International Conference on Health and Biological Science
ICHBS, Page 232-246, December 2024

Formulation and iritaso test of ethanol extract gel of kersen bark (muntingia calabura l.)

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Abstract. Skin disease is an infection of microorganisms in the tissue. If not treated properly, it causes poor health. In developing countries, natural ingredients are used to treat skin problems such as cherry bark because it contains flavonoids, saponins, polyphenols and tannins in gel preparations. The purpose of this study was to test the physical properties and irritation of ethanol extract gel preparations of cherry bark (Muntingia calabura L.). Cherry bark was extracted by maceration method for 3 days using 70% ethanol solvent, then formulated with gel material made with extract concentrations F1 (2.5%), F2 (5%) and F3 (7.5%). The results of the physical properties test of all gel preparations met the requirements, namely dispersion diameter 3-7 cm, adhesion> 1 second, pH value according to skin pH, namely 4.5-7.5 and viscosity 2000-50,000 Cps. The results of irritation tests of all in vivo gel preparations with rabbits showed no erythema or edema after treatment for 24, 48 and 72 hours, so the gel preparations were considered safe and did not cause irritation. It can be concluded that all gel preparations of ethanol extract of cherry bark (Muntingia calabura L.) meet the standards for physical properties and irritation tests.

1 Introduction

Skin disease is an infection caused by the presence of microorganisms (fungi,parasites, bacteria, and viruses) that attack tissues (2). Improper treatment results in worsening health conditions. (1). Skin diseases are one of the things that affect the quality of life of adults and adolescents (14). In developing countries, local communities usually rely on natural products or traditional remedies for skin health care and addressing problems with their skin (45). Herbal remedies havebecome very important as an alternative to curing skin diseases due to fewer sideeffects at a low cost and high effectiveness (17). One of the plants that can treat various skin diseases is the kersen plant (*Muntingia calabura* L.). This plant is traditionally used to treat bleeding in open wounds, purulent wounds, burns, inflammation of the walls of blood vessels accompanied by blood clots (4).

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Kersen bark (*Muntingia Calabura* L.) is a plant that is often found on the sideof the road as a barrier plant. The use of the kersen plant is stated to have high benefits as a medicinal plant. The bark of the kersen stem contains flavonoid compounds, saponins, polyphenols and tannins (31). Empirically, this plant has the ability to be antibacterial, antioxidant, antidiabetic, anticancer, and anti- inflammatory (26). The ability of kersen plants as an antibacterial is evidenced byinhibited growth *Staphylococcus epidermidis* and *Edwardsiella tarda* (13).

Based on previous research, it has been shown that kersen leaf extract can inhibit bacteria *Staphylococcus aureus* at a concentration of 2.5% it can inhibit by 16.55 mm, a concentration of 5% can inhibit about 17.82 mm, and a concentration of 7% can inhibit 19.33 mm. So that kersen leaves can be formulated as an active ingredient in liquid soap preparations (21). The kersen plant can be used as an anti-acne in the form of easy-to-use preparations such as preparations in gel form.

Gel preparations are widely chosen because they are very easy to apply (easy to evenly distribute, absorb and clean) and more attractive than other topical preparations, for example, preparations in the form of lotions, creams and ointments (15). In gel preparations, it does not cause a sticky feeling, can providea cold sensation and is relatively stable so that it has better potential for topical formulations, besides that gel preparations also have several advantages, namely good absorption, gel is not transparent, soft, easy to apply and does notcause dry skin (24).

Evaluation of the physical properties and irritating properties of topical preparations needs to be carried out, which is intended so that the preparation can provide a guarantee, namely that the physical can be known whether there are changes that have occurred in the preparation in terms of its physical aspect(such as the preparation smells bad, the color changes) and can provide a guarantee that it will not irritate the skin when used (22). The physical propertiesof the preparation affect the achievement of the expected pharmacological effect(15).

