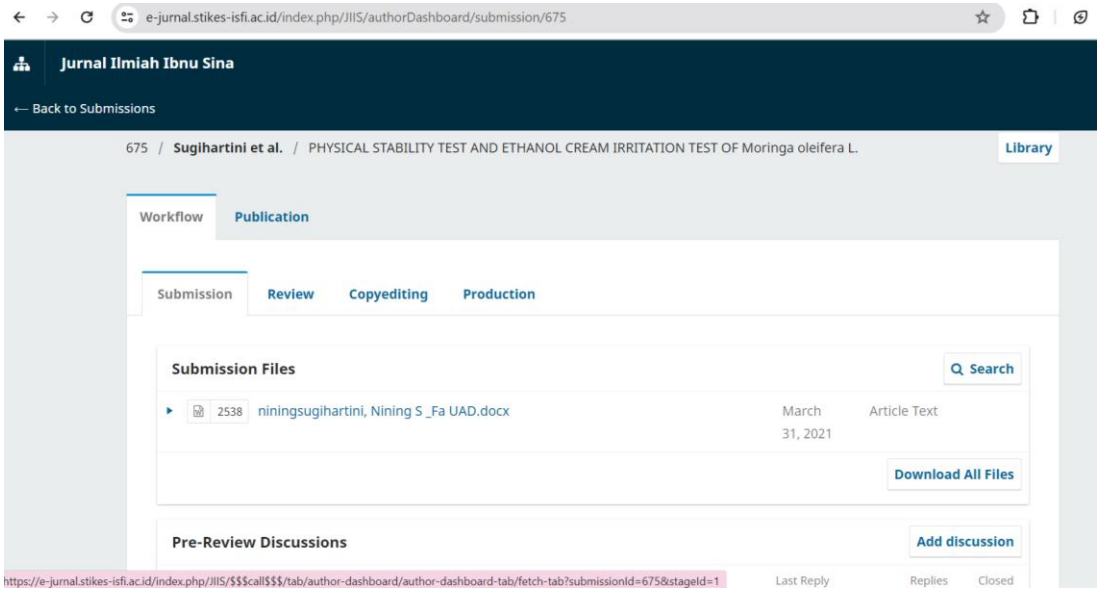
Tahap accepted

Editor menyatakan artikel telah dapat diterima pada tanggal 15 September 2021 seperti tersaji pada lampiran 5.

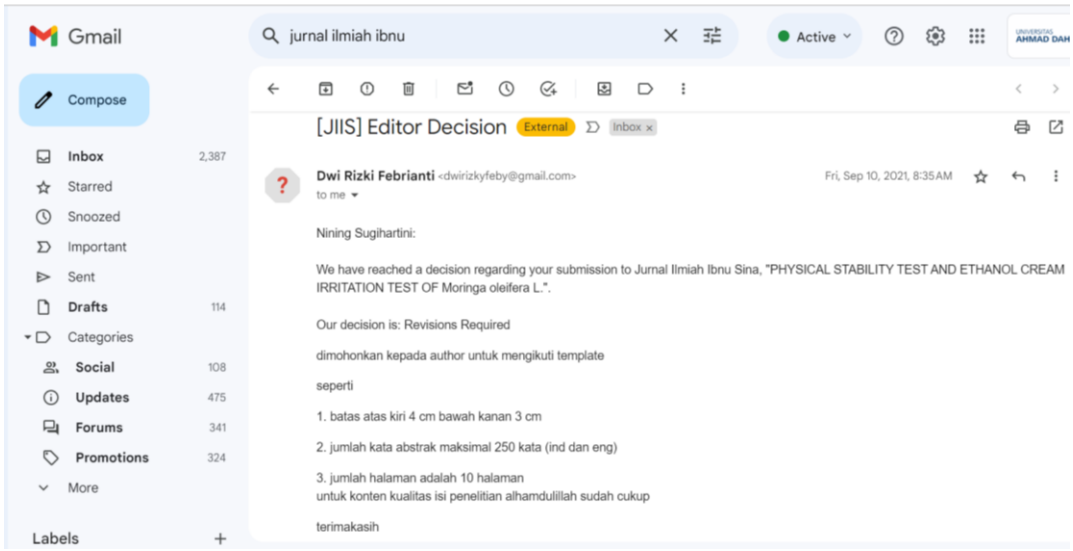
Tahap cetak

Editor menyatakan artikel telah dapat diterima pada tanggal 25 Oktober 2021 seperti tersaji pada lampiran 6.

