Gel Formulation of Kersen Leaf Ethanol Extract (*Muntingia calabura* L.) as Anti-inflammatory in Mice (*Mus musculus*)

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Abstract

Kersen (*Muntingia calabura* L.) is a type of neutropic plant that can grow well in the tropics. Its leaves have pharmacological effects such as antipyretic, antibacterial, antidiabetic, cardioprotective, antiulcer, antioxidant and anti-inflammatory. The chemical compounds contained in kersen leaves are saponins, tannins and flavonoids. Anti-inflammatory is the name for drugs or agents that work against or suppress the inflammatory process. Gel is a semi-solid preparation that is clear, contains active substances, is translucent and is a colloidal dispersion that has a strength caused by a network that binds together in its dispersed phase. The method used in this study was experimental using mice as experimental animals induced by egg albumin. The results showed that the three formulas had activity as anti-inflammatory and the gel preparation was stable during storage which was characterized by no significant changes in the preparation. So it can be concluded that the kersen leaf ethanol extract gel preparation that provides the best activity is F3.

Keywords: Anti-inflammatory; Kersen Leaf; Ethanol Extract; Gel; *Muntingia calabura L.*

1. Introduction

Kersen (*Muntingia calabura* L.) is a shrub that has fruit, can grow on less fertile soil, and is able to tolerate alkaline, acidic, and drought conditions. Its leaves have pharmacological effects such as antipyretic, antibacterial, antidiabetic, cardioprotective, antiulcer, antioxidant and anti-inflammatory (Puspitasari et al., 2018). The chemical compounds contained in kersen leaves are saponins, tannins and flavonoids. The content of flavonoid compounds has received a lot of attention because this group of compounds has many activities, one of which is its anti-inflammatory activity (Athaillah & Lianda, 2021).

In research conducted (Nugrahaeni et al., 2022). Stating that the concentration of ethanol extract of kersen leaves can provide an anti-inflammatory effect of more than 50% at a concentration of 5% with a value of 70, 27% and 10% with a value of 95.83%. According to Amdeekar, anti-inflammatory treatment is successful if there is 50% inhibition of inflammation [1] (Amdekar et al., 2015).

Anti-inflammatory is the name given to drugs or agents that work against or suppress the inflammatory process (Dorlan, 2002). There are three mechanisms used to suppress inflammation, the first being the inhibition of the enzyme cyclooxygenase. The second mechanism to reduce inflammation involves inhibition of immune functions. In the inflammatory process, the role of prostaglandins is to stimulate the immune system. The third mechanism for treating inflammation is to antagonize the effects of chemicals released by immune cells (Olson, 2003).

Gels or commonly called jellies are semi-systems consisting of two colloidal phases, namely solid and water, mixed together in a liquid. Gel formulations consist of additives and main ingredients. The main ingredients of the gel consist of three kinds, namely synthetic, semisynthetic and natural polymers (Ministry of Health, 1995).

The potential of kersen (*Muntingia calabura* L.) leaf extract as anti-inflammatory encourages further research to formulate it in gel dosage form. So this study was conducted to determine the anti-inflammatory activity of ethanol extract of kersen leaves (*Muntingia calabura* L.) in gel dosage form against mice (Mus musculus).

2. Methodology

2.1 Tools and Materials

The tools used in this study are oven, hot plate, thermometer, fan, vortex, viscometer, blender, erlenmeyer, measuring cup, analytical balance, digital balance, 1ml spoit, mortar and stamper, knife, cutting board, lidded jar, laboratory glassware, universal pH and mice cage. The materials used in this study consisted of ethanol extract of kersen leaves (*Muntingia calabura* L), voltaren gelR, 70% ethanol, NaCl 0.9%, triethanolamine, propylenglycol, glycerin, carbopol 940, methyl paraben, menthol, distilled water, parchment paper, filter paper, egg albumin and mice (*Mus musculus*).

2.2 Research Procedures

2.2.1 Sample preparation and processing

The samples used in the study were old and fresh kersen leaves. Kersen leaves were collected then washed with running water and then dried by cold-angling. After that, dry sorting was carried out to separate from unwanted plant parts and then after drying, the sample was mashed by blending.