Based on the description above, researchers are interested in making a formulation and irritation test of gel preparations from ethanol extract of kersern bark (*Muntingia calabura* L.). because previously there was no research on making gel preparations with kersern bark extract (*Muntingia calabura* L.). To evaluate the preparation, physical tests were carried out which included organoleptic, homogeneity, viscosity, pH, dispersion and adhesion of the gel preparation of ethanol extract of Kersen bark bark (*Muntingia calabura* L.). Meanwhile, irritation tests are carried out on preparations with the aim of assessing the safety of the product.

2 Method

2.1. Types of Researchers

The research method used was laboratory experiment which included preparation of materials, plant determination, making of kersen bark extract (muntingia calabura L.), phytochemical screening and making gel formulation from kersen bark extract. Plants were taken from Ledug Village,Banyumas Regency. This research was conducted at the Harapan Bangsa University Research Laboratory and the Ahmad Dahlan University Biology Laboratory. This research has passed the research ethics permit where in this research the research ethics permit was carried out at Harapan Bangsa University. The results of the kersen bark ethanol extract gel preparation was tested for physical properties and irritation tests on albino rabbits. All data generated were then analyzed using the IBM SPSS 22 program with an SPSS program license, namely the code

9DNCAF2O3QVDV7FBI0696006GWLNXZ PPRYT which was tested using One Way Anova to determine the differences between each formula (36).

2.2. Tools and Materials

2.2.1. Tool

Laboratory glass equipment (pyrex), a set of evaporator equipment (biobase), waterbath (mammert), refrigerator (sharp), oven (mammert), hotplate, pH meter, homogeneity test equipment, dispersion test device,vision device, parselen dish, blender (cosmos) stirring rod, mortar spatula and stemper, water bath (cimarex), digital scale (kenko), animal scale, milligram scale, glass beaker (pyrex), measuring cup, spoon, spatula, napkin, knife, teleman, ointment pots, watch glasses and parchment paper.

2.2.2. *Material*

The ingredients used in the gel include kersen bark extract *(Muntingia calabura L.)*, 70% *ethanol (Technical),* carbapole 940, Na CMC *(Corboxymethlcellulose food greade),* Glycerin (vegetable glycerine), propylene glycol *(Phrmaceutecal grade),* methyl paraben *(Phrmaceutecal grade*), Triethanolamine (TEA), propyl paraben *(Phrmaceutecal grade)* and aquadest. The material used in the irritation test study was a gel preparation of kersen stem bark extract, an albino rabbit test animal with a body weight of about 2 kg.

2.3. Formulation

Table 1. Gel formulation

Material	**Uses**	**Composition (grams)**			**Range (%)**
		F1	**F2**	**F3**	
Extract	Active substance	2,5	5	7,5	
Carbopol 940	Gelling agent	0,5	0,5	0,5	0,5-1
Na-CMC	Fastener	5	5	5	2-10
Gliserin		10	10	10	10-15
Propylene glycol	Humektan	2,5	2,5	2,5	3-6
Tritolamin (TEA)	Alkazing agent	0,6	0,6	0,6	0,5-2
Methyl paraben	Preservatives	0,2	0,2	0,2	0,4-0,8
Propyl paraben	Preservatives	0,5	0,5	0,5	0,4-0,8
Aquadest	Solvent	Add 100	Add 100	Add 100	

The gel is made based on the formulation of the gel base. The process of making the gel begins to prepare the ingredients according to the formula.and all ingredients are weighed according to the formula. Each formulation has different patterns of extract concentration and aquadest volume. It was carried out by developing a CMC-Na base and 940 carbopolon a mortar with hot water. Carbopol 940 is added with TEA to neutralize the

pH of carbopol 940. After that, CMC-Na and carbopol 940 that have been added TEA *(Merck),*Crushed until homogeneous After expanding, methyl praben that has been dissolved with ethanol is grinded until homogeneous. Added with ethanol extract of kersen bark bark that has been dissolved withglycerin is gradually crushed until homogeneous. Finally, add the abrasive praben profile until homogeneous (20).