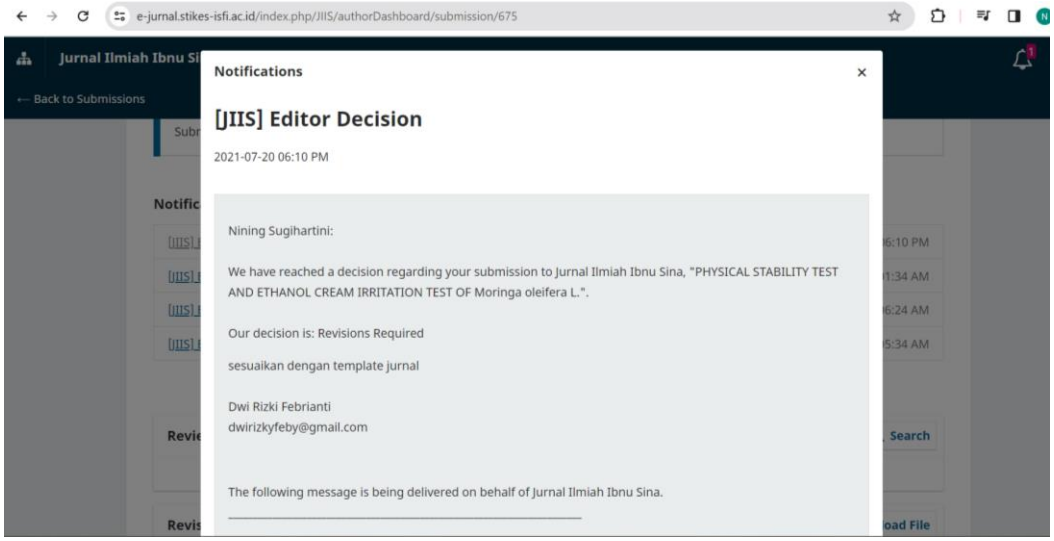
Lampiran 1. Bukti submit di OJS JIIS

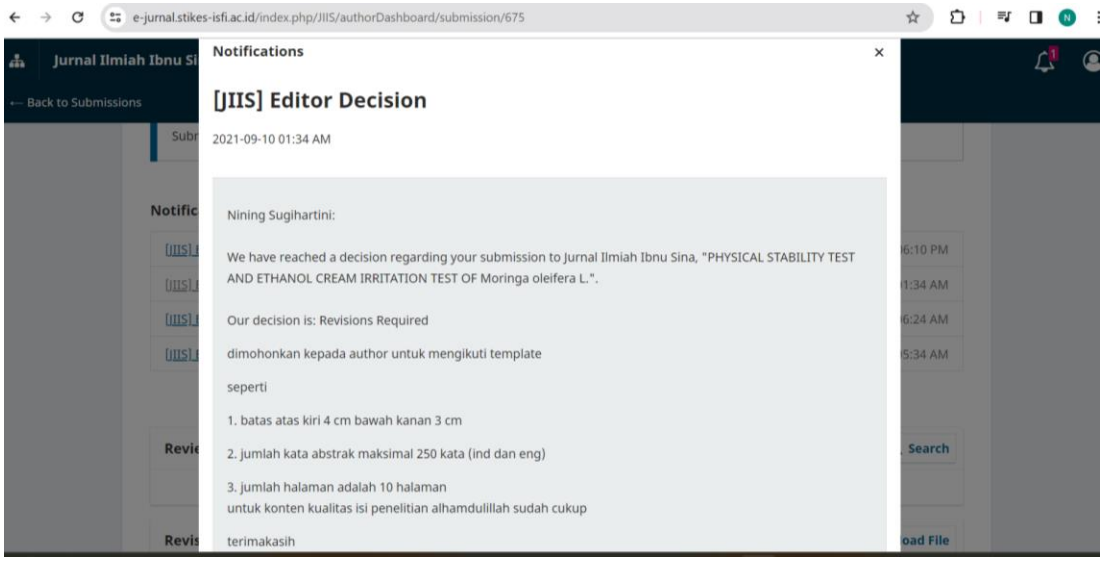


Lampiran 2. Email terkait permintaan revisi



Lampiran 3. Permintaan revisi di OJS





Lampiran 4. Artikel hasil perbaikan

***PHYSICAL STABILITY TEST AND ETHANOL CREAM IRRITATION TEST OF Moringa oleifera* L.**

UJI STABILITAS FISIK DAN UJI IRITASI KRIM EKSTRAK ETANOL DAUN KELOR (*Moringa oleifera* L.) DENGAN VARIASI KONSENTRASI EKSTRAK

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ABSTRAK

Pengembangan bentuk sediaan topikal krim ekstrak daun kelor telah dilakukan karena sangat bermanfaat dalam menjaga kesehatan kulit. Sebuah sediaan farmasi harus memenuhi persyaratan stabilitas dan tidak toksik Penelitian ini bertujuan untuk mengetahui pengaruh variasi konsentrasi ekstrak etanol daun kelor dalam bentuk sediaan krim basis M/A terhadap stabilitas fisik sediaan dan uji iritasinya.

Ekstrak etanol daun kelor (*Moringa oleifera* L.) diperoleh dengan metode maserasi dengan pelarut etanol 50%. Ekstrak kemudian diformulasikan dalam bentuk kirm basis M/A dengan konsentrasi 1% (F1), 3% (F2), dan 5% (F3). Krim dievaluasi stabilitas fisik meliputi uji mekanik (sentrifugasi) dan stabilitas fisik dalam suhu kamar (25±2^{o}C) dengan parameter pH, viskositas pada hari ke 1, 7, 14, 21 dan 28. Selain itu krim juga dievaluasi daya iritasi menggunakan hewan uji (kelinci) berdasar pedoman uji toksisitas nonklinik secara *in vivo,* Badan POM (2014). Data hasil uji pH dan viskositas dianalisis dengan menggunakan uji *oneway* ANOVA.

