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The effectiveness of butterfly pea hydrogel film (*Clitoria Ternatea)* with chitosan and poly-vinyl-alcohol based as diabetic wound healing

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ABSTRACT: Butterfly pea possesses antioxidant activities with IC_{50} 470 µg/ml of wound healing acceleration quality. Hydrogel for diabetic wound healing due to its physical characteristic that binds water, aside from its qualities that wets the surface and bio-compatibility in the body. This study aimed to identify effectivity test of hydrogel film preparation with butterfly pea (*clitoria ternatea l.*) Water extract for diabetic wound healing, employing true experimental method which involves 12 rats divided into 4 groups. The control group was treated without the Hydrogel (HET) or HET0 on hyperglycemic rats, HET1 (2%), HET2 (5%), and HET3 (7.5%). The findings revealed wounds treated with HET0 was healed in 9 days with 11.13 mm of diameter (100%), HET1 was healed in 6 days with 15.22 mm of diameter (100%), HET2 that was healed in 4 days with 11.29 mm of diameter (100%), and HET3 that was healed in 8 days with 9.05 mm of diameter (100%). It was concluded that the effectivity test revealed that the best formula is HET2 (5%) healed in 4 days with 11.29 mm of diameter in 100%.

KEYWORDS: Butterfly pea; Diabetic Wound Healing; hydrogel film.

INTRODUCTION

Butterfly pea *(Clitoria ternatea* L.) is a type of vine that is often found in the yards of people's houses which are usually used as ornamental plants and forests. This plant, which generally has bright blue, white, pink, and purple flowers, can be used for various purposes such as food coloring, cakes, and family medicinal herbs (TOGA) [1].

Butterfly pea (*Clitoria ternatea* L.) is one of the plants that has been studied and is efficacious for diabetic wounds [2]. The chemical constituents of butterfly pea include anthocyanins, saponins, flavonoids, alkaloids, co-oxalates, and triterpenoids. The chemical compounds that were successfully studied in butterfly pea petals contain 19 types of anthocyanins and 14 types of flavonol glycosides. Anthocyanins are effective for maintaining eye tissue, maintaining the immune system, and preventing platelet aggregation while also having antidiabetic, anti-inflammatory properties. In addition, butterfly pea also contains chemical compounds such as delphinidin, tri glucoside, and phenolics [3]. One gram of dry butterfly pea extract contains an average of 11.2 mg of flavonoids equivalent to catechins. Water extract of butterfly pea also has antioxidant activity IC_{50} of 470 μg/ml [4].

The hydrogel film is a cross-linked hydrophilic polymer network, which swells upon absorption of water or biological fluids but does not dissolve due to cross-linking. The advantage of hydrogel films is that they can deliver drugs transdermally as well as provide controlled and constant drug release, are easy to use, increase low drug bioavailability and avoid fist-pass, as well as create a cold feeling when used.

MATERIALS AND METHODS

Material

Butterfly pea Flower retrieved from Gorontalo city, Chitosan (Phy Edumedia), PVA (Phy Edumedia), PEG 400, PBS (Muda Berkah), Glyserin (Phy Edumedia), Nipagin (Phy Edumedia), Acetic Acid (Phy Edumedia), and Aquadest.

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Preparation of butterfly pea extract infusion

100 mL Aquadest was included into 10 g of butterfly pea simplicia and strirred until homogenous. The blend was bubbled at 90 ^{0}C for 15 min, blended. At that point, the infused blend was sifted utilizing channel paper. The butterfly pea flower extract solution is then put into an automizer to be processed into powder extract.

Preparation of butterfly pea hydrogel

The components for hydrogel films preparation were taken from the best formulation in the pre-formulation (Table I) which produced the best base with a concentration of Chitosan 2%, PVA 5%, PEG 400 1%, Glycerin 1%, Nipagin 0.18% and Acetic Acid 1 ml. Based on the formula, the concentration of water butterfly extract was varied by 2%, 5% and 7.5%. Stir until homogeneous and let stand to remove bubbles. The homogeneous film fabric was poured into a petri dish and oven at 60°C for 6 hours. At that point it was defrosted for 2 hours at 25 ^{o}C. The layer is dried at room temperature [9], [10], [11].