3 Result And Discussion

3.1 Plant Determination

Plant determination is carried out to determine the authenticity and correctness of kersen plants (*Muntingia calabura* L.) used in the study. Plant determination is carried out by comparing or equating the characteristics of the plant to be studied with other plants whose identity is already known (44). Kersen stems were obtained from Ledug Village, Banyumas District. Plant determination was carried out in the Environmental Laboratory of the Facultyof Biology, Jenderal Soedirman University, emphasizing that the plants usedwere indeed kersen plants that belonged to the *Muntingiaceae,* Genus *Muntingia* and species *Muntingia calabura* L.).

3.2 Extraction Of Kersen Bark

In this study, the manufacture of extracts Kersen bark (*Muntingia calabura* L.) done by simplicia Kersen bark as much as 300 grams in maceration for 3 days with a 70% ethanol solvent which does not cause irritation when used on the skin so that it is safer and more maceration results because flavonoid compounds that are polar will be maximallymacheted if dissolved with a solvent that is polar so that ethanol 70% is a solvent that is polar (27).

Randement calculations are carried out to determine the percentage of the amount of extraction and to determine the level of effectiveness of the resulting process (41). The results of the rendement can be seen in Table 2.

Table 2. Randement of kersen bark extract (Muntingia calabura L.)

Sample	Simplisia Weight	Extract Weight	% Randemen
Kersen bark (*Muntingi calabura* L.)	*a* 300 grams	79,322 grams	26,40%

In simplicia Kersen bark (*Muntingia calabura* L.) with a weight of 300 grams which was macerated for 3 days with 70% ethanol, a thick extract of 79.322 grams was obtained with a percentage of 26.4%. The result meets the requirements of the Indonesia Herbal Pharmacopoeia, which is not lessthan 10% (11). This is in line with previous research that maceration for 3 days with 96% ethanol at 500 grams of simplicia Kersen bark (*Muntingia calabura* L.) produced a randement of 11.71% (4).The results of this study show that the type of solvent in the extraction will affect the high yield obtained (27).

3.3 Skrining Fitokimia

Phytochemical screening is a simple method for the qualitative analysis of compounds contained in plants, by observing the color response or discoloration that occurs after the addition of reagents (Melati and Parbuntari 2022). The phytochemical test carried out in this study is a flavonoid test, which is intended to find out whether the compound is contained in the kersen bark extract because of flavonoid compounds that are useful for antibacterial and anti-acne (26).

The identification of flavonoid compounds in this study was carried out using kersen bark extract which was reacted with Mg (magnesium powder) and HCl (hydrochloric acid) powders and was observed qualitatively the color change reaction that occurred. The results of this study were tested positive for containing flavonoid compounds in kersen stem extract because there was a color change to orange (23). The color change occurs because of a reaction when the addition of magnesium powder and hydrochloric acid to the flavonoid test will cause a decrease in the existing flavonoid compounds so that it causes a red reaction which is characteristic of the flavonoid content (23). In the flavonoid test, the results were positive because there was a change in the color of the solution to reddish. The addition of Mg and HCl to flavonoid-containing samples will form flavium salts that are red or orange (37).