Hasil uji stabilitas fisik menunjukkan peningkatan konsentrasi ekstrak daun kelor menyebabkan peningkatan viskositas ($P<0,5$) dan penurunan pH ($P<0,5$) namun tidak mempengaruhi kestabilan fisik (uji mekanik) dan efek iritasinya. F2 (1%) memiliki pH sebesar 7,61 sedangkan F4 (5%) sebesar 7,01.Berdasarkan hasil penelitian disimpulkan variasi konsentrasi ekstrak etanol daun kelor dapat mempengaruhi stabilitas fisik krim dan tidak mempengaruhi sifat iritasinya. Krim ekstrak etanol daun kelor dengan konsentrasi 5% (F4) memiliki stabilitas fisik dan tidak mengiritasi kulit yang lebih baik dibanding formula lainnya.

Kata Kunci: uji stabilitas fisik, uji iritasi, ekstrak daun kelor, krim

ABSTRACT

The development of topical dosage forms of Moringa leaf extract cream has been carried out because it is very useful in maintaining skin health. A pharamceutical preparation must meet the

requirements of stability and non toxic. The study aimed to determine the effect of variations un the concentration ethanol extract cream of Moringa leaf of physical stability and irritability.

Ethanol extract of Moringa leaves was obtained by maceration method with 50% ethanol solvent. The extract was then formulated in the form of O/W base scarring with concentrations of 1% (F1), 3% (F2), and 5% (F3). Creams were evaluated for physical stability including mechanical tests (centrifugation) and physical stability at room temperature (25±2^{o}C) with parameters pH, viscosity on days 1, 7, 14, 21 and 28. In addition, creams were also evaluated for their irritability in vivo with using test animals (rabbits). The data obtained were analyzed using one-way ANOVA test.