Table 1 shows the component ingredients in the dosage formula, which will be tested for their effectiveness in wound healing. In this case, it was made in 4 formulas, namely HET 0, HET 1, HET 2, and HET 3, each of which contains the same components but differs only in the variation in the concentration of the butterfly pea water extract.

Table 1. Formulation of butterfly pea hydrogel film.

No	Materials	Formulas			
		HET0[a]	HET1[b]	HET2[c]	HET3[d]
1	Butterfly pea flower extract	-	2	5	7,5
2	Chitosan	2	2	2	2
3	PVA	5	5	5	5
4	PEG 400	1	1	1	1
5	Glyserin	1	1	1	1
6	Nipagin	0.18	0.18	0.18	0.18
7	Acetic Acid	1	1	1	1
8	Aquadest	100	100	100	100

a: Formula without butterfly pea water extract
b: 2% extract
c. 5% extract
d. 7.5% extract.

Organoleptic test

Observe the color and consistency of the hydrogel film for each formula. Judging from the film's transparent color, elastic consistency, and film odor [12].

Morphology test

Cutting the sample with a estimate of 1cm x 1cm, then placing the sample on a glass preparation and observing it using a microscope with a magnification of 100x and looking at the surface topography of a material to analyze the hydrogel surface, from texture and shape. The test results must have a film structure that is smooth, flexible, without pores [12].

Water resistance test

Measured the starting weight (Wo) of the sample 1cm x 1cm and after that splashing it in distilled water for 15 minutes. The sample that has been soaked is then filtered using filter paper and the final weight (Wa) is measured again so that the percentage of water absorbed will be obtained by using the following equation:

$$\% \text{ Absorbed Water } = \frac{Wa-W0}{W0} \text{ x } 100\% \qquad \text{(equation 1)}$$

While the higher the water absorption, the more susceptible the membrane properties will be, the lower the water absorption value, the better the film properties. [13].

Hydrogel absorption ability test

Incubating the hydrogel in phosphate-buffered saline (PBS) at pH 7.4 at room temperature. PBS solution was prepared by mixed 100 mL Aquadest with 1 tablet of PBS and stirring until dissolved.. The test was carried out by measuring the initial mass (m0) of a 1 cm x 1 cm sample and then soaking it in PBS solution

for 3 hours [14]. The drenched test was at that point sifted utilizing filter paper and the ultimate weight (me) was measured once more. The formula of amount of water absorbed within the hydrogel [15] :

$$\% \text{ absoption} = \frac{m0-me}{m0} \times 100\% \qquad \text{(equation 2)}$$

Hydrogel is a network of interconnected hydrophilic polymer chains with high absorbency from 00.0% to 99.9%, high absorption rate indicates better hydrogel [16].

Hyperglycemic rats

The samples used were 15 male Wistar rats weighing 250-300 grams and 2-3 months old. First, the rats weight was measured. Glucose levels were measured using a glucometer. The rats were fasted for 16-18 hours and then the initial blood glucose levels were measured. Furthermore, the rats were induced intraperitoneally with150 mg/kg BW alloxan. The rats' glucose levels were measured after 3 days. Diabetic rats if glucose levels ≥126 mg/dL [17-18].

Making wounds in rats with hyperglycemia conditions

Check blood sugar before suturing the wound. Hyperglycemic ulcers occur when fasting blood sugar has reached 126 mg/dL or higher. The size of the wound area was 2x1 cm and <2 mm rat backs were shaved to a size of 5x3 cm. General anesthesia of rats given 0.2 mL ketamine hydrochloride intramuscular. Rats were placed in cages and unconscious for 5 minutes. Then disinfect using povidone iodine on the part that will be injured. Pinch the skin with tweezers then excise the marked skin using surgical scissors. After the wound is made, do wound care with a predetermined procedure. The mouse was put in the cage and allowed to regain consciousness [17].