2.2.2 Simplisia Powder Water Content Test

Simplisia powder water content test was carried out by first, porcelain cup was oven first for 30 minutes at 105oC with the aim of removing some of the water from the cup by using heat energy. Then, the cup is weighed 3 times to get the constant weight of the cup. Next, weighed 10 gran of simplisia in a cup that has known its constant weight then oven for 5 hours at 105oC.

2.2.3 Drying Shrinkage Determination Test

The drying shrinkage determination test is carried out by means of an empty cup in the oven first for 30 minutes at 105oC with the aim of removing or removing some of the water from the cup by heating the cup in the oven or by using heat energy.

2.2.4 Examination of Extract Yield

Extract yield is one of the parameters to assess the quality of an extract. Extract yield is the ratio between the extract obtained and the initial simplisia.

2.2.5 Sample Extraction

The sample is extracted by maceration method. The sample was macerated as much as 300 grams with 70% ethanol as much as 4000 ml. The sample was macerated for 3x24 hours while stirring occasionally then the sample was filtered. The resulting sample is concentrated until a thick extract is obtained by fanning using a fan until a thick extract is obtained.

2.2.6 Gel Preparation

Prepare the tools and materials to be used. Weigh the ingredients according to the calculation. Develop carbopol 940 in hot water at 70oC for 15 minutes. After expanding add little by little TEA then stir until homogeneous. After homogeneous add menthol then grind again until homogeneous (mixture 1). Combine glycerin and propylenglycol and add methyl paraben then mix until homogeneous (mixture 2). After homogeneous, add mixture 2 into mixture 1 and then grind until homogeneous then add kersen leaf extract and the remaining aquadest then grind until homogeneous and a gel mass is formed.

2.2.7 Gel Evaluation

Organoleptic

Organoleptic tests are carried out by visually observing the color, odor and shape of the preparation. A good gel preparation is characterized by a semi-solid concentration and a preparation that is usually clear (Astuti et al., 2017).

Homogeneity

The homogeneity test of the gel preparation is carried out by spreading the preparation evenly on transparent glass or a glass object and then observing it. A homogeneous preparation is characterized by the absence of coarse grains on the glass object (Astuti et al., 2017).

Test pH

The pH test was carried out using the colorimetric method. The pH of the preparation is measured based on the color change observed visually. This method is usually carried out using litmus paper, universal indicator paper, and indicator solutions for acid-base titrations. The pH test is carried out using universal pH by applying the preparation to litmus paper, then observing the color change and comparing it with the pH indicator used. The pH test results should be 4.5-6.5, which is the physiological pH of the skin (Astuti et al., 2017).

Spreadability Test

The gel was weighed at 0.5 gram, placed in the middle of the watch glass, covered with another glass, left for 1 minute, then the diameter of the gel spread was measured, then a weight of 150 grams was given and left for one minute, then the diameter of the gel spread was measured. The spreadability of 5-7 cm indicates the consistency of a semisolid preparation which is comfortable to use (Yati et al., 2018).

Adhesion Test

The gel was weighed at 0.25 grams, placed on a glass object, covered with another glass object on top of the gel, then pressed with a weight of 500 grams for 15 minutes (Bryce et al., 2018). Good adhesion for preparations is > 1 second (Cahyaningsih, 2018).

Viscosity Test

The viscosity test is carried out using a viscometer. Put 100 ml of the gel preparation into the pot. The requirements for a good gel viscosity test are 2000-4000 cPs (Martin et al., 1990).

Preparation of 1% w/v

Egg albumin suspension is made by weighing 0.1 gram of egg white and then dissolving it with 10 ml of 0.9% NaCl.

Selection and Preparation of Test Animals

The test animals that will be used are healthy male mice aged 2-3 months with a body weight of 20-40 grams. There were 25 test animals divided into 5 groups consisting of 5 animals in each group. Before treatment, the mice were adapted for 7 days by being given enough food and drink and did not show significant weight loss.