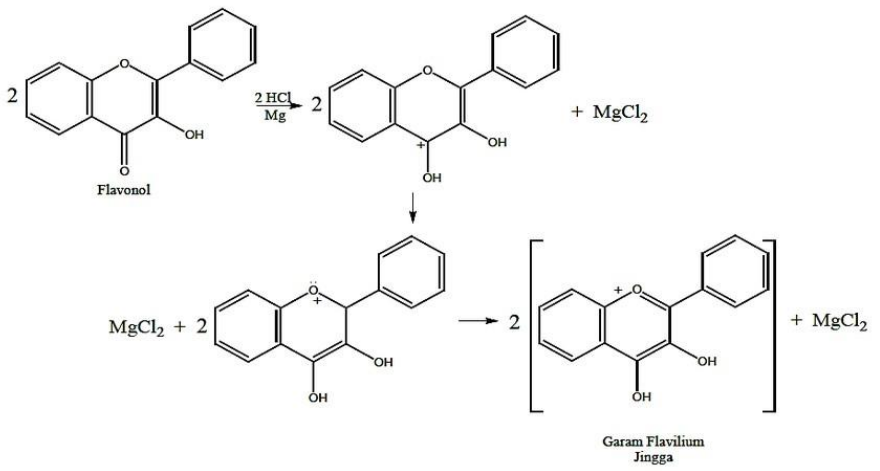

Figure 1. Flavonoid Reaction with HCl and Mg Metal [39].

Table 3. Results Of Flavonoid Screening of Kersen Bark Extract

Compound	Observation	Result
Flavonoid	The solution changes color to orange	(+)

Information:
(+) Contains such secondary metabolite compounds
(-) Does not contain such secondary metabolite compounds

The results of the flavonoid test from the bark extract of the kersen stemwere positive for flavonoids (23). This result is in accordance with previousresearch that states that flavonoids are found in the bark of kersen stems (7)(33)(40). The total flavonoid content at a concentration of 100 ppm ofkersen bark is 10.82 mg/kg (42).

3.4 Formulation Of Gel Preparations

In this study, ethanol extract of kersen bark was formulated in the form of a gel preparation. The gel formulation was made as much as 100 grams with variations in extract concentrations of 2.5%, 5% and 7.5% using a gel base in the form of Karbopol 940. Gel preparations need a base to get a preparation that has high stability and compatibility, low toxicity and is able to increase the contact time with the skin (2).

The manufacture of kersen bark ethanol extract gel began by developinga carbopol base of 940 and CMC-Na as a binder on the mortar with hot water. Carbopol 940 is added with TEA to neutralize the pH of carbopol 940.After that, CMC-Na and carbopol 940 that have been added TEA *(Merck),* are ground until homogeneous. After expansion, methyl praben that has been dissolved with ethanol is crushed until homogeneous. Added with ethanol extract of kersen bark bark that has been dissolved with glycerin is gradually crushed until homogeneous. Finally, add the gerroded propyl praben until homogeneous (20).

Carbopol 940 or carbomer is a high molecular weight synthetic polymer of acrylic acid cross-linked with allyl sucrose or allyl ether pentaerythritol, Carbomer in pharmaceutical formulations of liquid or semi-solid preparationscan increase viscosity (2). The concentration of carbopol 940 as a gelling agent can be varied to produce a preparation that meets the criteria of the physical properties of the gel, namely pH, viscosity, adhesion, dispersibility, organoleptic and stability. Carbopol 940 can affect the release of the active substance of the drug through its viscosity value (32). Carbomer has acidic properties so in its use it requires other additives such as TEA which functions as a pH regulator so that the pH is close to the pH of the skin (2).

Sodium Carboxymethyl Cellulose (Na CMC) is widely used in oral and topical pharmaceutical formulations mainly because it can improve its viscosity properties (2). Propylene glycol and glycerin function as humectants, aquades as solvents, while methyl parabens as preservatives so that preparations do not mold quickly (38).

The gel that has been made is tested for the physical properties of the preparation to determine the quality of the gel preparation made. Physical properties tests of gel preparations include organoleptic tests, homogeneity tests, viscosity tests, pH tests, dispersion tests, adhesion tests and irritationtest evaluations.

3.5 Evaluation Of the Physical Properties Of The Gel

3.5.1 Organoleptic Test

Organoleptic examinations include odor, colour and texture, and these examinations involve respondents with certain criteria (12). This test is carried out to see the physical stability of the preparation by observing color, turbidity and homogeneity (41).