In vivo effectiveness test of butterfly pea hydrogel as diabetic wound healing

Rats were divided into 4 groups. The research did not utilize a positive control group; it only compared negative controls and differences in extract concentration. A control group was treated without butterfly pea extract, and the first, second, and third treatment groups used 2%, 5%, and 7.5% hydrogel films, respectively. The treatment was given the day after the wound was made by attaching a hydrogel film to the diabetic wound once a day, after which visual observations were made daily by measuring the diameter of the wound and carried out for 20 days. Then calculate the percentage of wound closure for each group [18], [19].

$$\%\text{Wound Healing} = \frac{Wound\ Diameter\ H1 - Wound\ Diameter\ on\ the\ n-day}{Wound\ Diameter\ H1} \times 100\% \qquad \text{(equation 3)}$$

Irritation test

Groups of animals were subjected to irritation testing and observed 24, 48, and 72 hours after hydrogel treatment. Parameters Observations were based on the degree of inflammation (edema) and redness reaction (erythema) that occurred [20].

Irritation test was carried out in vivo with closed patch test method. Hair on the back is shaved first. This shaving was carried out 24 hours before being treated in the test area. Material sample is applied to the test area, then covered with a non-reactive dressing and plastered. After 24 h of treatment, the dressing was removed and the test area cleaned with water to remove any residual test material. At 24, 48, and 72 hours after reagent application, the test area is examined and changes in the skin's response to the reagent are observed and scored by giving a score from 0 to 4 depending on skin reactivity watched [21].

▪ RESULTS

Results of organoleptic test

Organoleptic examination of hydrogel water extract of butterfly pea (HET) was carried out visually, including shape, color, and smell. Based on the organoleptic test, the preparation met the criteria of all tests, namely at HET1, the consistency of the preparation was elastic, not brittle, transparent in color, and elastic. In HET2, the consistency of the elastic preparation is transparent white. In HET3, the consistency of the preparation is stiff, slightly brittle, and transparent in color. In HET4, the consistency of the preparation is stiff, slightly brittle, and transparent white in color.

Results of morphology test

The HET surface morphology test was done using the Microscope test with 100x magnification. Characterization is used to see the surface topography of a material to analyze the surface, texture, shape, and size of the wound-covering membrane. Microscope test results showed the best results, namely HET 1 has a film structure that was smooth, flexible and had no pores. The results on HET2 has a smooth, flexible film structure but small bubbles are in the preparation. HET3 has a somewhat coarse, flexible, and porous structure. HET4 has a structure that is not too smooth and porous. With the addition of the glycerin plasticizer to the hydrogel film, the surface structure also looks smoother than without glycerin added and the addition of chitosan at a concentration of 2% makes the hydrogel film more flexible than using 1% chitosan [22].

Results of water resistance test

Table 2. Results of average hydrogel film resistance measurement.

Formulas	Initial weight(w0)	Final weight (wa)	Water absorbed (%)
HET 1 (2:5)	0.0650	0.1275	0.96
HET 2 (2:10)	0.0601	0.1527	1.54
HET 3 (1:5)	0.0670	0.2592	2.96
HET 4 (1:10)	0.0638	0.2658	3.06

The Table 2 presents data on the resistance properties of hydrogel films. The initial weight column denotes the starting weight of each hydrogel film sample prior to exposure to specific environmental conditions. Conversely, the final weight column records the concluding weight after exposure to said conditions. Water absorbed column quantifies the amount of water absorbed by the hydrogel film during the measurement period. This data elucidates the hydrogel film's capacity for water retention or absorption, crucial in diverse applications such as biomaterials and pharmaceuticals. The percentage of water absorbed is calculated using equation 1 and the results obtained are as follows: HET1 0.96%, HET2 1.54%, HET3 2.96%, HET4 3.06%.

Results of hydrogel absorption ability

Table 3. Results of measuring average hydrogel absorption capability.

Formulas	Initial weight(m0)	Final weight(me)	Hydrogel Absorption (%)
HET 1 (2:5)	0.0427	1,6832	38.4
HET 2 (2:10)	0.0447	1.6453	35.8
HET 3 (1:5)	0.0440	1.5092	33.3
HET 4 (1:10)	0.0484	1.5394	30.8

The table above presents the results of measuring average hydrogel absorption capability, where the percentage of hydrogel absorption is calculated using equation 2, yielding the following results: HET1 38.4%; HET2 35.8%; HET3 33.3%; HET4 30.8%.