Treatment of Test Animals

Mice that have met the criteria have their body weight weighed and then divided into 5 groups, each group consisting of 5 mice. Each group measured the volume of the mice's legs before being induced. After that, each group was induced with 0.1 ml of egg albumin on the sole of the right foot intraplantarly, then the volume of the mice's feet was measured again after induction. In group I: Giving cherry leaf ethanol extract gel with a concentration of 5%. Group II: Giving cherry leaf ethanol extract gel with a concentration of 10%. Group III: Giving cherry leaf ethanol extract gel with a concentration of 15%. Group IV: Administration of control (+) branded anti-inflammatory gel. Group V: Administration of control (-) gel base. After treatment, the mice's leg volume was measured every 15 minutes for 180 minutes using a digital caliper.

3. Results And Discussion

3.1. Results

3.1.1 Dry Shrinkage Results and Water Content

Table 1. Dry Shrinkage Results and Water Content

Simple Weight	Cup+Simplisia Before in the Oven	Cup+Simplisia After being in the Oven	% Dry Shrinkage	% Water content
Dry Shrinkage (2 gr)	43.1 gr	42.9 gr	2.32%	2.14%
Water Content (10 gr)	65.3 gr	63.9 gr		

3.1.2 Results of Soaking Kersen Leaf Extract (*Muntingia calabura*L.)

Table 2. Results of Soaking Kersen Leaf Extract (*Muntingia calabura*L.)

Sample	Simple Weight	Extract Weight	% Yield
Kersen leaf simplicia	300 grams	37.9 grams	12.63 %

3.1.3 Physical Quality Test of Kersen Leaf Ethanol Extract Gel Preparation (Muntingia calaburaL.)

Table 3. Physical Quality Test of Kersen Leaf Ethanol Extract Gel Preparation (*Muntingia calabura* L.)

Formulas	F1 5%	F2 10%	F3 15%	F4 (Base)	Standard
Color	Special chocolate extract	Special chocolate extract	Special chocolate extract	Clear white	According to the color of the extract
Form	Semi solid (Gel)	Semi solid (Gel)	Semi solid (Gel)	Semi solid (Gel)	Semi solid (Gel)
Aroma	Typical aromatic extract	Typical aromatic extract	Typical aromatic extract	Typical aromatic menthol	Distinctive aromatic and does not smell rancid
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Spread Power	5.2	4.4	4.6	3.8	5-7 cm
Stickiness	6	7	10	7	>1 second
pH	6	6	6	6	4.5-6.5
Viscosity	2634	2882	3357	3612	2000-4000 cPs

3.1.4 Average Inflammation Volume of Mice Feet Before and After Egg Albumin Induction

Table 4. Average Inflammation Volume of Mice Feet Before and After Egg Albumin Induction

Treatment	Before Induction (mm)	After Induction (mm)
F1	0.119	2,826
F2	0.126	2,733
F3	0.306	2,946
K+	0.153	2,819
K-	0.126	2,959

Information :
F1: 5% ethanol extract gel
F2: 10% ethanol extract gel
F3: 15% ethanol extract gel
K+ : Voltaren emulgel positive control
K- : Gel base

3.1.5. Data on Decreased Volume of Inflammation, and % Inhibition of Mice Feet (*Mus musculus*)

Table 5. Data on Decreased Volume of Inflammation, and % Inhibition of Mice Feet (*Mus musculus*)

Minute	Average Edema Volume (mm) 3rd Minute				
	F1	F2	F3	K+	K-
15	1,786	1,446	2,439	2,233	2,653
30	1,299	1,233	1,906	1,613	2,446
45	1,126	0.899	1,566	1,279	2,086
60	0.859	0.726	1,359	0.959	1,799
75	0.733	0.693	1,219	0.813	1,706
90	0.659	0.619	1,079	0.713	1,613
105	0.626	0.553	0.939	0.659	1,479
120	0.559	0.446	0.826	0.592	1,319
135	0.419	0.373	0.779	0.479	1,239
150	0.366	0.299	0.633	0.413	1,166
165	0.293	0.173	0.506	0.279	1,079
180	0.193	0.159	0.319	0.199	0.979
VR	52.40	40.35	26.90	45.75	119.33
(%) Inhibition	61.23	70.82	80.27	67.69	-