Table 4. Organoleptic Test Results

Formula	Organoleptic test results		Texture	Standard
	Color	Aroma		
F0	Light brown	Typical of kersen bark	Thick	A thick, tuda brown gel
F1	Brown	Typical of kersen bark	Thick	with a distinctive smell of kersen bark (41)
F2	Dark brown	Typical of kersen bark	Thick	

The results of organoleptic testing showed that each formula had a distinctive smell of kersen bark. The color of each gel formula is different starting from F1 with light brown, F2 brown and F3 dark brown. The texture of the gel preparation in each formula is thick. This is in line with previous research where gel preparations using carbopol 940 base have a thick texture with a color according to the color of the extract it contains, as well as a distinctive smell like a sample (20). Another study states that the gel of ethanol extract of kersen leaves has a semi-solid shape with a brown color (25). The addition of extracts to each formula has an effect on the level of color concentration (41). The higher the concentration of the extract used, the more concentrated or the higher the intensity of the color of the prepared preparation (30)(35)(43).

3.5.2 Homogeneity Test

The homogeneity test aims to determine the homogeneity of a preparation and identify the possibility of change. Homogeneous preparations are indicated by the absence of coarse particles or granules in the preparation (5). The homogeneity test was carried out by applying the above preparation which was taken on 3 parts of the preparation, namely the top, middle and bottom and then placed on a glass object (transparent glass), the early test was carried out with 3 replications.

Table 5. Homogeneity Test Results

Formula	Homogeneity test results	Standard
F1	No coarse and homogeneous granules	No coarse and homogeneous granules (5).
F2	No coarse and homogeneous granules	
F3	No coarse and homogeneous granules	

Based on Table 5, the homogeneity test results of the gel preparation of ethanol extract of kersen bark are F1, F2 and F3 providing a homogeneous arrangement as evidenced by the absence of coarse grains and lumps in the preparation. Homogeneous preparations can help the distribution of active substances optimally and provide a comfortable taste when applied to

the skin (20).

The results of this test are in accordance with previous research where mangosteen peel gel with various series of extract concentrations, namely 5%, 10% and 15% is homogeneous with the absence of coarse particles on the glass of the object (35). Research onkersen leaf extract sunscreen gel also stated that homogeneous gel preparations have an even distribution of particles in the object glass (25).

3.5.3 pH Test

The pH test aims to evaluate that the preparation made has a pH value in the appropriate range and is acceptable to the skin (20). The results of the pH test of the preparation show that the preparation mademeets the pH test requirements of gel preparations. The pH test of the preparation is said to be qualified because of the pH of the extract gel preparation Kersen bark (*Muntingia calabura* L.) made not to exceed the physiological pH of the skin which is 4.5-8.0 (41). The test results ofthe gel preparations made have a pH in the range of 4.7-5.4 which meets the physiological pH range of the skin.

Table 6. pH test results

Formula	Ph	Standard
F1	5.4±0.1	4,5-8,0
F2	5.0±0.1	(41).
F3	4.7±0.057	

The test results showed that there was a difference in the pH of eachpreparation which was influenced by the number of concentrations of extracts used, which the more it produced, the more acidic the pH. Increased concentration of extracts Kersen bark will lower the pH of the preparation where F3 has the highest concentration of extract so that the pH becomes the lowest. This is in line with Research states that the bark of the stem of the kersen plant has acidic properties (18).

Extract Kersen bark acidic, as stated in previous studies that pH of the extract Kersen bark ranges from 4.29 (18), so that with an increasein the concentration of the extract, the pH of the preparation will be lowered (28). F1 which has the lowest extract concentration has the highest pH compared to the pH value of F2 and F3 which is more acidicthan F1 (28)(18).