Results of glucose rate test results in rats

Table 4. Results of glucose rate test.

HET	Rat	Glucose rate			Average
		T0[a]	Average	T1[b]	
	1	59 mg/dL		146 mg/dl	
HET0	2	81 mg/ dL	55± 28.21	112 mg/dl	176± 84.29
	3	25 mg/ dL		272 mg/dl	
	1	97 mg/ dL		146 mg/dl	
HET1	2	95 mg/ dL	102± 10.44	514 mg/dl	409± 229.30
	3	114 mg/ dL		567 mg/dl	
	1	87 mg/ dL		234 mg/dl	
HET2	2	90 mg/ dL	94± 10.78	155 mg/dl	208± 46.19
	3	107 mg/dl		236 mg/dl	
	1	80 mg/ dL		441 mg/ddl	
HET3	2	102 mg/dl	86± 14	484 mg/dl	480± 37.63
	3	76 mg/dL		516 mg/dL	

a.T0: Initial Glucose Rate
b.T1: Final Glucose Rate

Table 4 describes the results of the Glucose Rate Test on each of the 3 rats in each group by calculating the average blood glucose levels before and after alloxan induction. The average blood glucose levels of rats before induction in HET0 were 55 mg/dL; in HET1, 102 mg/dl; in HET2, 94 mg/dL; and in HET3, 86 mg/dL. Blood glucose levels increased after alloxan induction with averages of HET0 at 176 mg/dL; HET1 at 409 mg/dL; HET2 at 208 mg/dL; and HET3 at 480 mg/dL.

Alloxan induction is capable of increasing the glucose levels in rats to a state of hyperglycemia, with blood glucose levels exceeding 135 mg/dL [23], [24]. Alloxan is one of the diabetogenic substances that are toxic, especially to pancreatic beta cells, and when administered to experimental animals, can induce diabetes. The mechanism of beta cell damage by alloxan begins with the oxidation of sulfhydryl groups and the formation of free radicals. Alloxan reacts with two -SH groups bound to the side portion of proteins or amino acids, forming disulfide bonds, thus inactivating the proteins, resulting in disruption of their functions [23], [24]. Subsequently, the glucose levels are measured during the research process for up to 10 days after the treatment using hydrogel film is completed.

Results the effectiveness test of accelerating wound healing

Table 5. Effectiveness test results.

No	Treatment	Wound closure diameter on day – (mm)									
		1	2	3	4	5	6	7	8	9	10
1	HET0	21.04	18.97	17.98	17.13	15.94	12.44	11.70	11.37	0	0
2	HET1	20.63	17.81	17.91	17.29	15.22	0	0	0	0	0
3	HET2	20.80	15.77	11.29	0	0	0	0	0	0	0
4	HET3	20.65	15.15	14.65	12.43	11.30	8.47	9.05	0	0	0

Table 6. Wound closure percentage results.

No	Treatment	Percentage of wound closure (%) / day									
		1	2	3	4	5	6	7	8	9	10
1	HET0	0	9	14	18	24	40	44	47	100	0
2	HET1	0	13	13	16	26	100	0	0	0	0
3	HET2	0	24	45	100	0	0	0	0	0	0
4	HET3	0	26	29	39	45	58	56	100	0	0

Table 5 and table 6 above illustrate the results of the effectiveness test of butterfly pea hydrogel film. Table 5 describes the wound closure results measured in millimeters, while Table 6 delineates the percentage of wound closure in rats under diabetic conditions. Throughout the 10-day treatment process, blood glucose levels of the rats were measured to ensure they remained in a hyperglycemic state.

▪ DISCUSSION

The resulting water content obtained in butterfly pea simplicia was 4% or ≤10% (meeting the quality requirement for simplicia water content according to the Indonesian Herbal Pharmacopoeia 2008). Too high a water content (>10%) induces bacterial growth, which will reduce the stability of simplicia and affect its preservation. [25]. Yield is the ratio between the dry weight of the produced sample and the weight of the raw material. The performance of a sample is very important as it determines the amount of extract obtained during extraction. In addition, the yield data are related to the active compounds in a sample, so if the yield increases, the amount of active compounds in the sample also increases [26].