Information :
F1: 5% ethanol extract gel
F2: 10% ethanol extract gel
F3: 15% ethanol extract gel
K+ : Voltaren emulgel positive control
K- : Gel base
VR (Inflammation Volume): The total volume of inflammation in various groups
% Inhibition: Percentage of total inflammation inhibition for each group

3.2. Discussion

This research was conducted to determine the anti-inflammatory activity of cherry leaf ethanol extract gel with varying extract concentrations. Apart from that, this research was also carried out to determine the stability of cherry leaf ethanol extract gel preparations and the concentration of a good base for use in making gel preparations. The extract concentrations used in this research were 5%, 10% and 15%. The cherry leaves (*Muntingia calabura* L.) used in this research were taken from Kaili Village, West Suli District, Luwu Regency, South Sulawesi. Cherry leaves are taken and then processed into simplicia. The process of making cherry leaf simplicia is carried out by airing it without being exposed to direct sunlight (Sentat & Pangestu, 2016).

After producing the simplicia, dry shrinkage and water content tests were carried out. The test results can be seen in table 4.1. In the drying shrinkage test the drying loss was 2.32% and in the water content test it was 2.14%, which means that in the drying shrinkage test the water content had met the requirements, this was in accordance with the dry shrinkage requirements, namely ≤ 11% and the water content requirements. that is, not ≥10% (Ministry of Health of the Republic of Indonesia, 2000 and BPOM, 2014). The results of the identification test of the chemical compound content of the ethanol extract of cherry leaves carried out by Sentat and Pangestu showed that the ethanol extract of cherry leaves contains flavonoids, saponins, steroids and tannins. This is reinforced by research conducted by (Vonna et al., 2021). Which states that the ethanol extract of cherry leaves contains flavonoids, saponins, steroids and tannins.

After drying shrinkage and water content tests were carried out, the simplicia was then extracted using the maceration method. 300 grams of cherry leaf simplicia was macerated using 4000 ml of 70% ethanol filter solution and a thick extract of 33.9 grams was produced. After a thick extract is produced, the yield value is tested. The yield value for cherry leaf extract is 12.63%, which can be seen in table 4.2, which means that it meets the requirements for the yield of thick extract, namely not less than 10% (Indonesian Herbal Pharmacopoeia, 2017). After producing a thick extract, a gel preparation is then made by weighing all the ingredients according to the calculations. Carbopol is developed with hot distilled water for 15 minutes then add TEA little by little and grind until homogeneous then add menthol then grind again until homogeneous (mixture 1). Mix glycerin and propylene glycol and add methyl paraben then stir until homogeneous (mixture 2). Add mixture 2 to mixture 1 and grind until homogeneous, then add cherry leaf extract and the remaining distilled water then grind again until homogeneous and a gel mass is formed.

After the gel preparation is finished, a physical quality test of the preparation and an anti-inflammatory test are carried out on the preparation. The preparation evaluation test consists of organoleptic test, pH test, spreadability test, adhesion test, and viscosity test. The test results can be seen in table 4.3. The results of the organoleptic tests from the four gel preparation formulas with different concentrations showed that for color, the higher the concentration, the more intense the gel color was and the preparation without extract produced a clear white color. The odor of the three formulas with extracts has a distinctive aromatic odor of cherry leaf extract and the formula without extracts produces a distinctive aromatic menthol odor. In the dosage form, a gel is generally produced, namely jelly. In the homogeneity test, the four gel formulas produced produced homogeneous preparations, which can be proven by the absence of coarse grains or particles when the gel is applied to the test glass. Based on the test results from week 1 to week 4, it can be concluded that the cherry leaf extract gel preparation is stable based on testing the odor, shape, color and homogeneity of the preparation. Based on the pH test results of the cherry leaf ethanol extract gel preparation, it showed that the four formulas were stable during the 4 weeks of testing, which was indicated by not experiencing significant changes during the test. The pH test results on all formulas produce a pH of 6, which means it meets the requirements and is in accordance with the skin's pH. pH evaluation is carried out to determine and see the suitability of the pH of the preparation to the pH of the skin. This measurement can

be seen whether the preparation made will not irritate the skin. With the pH of the preparation corresponding to the skin pH range of around 4.5-6.5 (Astuti et al., 2017).