The results of the physical stability test showed that an increase in the concentration of Moringa leaf extract caused an increase in viscosity (P<0.5) and a decrease in pH (P<0.5) but did not affect physical stability (mechanical test) and its irritating effect. F2 (1%) has a pH of 7.61 while F4 (5%) is 7.01. Based on the results of the study, it is concluded that variations in the concentration of ethanol extract of Moringa leaves can affect the physical stability of the cream and do not affect its irritation properties. Moringa leaf ethanol extract cream with a concentration of 5% (F4) has physical stability and does not irritate the skin better than other formulas.

Keywords: *extract of moringa leaves, cream, physical properties test, irritation test, stability*

INTRODUCTION

Previous studies have shown the active substance and activity of Moringa leaf ethanol extract. The leaves of Moringa (*Moringa oleifera* L.) has β carotene levels of 0.24±0.01µg/ml; total phenolic 122.26±1.49 mg/g extract, total flavonoids 2.51±0.06%; IC_{50} antioxidant with DPPH 155.58 µg/ml method; tyrosinase enzyme inhibition 143.99 µg/ml; SPF 24.75±0.11 (Sari, 2018). Moringa leaf ethanol extract has strong antioxidant and antithyrosinase activity because it contains flavonoids which can inhibit tyrosine activity in the process of melanin formation (Abidin *et al.,* 2018). The activity of ethanol extract of moringa as antioxidant, sunscreen and antielastase is higher than extract ethanol of papaya fruit (Alimsyah *et al*, 2020).

Research on the formulation of Moringa leaf extract has been carried out that Moringa leaf extract can be used in topical treatment in the form of a cream dosage as a sunscreen and prevents aging (Atif *et al.,* 2013). The other study (Nuryanti and Sugihartini, 2017) showed concentration of ethanol extract at 3% can be used to maintain evenness of skin. The concentration of ethanol extract affected the adhesivity, spreadability, pH and viscosity of cream in type O/W and W/O (Latif *et al*, 2020; Rikadyanti *et al*, 2020).

The choice of cream in this study has several advantages, including that it can be used to deliver drugs that show low water solubility, can be used to reduce irritation by formulating preparations in the form of an oil-in-water emulsion (Jones, 2008). The choice of cream type O/W is because it is easy to apply to the skin, is easy to wash off after being applied to the skin and has good spreading properties on the skin.

A cream preparation must meet physical stability requirements with pH and viscosity parameters because the stability of a substance is something that must be considered in making a formulation of a pharmaceutical preparation. This is important considering that a preparation is usually produced in large quantities and requires a long time to reach the consumer. Therefore, the stability of these preparations is tested according to a predetermined procedure. Cream preparations are stable, that is, if they are within acceptable limits during the period of storage and use, that is, their properties and characteristics remain the same as they had at the time of manufacture. The presence of active substances is thought to affect the physical stability of cream formulations (Rosmala, 2014).

In addition, the use of creams on the skin can cause several reactions such as irritation, phototoxicity, contact allergies and photocontacts as well as contact urticaria. Irritation that occurs on the skin is influenced by several factors, including frequency of use, product composition/formula, concentration of ingredients/composition, use of certain

ingredients, product penetration-enhancing compounds, location of product use, skin condition, contact time and the cumulative effect. To ensure that the cream preparations made do not irritate the skin, if used, it can be done by using an experimental animal irritation test. If there are signs of irritation on the skin of experimental animals, then there is the possibility of irritation to human skin (Windarwati, 2011).

Based on the description above, the basis for researchers to determine the effect of variations in the concentration of ethanol extract on physical stability with pH test parameters, viscosity test and to determine the in vivo irritation power of the ethanol extract cream of *Moringa oleifera* L. leaf.

METHODOLOGI

Sample

The samples used were Moringa oleifera L. leaves obtained from Pasar Beringharjo Yogyakarta and moringa leaf extract cream formulated in the Pharmacy Technology Laboratory of Ahmad Dahlan University Yogyakarta.

Material and Equipment

Materials in this study are extract leave of moringa, base of dosage form with pharmaceutical degree are cethyl alcohol, liquid parafin, stearic acid, Butylated Hydroxytoluene (BHT), glycerin, Trietanolamin (TEA), methyl paraben, prophyl paraben, aquadest. Six of rabbit was used in irritation test with 2 kg weight of body.