The yield of butterfly pea powder extract obtained 91.9%. According to the Indonesian Herbal Pharmacopoeia, the optimal extract has a yield of >10% [27]. The type of solvent factor affects this. The success of separation depends on the solubility of the components to be separated in the solvent. Polar compounds dissolve in polar solvents and vice versa. The solvent used in the extraction process is aquadest because the flavonoid compounds in the butterfly pea can dissolve in water [28].

Evaluation of preparations cinsist of results of organoleptic test

Organoleptic examination of hydrogel water extract of butterfly pea (HET) was carried out visually, including shape, color, and smell. Based on the organoleptic test, the preparation met the criteria of all tests, namely at HET1, the consistency of the preparation was elastic, not brittle, transparent in color, and elastic. In HET2, the consistency of the elastic preparation is transparent white. In HET3, the consistency of the

preparation is stiff, slightly brittle, and transparent in color. In HET4, the consistency of the preparation is stiff, slightly brittle, and transparent white in color.

The HET surface morphology test was done using the Microscope test with 100x magnification. Characterization is used to see the surface topography of a material to analyze the surface, texture, shape, and size of the wound-covering membrane. Microscope test results showed the best results, namely HET 1 has a film structure that was smooth, flexible and had no pores. The results on HET2 has a smooth, flexible film structure but small bubbles are in the preparation. HET3 has a somewhat coarse, flexible, and porous structure. HET4 has a structure that is not too smooth and porous. With the addition of the glycerin plasticizer to the hydrogel film, the surface structure also looks smoother than without glycerin added and the addition of chitosan at a concentration of 2% makes the hydrogel film more flexible than using 1% chitosan [22].

Table 2 shows the results of the water resistance tested for 15 minutes, and the test results show that HET1 has the best results because the water a: The is low, the hydrogel film resistance is high due to the concentration of chitosan in HET1 chitosan 2% and PVA 5%, in HET2 it becomes 2nd place because the PVA concentration of 10% is more significant than Chitosan 2%, the resistance level becomes slower, HET3 and HET4 experience a high decrease because the concentration of chitosan is only 1%, while PVA concentrations are very high, 5% and 10%.

From these results, it is known that HET1 is the best formula in terms of water resistance. This is influenced by chitosan, which has resistance and is not easily soluble in water at a concentration of 2%. 5% PVA has moisturizing properties, both in film formation and high concentrations of PVA will quickly absorb (water soluble). Chitosan/PVA blends have been extensively researched and developed for many different applications. Typical properties of the proven Chitosan/PVA blends include mechanical, thermal, morphological and pH-sensitive properties [29]. It can be seen that with increasing the concentration of chitosan added to the hydrogel, the resistance value of the hydrogel to water increases, and the higher the concentration of PVA, the easier it is to absorb water.

HET 1 absorption rate is high because the 2% concentration of chitosan can absorb liquids higher, and the PVA concentration of 5% is the best and fastest concentration. Compared to chitosan, the absorption rate becomes slower (Table 3). HET3 and HET4 experience a high decrease because the concentration of chitosan is only 1%, unable to absorb fluids, while the concentration of PVA HET3 is 5% and HET4 is 10%.

From these results, it is known that HET1 is the best formula in terms of the absorption rate of this hydrogel affected by the concentration of 2% chitosan being able to absorb liquids the higher and the concentration of 5% PVA being the best concentration and fast in absorption ability tested for 3 hours. Hydrogel films must have a network of hydrophilic polymer chains that are interlocked with a high absorption capacity of 00.0% to 99.9%. The higher the absorption rate, the better [16].