The results obtained were continued using SPSS version 26 which was carried out a normality test with Shapiro-Wilk and the homogeneityof variances test obtained a significance value of p-value>0.05 which means that the data is distributed normally and homogeneously, so thatthe Anova test can be carried out. Based on the One-Way Anova test,a significance value of 0.004<0.05 was obtained so that H1 wasaccepted, which means that there was a significant difference in the pHtest results based on the three test formulas, so a follow-up test was carried out for Post-Hoc Tukey HSD which aimed to determine the magnitude of the difference between the formulas (11).

The results of the Post-Hoc Tukey HSD test showed significant differences in F1 to F2 with a significance value of 0.015 (p-value<0.05),in F1 to F3 with a significance value of 0.000 (p-value<0.05) and in F2 to F3 with a significance value of 0.003 (p-value<0.05). Based on this, it can be interpreted that the concentration of kersen bark extract affectsthe pH parameters of the gel preparation.

3.5.4 Viscosity Test

Viscosity tests are carried out to determine the viscosity value of gelpreparations (10). In this study, the viscosity of each gel preparation wasobserved by measuring it using a viscometer (Atago®). The preparation is inserted up to 100 ml into a cup with a rotation of 12 rpm, then spindlenumber 3 is installed and the rotor is run. The viscosity value indicatedby the tool is observed. In this test, the viscosity value is said to meet the standard of a good preparation if the viscosity value is in the rangeof 2,000-50,000 Cps (11). Viscosity test gel Kersen bark The F1-F3 formula has met the requirements because it is in the value range of 17,441-18,681 Cps.

The viscosity value of the gel greater than 50,000 Cps will cause the gel preparation to become too thick so that it is difficult to apply and thepreparation cannot be spread evenly. Viscosity less than 2,000 Cps can cause the gel preparation to become too thin so that it adheres less to the skin and flows immediately after application (36). The results are inTable 7.

Table 7. Viscosity Test Results

Formula	Viscosity (Cps)	Standard
F1	17,441±3.60	2,000 – 50,000 Cps
F2	18.271±0.35	(February 2021)
F3	18.681±6.56	

Viscosity test results Kersen Stem Bark Extract Gel It can be knownthat from F1-F3 the viscosity value continues to increase which in F3 has the largest viscosity value among the other two gel formulas. The increase in the viscosity value of F3 occurs along with the increase in the concentration of kersen bark extract where the higher the concentration of the extract in F3 can increase the viscosity of the preparation which will form the gel base thereby increasing the viscosity(22).This result is in line with previous research where the viscosity value of the gel *hand sanitizer* Tongue-in-law leaf extract is increasing with an increase in the concentration of tongue-in-law extract (22).

The results obtained were continued using SPSS version 26 which was carried out a normality test with *Shapiro-Wilk* and the *homogeneity of variances test* obtained a significance value *of p-value>0.05 which means that the data* is distributed normally and homogeneously, so thatthe Anova test can be carried out. Based on *the One-Way Anova test,* a significance value of 0.000<0.05 was obtained so that H1 wasaccepted, which means that there is a significant difference in the viscosity test results based on the three test formulas, so a follow-up test *of Tukey* HSD was carried out which aimed to determine the magnitude of the difference between the formulas (11).

The results *of the Post-Hoc Tukey* HSD test showed significant differences in F1 to F2 with a significance value of 0.001 (*p-value<0.05), in F1 to F3 with a significance value of 0.000 (p-value<0.05) and in F2 to F3 with a significance value of 0.002 (p-value<0.05). Based on this, it can be interpreted that the concentration of kersen bark extract affects the viscosity parameters of the gel preparation.*

3.5.5 Adhesion Test

The adhesion test aims to determine the time it takes for the gel to adhere to the skin. The longer the gel sticks to the skin, the more active substances diffuse into the skin so that the more effective its use is so that it allows higher absorption of the drug by the skin, preferably if thebond between the gel and the skin is less optimal, the drug will be easilyremoved from the skin (2).