In the adhesive strength test, the four formulas met the adhesive strength requirements, namely ≥1 second (Cahyaningsih, 2018). The adhesion test was carried out to determine the ability of the gel preparation to adhere when applied to the skin. Based on the results of the tests that have been carried out, it can be seen that the cherry leaf ethanol extract gel preparation has stable adhesion during the storage period as indicated by the absence of significant changes in the adhesion test results from week 1 to week 4. Based on the test results, F3 entered a longer adhesion range, namely 10 seconds, compared to F1, F2, and F4, which showed lower adhesion results, namely 6, 7, and 7 seconds.

The spreadability test was carried out with the aim of determining the spreadability of the gel. A gel that has good dispersing power will provide good distribution of medicinal ingredients so that treatment is expected to be more effective. Based on the results of testing the spreadability of cherry leaf ethanol extract gel, the spreadability of the preparation from week 1 to week 4 showed results that were not much different. However, in the spreadability test for the four formulas, only F1 met the spreadability test, namely 5.2 cm, while for F2 it was only 4.4 cm, F3 4.6 and F4 3.8 while the spreadability requirement for gel preparations was 5-7 cm (Yati et al., 2018). Increasing the concentration of carbopol in F2, F3, and F4 causes the emulgel spreadability to be smaller. The decrease in spreadability value is caused by the viscosity of the preparation. The greater the viscosity of a preparation, the smaller its ability to spread (Tambunan & Sulaiman, 2018).

The viscosity test was carried out using a Brookfield viscometer. The viscosity test is carried out to determine whether or not a preparation is easy to apply. Viscosity describes the viscosity of a dosage form, which is related to spreadability and stickiness. In gel formulations, the desired viscosity is not too thick because if it is too thick it will be difficult to spread and will make it taste unpleasant when used (Thomas et al., 2023). A good viscosity is in the range of 2000-4000 cPs, because with this viscosity the gel is able to spread well when applied (Martin et al., 1990). The viscosity test results for the four formulas showed higher viscosity values in F4 with a carbopol concentration of 2% compared to F1, F2, and F3 with lower carbopol concentrations. This is because the higher the concentration of gelling agent, the higher the viscosity of the preparation. This is in accordance with Thomas' research, which states that the higher the concentration of gelling agent, the viscosity of the preparation will increase. However, the four formulas have met the viscosity requirements.

Anti-inflammatory test research on the ethanol extract of cherry leaves was carried out using the method of creating artificial inflammation on the soles of the feet of mice using 1% egg albumin as an inducer. It can be seen in table 4.4 that all treatment groups experienced an increase in inflammation volume. Inducing egg albumin can cause inflammation because the albumin in egg whites will stimulate the immune system to produce antibodies and sensitize mast cells, resulting in inflammation, which is characterized by swelling on the soles of the mice's feet. Test animals were grouped into five groups with five mice per group. Groups 1, 2, and 3 were groups that were given cherry leaf ethanol extract gel preparations with different extract concentrations, namely 5%, 10%, and 15%. This difference in concentration aims to see

at what dose the activity of the cherry leaf ethanol extract gel is best in inhibiting inflammation. Group 4 is the negative control group, namely the control that was given the gel base only and group 5 is the positive control group that was given the diclofenac voltaren gel preparation. The use of Voltaren gel as a positive control is because it contains diclofenac sodium, which is a non-steroidal anti-inflammatory drug (NSAID) which has analgesic and anti-inflammatory effects (Mangampa & Nugroho, 2015). The mechanism of action of diclofenac sodium is to inhibit the formation of cyclooxygenase (COX) so that it does not form prostaglandins, thereby reducing the formation of pain mediators in the peripheral nervous system (Nurhidayati et al., 2020).