Equipment in this study are rotary evaporation (Heidolph), vacuum pump (Rotary vanewater bath (Memmert), analytrical balance (Wiggen Hauser), pH meter semisolid (Laqua act), viskometer (Rheosys Merlin VR), mettler toledo HB43 moisture analyzer, glassware (Pyrex).

Table I. Formulation of extract leave moringa in cream type o/w (Suryati, 2015)

Ingredients	Formula 1 (%)	Formula 2 (%)	Formula 3 (%)	Formula 4 (%)
Extract leave of moringa	-	1	3	5
Liquid parafin	10	10	10	10
Cethyl alcohol	1,5	1,5	1,5	1,5
Stearic acid	3	3	3	3
BHT	0,02	0,02	0,02	0,02
Glycerin	2	2	2	2
TEA	2	2	2	2
Methyl paraben	0,1	0,1	0,1	0,1
Prophyl paraben	0,05	0,05	0,05	0,05
Aquadest ad	100	100	100	100

Formulation of cream

Cream is made by the melting method. In the first stage, materials in the water phase were carried out, namely Butylated Hydroxytoluene (BHT), glycerin, Triethanolamine (TEA), and methyl parabens. The ingredients are mixed and heated in aquadest at 75^{o}C until dissolved. Then the next step is carried out for materials with an oil phase, namely stearic acid, cetyl alcohol, liquid paraffin, and propyl paraben. The materials are mixed and heated at a temperature of 75^{o}C. The third stage is mixing by mixing the oil phase gradually into the water phase mixture at a temperature of 75^{o}C while stirring in a warm mortar. Stirring constantly. After the two phases are mixed, then the Moringa leaf extract is added gradually into the mixture while stirring until it is homogeneous and cooled at room temperature and forms a stable emulsion. Cool the cream while continuing to stir until homogeneous. The cream preparations that are formed are put into a closed container (Nuryanti, 2016).

Physical stability of cream

Sentrifugation test

The cream sample is put into a centrifugation tube then put into a centrifugator. Samples were centrifuged at 3750 rpm for 5 hours. After centrifugation, it was observed whether there was separation or not. Testing is only done at week 0 (Rieger, 2000).

Physical stability at room temperature (25±2^{o}C)

Cream samples were stored at room temperature (25±2ºC), then carried out organoleptic observations (changes in color, smell, and syneresis), pH measurements, and viscosity measurements. Observations were made on days 1, 7, 14, 21, and 28 (Iswandana and Sihombing, 2017).

Test of pH

pH determination is carried out using a pH meter. The trick: the tool is first calibrated with a standard buffer solution (pH 7.01) and an acid buffer solution (4.01) until the tool shows the pH value. Then the electrodes are washed with water and dry with a tissue. The electrode is immersed in the solution. Let the tool show the pH value until constant. PH observations were carried out on days 1, 7, 14, 21, and 28. Measurements were made three times for each preparation (Erawati *et al.,* 2016).

Viscosity test

The viscosity test of the cream preparations was measured using a Brookfield LV viscometer and a Rhemoeter (Rheosys merlin VR) with a 1/30 mm spindle parallel plate. The viscosity test was carried out by placing 50 mg of the preparation on a plate and closed in parallel. Then run through a computer device with the Rheosys micra application. The measurement system, spindle speed, number of measuring points, measurement time intervals

between points, and temperature are set to “test definition”. Viscosity measurement starts with pressing start and lasts a certain time. The measurement parameters are set so that the formula experiences the same treatment (Hendriana, 2016).

Irritation test

The irritation cream test was carried out on rabbit test animals. Testing for acute dermal irritation is based on the provisions of the BPOM (2014) regarding guidelines for in vivo non-clinical toxicity testing. This test is used to determine the presence of an irritating effect on the skin as well as to assess and evaluate the characteristics of a substance when exposed to the skin. The test animals used were healthy and adult male or female albino rabbits, weighing about 2. The dosage used for liquid test preparations is as much as 0.5 mL and for solid or semi-solid test preparations as much as 0.5 g while the preparations in the form of containers and medical devices are extracted and prepared for test preparations. The test preparation is exposed to a skin area of ± 6 (2 x 3) cm^2, then the location of exposure is covered with gauze and plastered with a non-irritant plaster. All test animals should be observed for the presence or absence of erythema and edema, response assessment carried out at 1, 24, 48, and 72 hours after opening the patch (for non-corrosive/irritant test preparations).