Table 8. Adhesion Test Results

Formula	Adhesion (seconds)	Standard
F1	2.96±0.66	>1 seconds (41).
F2	3.49±1.15	
F3	4.04±1.06	

The results of observations made on the F1-F3 formula have an adhesion of >1 seconds. It is said to have good adhesion of the preparation, namely when the object glass is removed >1 second, so that the preparation Kersen Stem Bark Extract Gel has adhesion that meets standards (41). In F1-F3 has a greater adhesion, this is because the adhesion value is directly proportional to the viscosity value, where the higher the viscosity value of F1-F3, the adhesion value of F1-F3 also increases (8)(32)(36).

Dosage Kersen Stem Bark Extract Gel that meets adhesion standards means that when applied to the skin, the preparation can adhere well. This test is quite important to be carried out especially for gel preparations. The preparation must meet the requirements of a good gel, one of which can adhere well but not stick to the surface of the body (8). The results of this study are in line with the previous research, namely the preparation of Brandish extract gel where the more extracts from F1 (5%) to F3 (15%), the higher the adhesion value, namely at F1 26.07 seconds and F3 to 33.61 seconds (36).

The results obtained were continued using SPSS version 26 which was carried out a normality test with Shapiro-Wilk and the homogeneity of variances test obtained a significance value of p-value>0.05 which means that the data is distributed normally and homogeneously, so that the Anova test can be carried out. Based on the One Way Anova test, a significance value of 0.000<0.05 was obtained so that H1 was accepted, which means that there was a significant difference in the results of the adhesion test based on the three test formulas, so a follow-up test of Tukey HSD was carried out which aimed to determine the magnitude of the difference between the formulas (11).

The results of the Tukey HSD Post-Hoc test showed significant differences in F1 to F2 with a significance value of 0.043 (p-value<0.05), in F1 to F3 with a significance value of 0.001 (p-value<0.05) and in F2 to F3 with a significance value of 0.033 (p-value<0.05). Based on this, it can be interpreted that the concentration of kersen bark extract affects the adhesion parameters of the gel preparation.

3.5.6 Spread Power Test

The dispersibility test is carried out to determine the distribution of the preparation on the surface of the skin so that the active compounds can spread evenly and be absorbed by the skin so that it can provide a therapeutic effect (29). Good base spreading ability will provide ease of application to the skin surface. Good spreadability test standards are 3-7 cm (20). Spread test results Kersen stem culotte extract gel has met the standard with F1-F3 values

ranging from 3.9 cmto 5.1 cm.

Table 9. Spread Test Results

Formula	Spread power (cm)	Standard
F1	5.1±0.3	3-7 cm
F2	4.2±0.2	(20).
F3	3.9±0.1	

The dispersibility value of the gel preparation showed a smaller value from F1-F3 which was caused by the increase in the concentration of kersen bark leaf extract in the gel. The larger the concentration of the extract, the thicker the preparation so that the dispersion value is smaller. So, it can be seen that the results of the dispersion test are inversely proportional to the value of viscosity and adhesion (28). This is in line with the study on cardamom fruit extract gel preparations which experienced an increase in extract concentration from F1 (5%) to F3 (15%) which had a decreasing dispersion from F1 by 6.46 cm to 4.23 cm at F3.

The results obtained were continued using SPSS version 26 which was carried out a normality test with *Shapiro-Wilk* and the *homogeneityof variances test* obtained a significance value *of p-value>0.05 which means that the data* is distributed normally and homogeneously, so that the Anova test can be carried out. Based on *the One-Way Anova test*, a significance value of 0.003<0.05 was obtained so that H1 was accepted, which means that there is a significant difference in the results of the spreadability test based on the three test formulas, so a follow-up test *of Tukey* HSD was carried out which aimed to determine the magnitude of the difference between the formulas (11).