The reduction in inflammation in the mice's feet was measured every 15 minutes for 180 minutes using digital calipers.The results of the study showed that when administering F1 gel, ethanol extract of cherry leaves (*Muntingia calabura* L.) with a concentration of 5%, an average reduction in inflammation volume of 61.23% was obtained. In F2 with a cherry leaf ethanol extract concentration of 10%, the results showed an average reduction in inflammation volume of 70.82% and in F3 with an extract concentration of 15%, the results showed an average reduction of 80.27%. From the percentage reduction in inflammation volume, it can be seen that an extract concentration of 15% showed the best reduction. In all three formulas, inflammation was reduced by more than 50%, which means that the active substance at that concentration already has anti-inflammatory activity. This decrease occurred due to the flavonoids contained in the ethanol extract of cherry leaves. The anti-inflammatory mechanism of flavonoids is through several pathways, namely by inhibiting COX and lipoxygenase enzyme activity, inhibiting neutrophil degranulation, inhibiting leukocyte accumulation, and inhibiting histamine release. Inflammatory activity of flavonoids by inhibiting COX and lipoxygenase which can cause inhibition of leukotriene and prostaglandin synthesis. Inhibition of leukocyte accumulation during The inflammatory process will cause a decrease in the body's response to inflammation. Inhibition of leukocyte accumulation occurs due to inhibition of COX so that thromboxane will be inhibited. Inhibition of neutrophil degranulation will interfere with the release of arachidonic acid by neutrophils. Inhibition of histamine release occurs because flavonoids can inhibit histamine release in mast cells (Nijveldt et al., 2001). Apart from flavonoid compounds which provide anti-inflammatory effects, there are also saponin compounds. Saponin works by inhibiting the increase in blood vessel permeability so that inflammation does not occur (Audina et al., 2018).

In the comparison group, namely Voltaren Emulgel, the average result of reducing edema was 67.69%, which means that the percentage reduction in edema volume in the positive control was greater than in F1 with an average reduction in edema of 61.23%, which means that the potential for inhibiting Voltaren Emulgel. better than F1. In F2 and F3 the average percentage reduction in edema was greater compared to the positive control.

4. Conclusion

From the research results it can be concluded that :

1. The extract concentration with the best anti-inflammatory activity is F3 with inflammation inhibition of 80.27%.

2. The results of the organoleptic tests, pH, spreadability, adhesiveness and viscosity, resulted in the four preparations being stable for 1 month of storage, which was indicated by no significant changes occurring in the preparations during the test.
3. From the results of the base concentration test, it can be concluded that the best base concentration as an anti-inflammatory gel is a base concentration of 0.5% or F1.

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6. Reference

[1] Amdekar, S., Roy, P., Singh, V., Kumar, A., Singh, R., & Sharma, P. (2015). Anti Inflammatory Activity Of Lactobacillus On Carrageenan-Induced Paw Edema In Male Wistar Rats. *International Journal of Inflammation, 2015*. https://doi.org/10.1155/2012/752015.

[2] Astuti, D.P., Husni, P. & Hartono, K. (2017). Formulation and Physical Stability Test of Antiseptic Hand Gel Preparation of Lavender Flower Essential Oil (Lavandula angustifolia Miller). J Farmaka. 15(1):176–84.

[3] Athaillah, A., & Lianda, S. O. (2021). Formulation and Evaluation of Stick Balm Preparations from Red Ginger Oleoresin (Zingiber officinale Rosc) as a Muscle and Joint Pain Reliever. Journal Of Pharmaceutical and Sciences, 4(1), 34-40. https://doi.org/10.36490/journal-jps.com.v4i1.62

[4] Audina, M., Yuliet, & Khaerati, K. (2018). Anti-inflammatory Effectiveness of Ethanol Extract of Sumambu Leaves (Hyptis capitata Jacq.) in Male Rats (Rattus norvegicus L.). Bocelebes. Biocebeles, 12(2), 17–23.

[5] BPOM RI, (2014). Quality Requirements for Traditional Medicines, Regulation of the Head of the Republic of Indonesia Food and Drug Supervisory Agency, Indonesia, p. 1–25.