Blood glucose is the amount of glucose present in the blood. Blood sugar comes from carbohydrates in food and is stored as glycogen in the liver and skeletal muscle. Blood glucose levels will increase when there is a lack of insulin, both absolute and relative. In normal animal conditions, blood glucose levels will increase after eating and remain for a short time [30]. The test results in table 4 above shows the results of the glucose level test before induction for HET0 with an average of 55 mg/dL, HET1 with an average of 102 mg/dL, HET2 with an average of 94 mg/dL and HET 3 with an average 86 mg/dL, after induction using alloxan is a chemical compound used to induce diabetes in laboratory animals. Alloxan is an unstable hydrophilic compound that is selectively toxic to the liver and kidneys. Pure alloxane is obtained from the oxidation of uric acid with nitric acid. The half-life of alloxane at pH 7.4 and temperature 37 °C is 1.5 minutes and is very susceptible to oxidation [31]. After induction, glucose levels were rechecked, which showed the results of HET0 with an average of 176 mg/dL, HET1 with an average of 409 mg/ dL, HET2 with an average of 208 mg/dL, and HET 3 with an average of 480 mg/dL, etc. Rats are considered diabetic if their glucose levels are≥126 mg/dL [32]. After the glucose level increased, a wound was made on the rat's back.

Butterfly pea (*Clitoria ternatea* L.), used as herbal therapy made in topical dosage forms, are indicated to repair skin tissue damage due to diabetic wounds [33], [34]. Therefore, the effectiveness test of preparations that have been made in the form of hydrogel films was carried out. Hydrogel film has several advantages over other wound drug preparations, namely having a longer contact time and having more availability of active substances due to the film's structure, which is a layer that tends to bind many active substances [35]. Compared to a gel that is too dilute, it will reduce its adhesive power so that the contact time of the active substance with the application site is also reduced [36]. Rats with hyperglycemia conditions had wounds on

their backs and were treated using butterfly pea extract hydrogel films. Observations were made every day for 10 days by continuing to observe a decrease in wound diameter.

The effectiveness test of the hydrogel film formula of water butterfly pea extract was carried out to see the wound-healing effect of each formula. The observation shows the wound that was given HET0 healed on day 9 with a wound diameter reduction of up to 11.13 mm and wound closure rate of 100%, while HET1 recipients healed on day 6 with a wound diameter reduction of up to 15.22 mm and a percentage of 100%, then for HET2-treated wounds healed on day 4 with wound diameter reduced to 11.29 with a wound closure ratio of 100 %, then those who received HET3 healed on day 8 with a wound diameter reduced to 9.05 mm with a wound closure rate of 100%. Factors affecting wound healing time are high glucose levels[37]. This observation was carried out for 10 days.

Based on previous studies using the same base but with the expansion of fluid smoke and vitamin K, it showed effective results by reducing the diameter of the wound by 2.6 mm and healing on day 14 with a healing percentage of 98.3% [10]. Chitosan has the ability as a hemostat that can help blood clotting, but this material is easily broken and torn, so to optimize its use, a crosslink modification can be made with Poly-Vinyl-Alcohol (PVA). Chitosan and PVA can produce a hydrogel film that has a surface morphology that appears flat and smooth and has been shown to help active ingredients accelerate wound healing [10].

Table 5 above shows the test results of the fastest wound healing effect on day 4 with a wound diameter reduction of 11.29 mm with a wound closure rate of 100% and with perfect healing, namely the hydrogel film formula with the addition of 5% water butterfly pea extract. The decrease in wound diameter can be seen in Table 6. Based to previous studies using the same base but with the addition of liquid smoke and vitamin K, it showed effective results with a decrease in wound diameter of 2.6 mm, healed on day 14 with a healing percentage of 98.3 % [10]. Based on statistical tests, the results of the T-test shows that HET0 obtained a value of 0.07, HET1 obtained a value of 0.00, HET2 obtained a value of 0.00, and HET3 obtained a value of 0.26. based on these results, there are 2 values obtained less than a significance value of 0.05 this is due to the different wound healing processes. Whereas the One way ANOVA test was carried out to see if there was a significant effect between the hydrogel film and the concentration of butterfly pea extract on wound healing, the results obtained were less than a significance value of 0.05, so it can be concluded that concentration and blood sugar levels can affect wound healing time.