The results of the *Tukey HSD Post-Hoc* test showed significant differences in F1 to F2 with a significance value of 0.009 (*p-value<0.05), in F1 to F3 with a significance value of 0.000 (p-value<0.05) and in F2 to F3 with a significance value of 0.004 (p-value<0.05). Based on this, it can be interpreted that the concentration of kersen bark extract affects the dispersibility parameters of the gel preparation.*

3.6 Evaluation of irritation

In this study the irritation test was carried out by shaving the fur on the rabbit's back, using 3 adult New Zealand albino rabbits (1 male, 2 female) who weighed about 2 kg. The test animals were acclimated for 5 days, then the rabbit's back was shaved approximately 10 x 15 cm or at least 10% of the body surface, divided into 5 parts. Next, as much as 0.5 g of gel preparation is applied to the shaved rabbit's back area. The rabbit's back is covered with gauze and plaster. The test preparation is exposed to a skin area of ± 6 (2 x 3) cm of plaster is removed and the skin is rinsed with water after a 4-hour exposure period (6). After testing the rabbit's back area, the surface of the rabbit's skin was observed. For each change such as erythema (redness) and udema (swelling) starting at the 1st, 24th, 48th, and 72nd hour after the attachment surface.

The results of the irritation test of the Kersen bark ethanol extract gel formula are non-irritating, the test in vivo concluded the safety of each formula (F1, F2 and F3) Kersen bark extract gel, this is evidenced by the absence of erythema and edema reactions in rabbit subjects. Observation of irritation test is carried out based on two parameters, namely erythema and edema. Erythema is redness of the skin caused by dilation of blood vessels and is the body's response to irritation. Meanwhile, edema is the body's response to irritation which is characterized by an enlargement of plasma that freezes in the injured area (16).

Table 10. Irritation Test Results of Gel Preparations

Formula	The Hour	Result	Information	BPOM Standards, 2020	
				Average score	Information
	24	0	Non-irritating	0,0-0,4	Very mild irritation (*negligible*)
F1	48	0	Non-irritating	0,0-0,4	Very mild irritation (*negligible*)
	72	0	Non-irritating	0,0-0,4	Very mild irritation (*negligible*)
	24	0	Non-irritating	0,0-0,4	Very mild irritation (*negligible*)
F2	48	0	Non-irritating	0,0-0,4	Very mild irritation (*negligible*)
	72	0	Non-irritating	0,0-0,4	Very mild irritation (*negligible*)
	24	0	Non-irritating	0,0-0,4	Very mild irritation (*negligible*)
F3	48	0	Non-irritating	0,0-0,4	Very mild irritation (*negligible*)
	72	0	Non-irritating	0,0-0,4	Very mild irritation (*negligible*)

The resulting gel has a very mild irritation category (negligible). The skin may show a low reaction or even no reaction at the first contact with the material. Therefore, the observation of irritation was carried out after 24, 48, and 72 hours after the animal was given the preparation. Erythema observation is determined when redness is seen on the skin. Meanwhile, edema can be seen at the height of the skin surface that is raised/swollen compared to normal skin. The irritation index is classified into very mild irritation (0-0.40), mild irritation (0.50-1.90), moderate irritation (2-4.90), and severe irritation (5-8) (16).

4 Conclusion

Based on the research on the gel extract of kersen bark (Mutingia calabura L) carried out, it can be concluded, the results of the physical properties test of Kersen bark extract gel showed that the preparation had a distinctive odor with a homogeneous light brown to dark brown gel shape because it did not show any coarse grains. It has a pH value of 4.7-5.4 and has a viscosity value of 17,441- 18,681 cps which can be attached for more than 1 second with a dispersion power between 3.9-5.1 cm. The gel preparations produced from this study did not irritate the skin, as evidenced by in vivo testing using rabbit subjects. In the subjects, no erythema or edema was found after treatment for 24, 48 and 72 hours, so the gel preparation of ethanol extract of kersen bark was considered safe and did not cause irritating effects

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