[6] Cahyaningsih, N. (2018). Formulation and Evaluation of Kaffir Lime (Citrus hystrix) Leaf Essential Oil Gel Preparation with HPMC Base as an Antibacterial Against Staphylococcus Aureus. Thesis, 1–15.

[7] Directorate General of POM, Directorate of Traditional Medicine Supervision. Republic of Indonesia Ministry of Health. (1995). Indonesian Pharmacopoeia Edition IV. Jakarta: Ministry of Health of the Republic of Indonesia.

[8] Mangampa, I., & Nugroho, T. E. (2015). The Effect of Giving Diclofenac Sodium Doses of 1.4 mg/Kg BW and 2.8 mg/KgBB on Serum Creatinine Levels in Wistar Rats. Main Medical Media, 4(4), 1004-1012.

[9] Martin, A., Swarbick, J., & Cammarata, A., (1990). Basic Physical Pharmacy and Physical Chemistry translated by Yosita, Third Edition, Pages 141-142, University of Indonesia

Press, Jakarta.

[10] Nijveldt, R. J., Nood, E. V., Hoorn, D. E. C., Boelens, P. G., Norren, K. V., and Leeuwen, P. A. M. (2001). Flavonoids: A Review Of Probable Mechanisms Of Action And Potential Applications. Am J Clin Nutr., 74, 418-425.

[11] Nurhidayati, L. G., Nugroho, B. H., & Indrati, O., (2020). Formulation and Physical Properties Test of Diclofenac Sodium Nanoemulsion Preparation with a Combination of Tween 80 and Transkutol. Saintex, 17(1), 33.

[12] Olson, J., (2003). Easy Learning Pharmacology. Jakarta: EGC Medical Book Publishers.

[13] Puspita, A. D. Mulangsri, D.A.K., & Herlina, H. (2018). "Sunscreen Cream Formulation of Ethanol Extract of Kersen Leaves (Muntingia calabura L.) for Skin Health" Media Researcher and Manager. Health., vol. 28, no. 4, pp. 263-270.

[14] Republic of Indonesia Ministry of Health. (2000). General Standard Parameters for Medicinal Plant Extracts, First Printing, 3-11, 17-19.

[15] Republic of Indonesia Ministry of Health. (2017). Indonesian Herbal Pharmacopeia II Edition. In Indonesian Herbal Pharmacopoeia.

[16] Sentat, T., & Pangestu, S. (2017). Test of the Analgesic Effect of Ethanol Extract of Kersen Leaves (Muntingia calabura L.) on Male White Mice (Mus musculus) Using Acetic Acid Pain Induction. Manuntung Scientific Journal, 2(2), 147. https://doi.org/10.51352/jim.v2i2.59.

[17] Tambunan, S. & Sulaiman, T. N. S., (2018). Lemongrass Essential Oil Gel Formulation with HPMC and Carbopol Base, Pharmaceutics Magazine, vol. 14, no. 2, pp. 87-95.

[18] Thomas, N. A., Tungadi, R., Latif, M. S., & Sukmawati, M. E., (2023). Effect of Carbopol 940 Concentration as a Gelling Agent on the Physical Stability of Aloe Vera Gel Preparations. 3(2), 316-324.

[19] Vonna, A., Desiyana, L. P., Hafsyari, R., & Illian, D. N., (2021) 'Phytochemical Analysis and Characterization of Ethanol Extract of Kersen Leaves (Muntingia calabura L.)', 5(1), pp. 8–12.

[20] Yati, K., Jufri, M., Gozan, M., & Putri Dwita, L. (2018). The Effect of Varying Hydroxy Propyl Methyl Cellulose (HPMC) Concentrations on the Physical Stability of Tobacco Extract Gel (Nicotiana tabaccum L.) and Its Activity Against Streptococcus mutans The Effect of Hydroxy Propyl Methyl Cellulose (HPMC) Concentration Va. Original Article Pharmaceutical Sciences and Research (PSR), 5(3), 133–141.