RESULT AND DISCUSSION

Making moringa leave extract

Moringa leaf extract was made by maceration with 50% ethanol. The viscous extract of moringa leaves was obtained as much as 710 g and the yield of the extract obtained was 23.67%.

Making of cream

The active ingredient used in this sunscreen cream is the extract of Moringa oleifera L. leaves with its constituent ingredients consisting of cetyl alcohol, liquid paraffin, stearic acid, Butylated Hydroxytoluene (BHT), glycerin, Triethanolamine (TEA), methyl paraben, propyl. parabens, and distilled water (Suryati, 2015), where these ingredients are often used in cream formulations. In making cream, Moringa leaf extract is added after the cream base is formed and the base temperature has started to decrease, with the aim that the active antioxidant compounds contained in the extract are not lost or damaged.

The oil phases in this formulation are stearic acid, cetyl alcohol, liquid paraffin, and propyl paraben because they have good base-forming and emollient characteristics in cream making. For the water phase selected Butylated Hydroxytoluene (BHT), glycerin, Triethanolamine (TEA), methyl paraben. TEA is used as an emulgator because TEA will form an O/W emulsion which is very stable when combined with free fatty acids. A suitable free fatty acid is stearic acid because stearic acid will react with TEA in situ to produce a salt, namely

triethanolamine stearate which functions as an emulgator (Aulton, 2002).

The use of emulgators to prevent coalescence, namely the union of small droplets into one separate single phase (Pratama and Zulkarnain, 2015). Liquid paraffin is used as an emollient that maintains the stability of the mixture of oil and water phases in cream preparations, cetyl alcohol in the O/W emulsion can increase stability and can increase the consistency of cream (Unyala, 2009). Methyl paraben and propyl paraben function as preservatives and antimicrobials. Glycerin is used as a humectant that keeps the skin moist when the preparation is applied to the skin and BHT is used as an antioxidant to delay or prevent the oxidation of fat in creams (Anonim, 1995). After forming a cream with various extract concentrations of 1%, 3%, 5% and base (without extract), then the physical stability and irritation tests were carried out. Physical stability test was performed to determine the occurrence of physical changes in cream preparations during the storage period. Physical stability tests include mechanical testing (centrifugation) and physical stability at room temperature (25±2ºC) with parameters of pH, viscosity on days 1, 7, 14, 21 and 28.

Result of Sentrifugation test

Result of sentrifugation test showed that the cream is stable as shown in Table II.

Tabel II. Result of sentrifugation test 25ºC

Cream	Beginning	Final
F1	There is no phase separation	There is no phase separation
F2	There is no phase separation	There is no phase separation
F3	There is no phase separation	There is no phase separation
F4	There is no phase separation	There is no phase separation

Result of pH test

The increasing the concentration of the ethanol extract of Moringa leaves in the cream, the lower the pH value of each cream. This is related to the active substance contained in the ethanol extract of Moringa leaves, namely compounds such as polyphenols, flavonoids, and ascorbic acid which are acidic (Sugihartini *et al*, 2020). The decrease in pH during storage can occur due to the influence of CO_2, because CO_2 can react with the water phase to produce acids. This can be due to the fact that the packaging or container used is not impermeable, allowing air or gas to enter. An airtight closed container is a container that can prevent the passage of air or gas during handling, transportation, storage, and distribution (Anonim, 1995).

Result of Viscosity test

Viscosity and flow properties are a statement of the pressure of a liquid to flow, the higher the viscosity the greater the pressure. The viscous value is influenced by the thickening agent, the selected surfactant, the proportion of the dispersed phase and the size of the particles

(Martin *et al.,* 2008). The purpose of conducting a viscosity test on the preparation is to determine the properties of the flow rate and the viscosity level of the cream. When the preparation has a consistency that is too thick, the active substance will be difficult to separate from the base of the preparation and difficult to apply to the skin because it is difficult to stick, while if the preparation is too watery it will reduce contact with the skin so that drug absorption is not optimal. According to SNI 16-4399-1996, the ideal viscosity value of a sunscreen cream is 2.000-50.000 cps. The viscosity test of the Moringa oleifera L. leaf extract cream was performed using a Brookfield LV viscosimeter. Determination of the viscosity of the cream preparations was carried out for 28 days with the observation time, namely the 1st, 7th, 14th, 21st and 28th day.

Result Irritation test

Based on the data on rabbit skin smeared with formula I, formula II, formula III, formula IV and control gauze, the irritation score (primary irritation index) is 0. The score can then be compared with the irritation response category in rabbits. From these data, it can be seen that the cream and cream bases with the addition of 1%, 3%, 5% Moringa leaf extract made in this study did not cause irritation to rabbit skin and were included in the negligible response category (very mild). It is hoped that with the irritation test, the ethanol extract of Moringa leaves cream on human skin is safe and comfortable and does not cause irritation to the skin when used.

CONCLUSION

1. The variation in the concentration of ethanol extract of 50% *Moringa oleifera* L. leaf has an effect on the physical stability of the cream preparations. The greater the extract concentration added, the greater the viscosity but the smaller the pH value of the cream preparations.
2. The variation in the concentration of ethanol extract of 50% Moringa oleifera L. leaves did not affect the skin irritation of experimental animals. The greater the concentration of Moringa leaf extract added to the preparation did not cause irritation to the skin of the test animals.
3. Formula III, namely cream with the addition of *Moringa oleifera* L. leaf extract 3% has a good physical stability of the cream compared to other formulas with organoleptic test parameters, vsicosity, pH and centrifugation test (mechanical) and does not cause irritation to the skin of the test animals.

AKNOWLEDGEMENT

This study was supported by grant from LPPM UAD in 2020.

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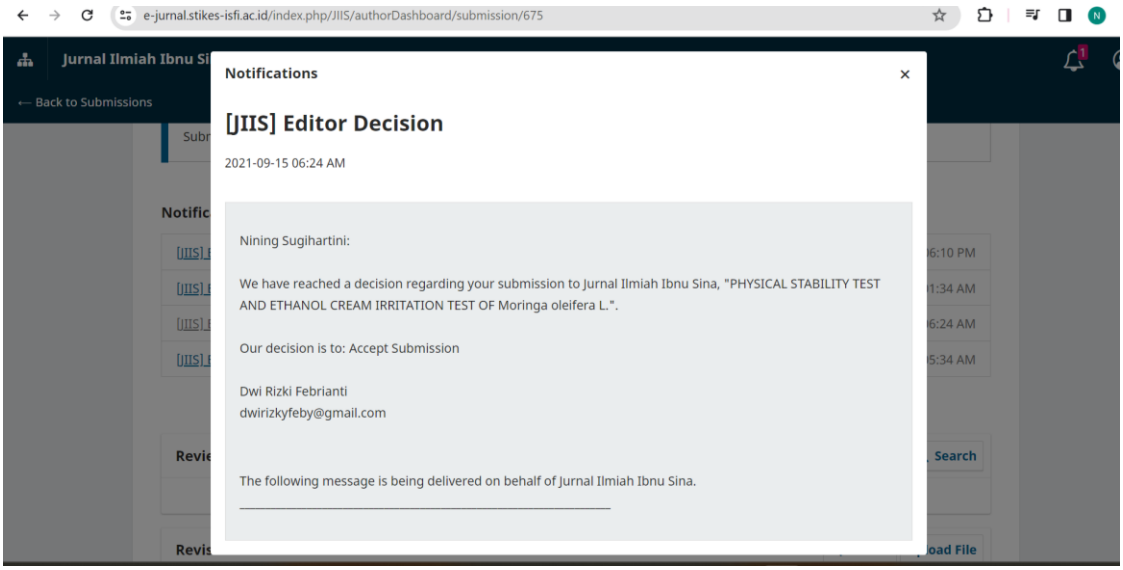
Lampiran 5. Informasi artikel diterima



Lampiran 6. Informasi bahwa artikel siap cetak