The Irritation test was carried out in vivo with a closed patch test. Prior to treatment, the hair on the back was shaved first. This shaving was carried out 24 hours before being treated in the test area. The test material was applied to the test area, then covered with a non-reactive bandage and plastered. After 24 hours of treatment, the bandages were removed, and the test area was cleaned with water to remove residual test material. At 24, 48, and 72 hours after administration of sample, the test area was then examined and observed for changes as a skin reaction to the test material and assessed by giving a score of 0 to 4 depending on the degree of skin reaction seen [21]. Based on the results obtained from this irritation test, it showed no hypersensitivity reactions, redness, or allergic reactions caused. Based on these data, it was also concluded that the preparation fulfilled the safety test statement. The irritation test was conducted on test animals using 12 white rats aged 2-3 months. Rat hair was shaved on the back until it was clean. Veet® is used to help remove fine hairs. The backs of rats that had been wounded were treated with HET0, HET1, HET2, and HET3 hydrogel film preparations by increasing the size of the hydrogel film to observe whether irritation occurred on the rat's back. The irritation test showed no hypersensitivity reactions in the form of erythema and edema in the experimental animals that were treated with each formula, the irritation test was carried out for 3x 24 hours.

▪ CONCLUSION

The results of the effectiveness test showed that the best formula was HET2 (hydrogel film with an extract concentration of 5%) with a decrease in wound diameter of 11.29 mm with a percentage of 100% on day 4. It was concluded that there was significantly different when compared with the negative control group on the butterfly pea extract formulated in dosage form hydrogel film on its effectiveness in wound healing. Based on the results of statistical analysis (T Test) obtained a value of 0.00 <0.05, indicating that there is a significant effect between the concentration of the butterfly pea extract and blood sugar levels on wound healing.

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REFERENCES

[1] E. C. Purba, "Kembang telang (*Clitoria ternatea L.*): pemanfaatan dan bioaktivitas," *EduMatSains*, vol. 4, no. 2, pp. 111–124, 2020.

[2] A. E. Al-Snafi, "Pharmacological Importance of *Clitoria Ternatea* – A Review Pharmacological Importance Of Clitoria Ternatea – A Review," *Iosr J. Pharm.*, vol. 6, no. 3, pp. 68–83., 2016.

[3] M. B. Lijon, N. S. Meghla, E. Jahedi, M. A. Rahman, and I. Hossain, "Phytochemistry and Pharmacological Activities of Clitoria ternatea," *Int. J. Nat. Soc. Sci.*, vol. 4, no. 1, pp. 1–10, 2017.

[4] A. M. Marpaung, "Tinjauan Manfaat Bunga Telang (*Clitoria ternatea L* .) Bagi Kesehatan Manusia," *J. Funct. food Nutraceutical*, vol. 1, no. 2, pp. 1–23, 2020, doi: 10.33555/jffn.v1i2.30.

[5] Y. Harliatika, "Formulasi dan Evaluasi Hidrogel Ekstrak Etanol Daun Gaharu (Aquilariamalacensis lamk.) Dengan Kombinasi Basis Karbopol 940 dan Hpmc," *J. Pharm. Sci.*, vol. 6, no. 1, 2021.

[6] E. Marin, Rojas, and Ciro, "A Review Of Pva Derivatives: Promising Materials of Pharmaceutical and Biomedical Applications," *African J. Pharm. Pharmacol.*, vol. 8, no. 24, 2014.

[7] U. K. Parida, A. K. Nayak, and Binhani, "Synthesis and Characterization of Chitosanpolyvinyl Alcohol Blended With Cloisite 30b For Controlled Release of The Anticancer Drug Curcumin," *J. Biomater. Nanobiotechnology,* vol. 2, pp. 414–425, 2011.

[8] A. Marpaung, N. Andarwulan, P. Hariyadi, and D. N. Faridah, "The Wide Variation of Color Stability of Butterfly pea (*Clitoria ternatea L.*) Flower Extract at pH 6-8.," *Southeast Asian Food Agric. Sci. Technol.*, pp. 283–291, 2018.

[9] B. Piluharto, A. Sjaifullah, I. Rahmawati, and E. Nurharianto, "Membran Blend Kitosan/Poli Vinil Alkohol (Pva): Pengaruh Komposisi Material Blend, Ph, Dan Konsentrasi Bahan Pengikat Silang," *J. Kim. Ris.*, vol. 2, no. 2, pp. 77–85, 2017.

[10] S. A. . Saputra, T. Dewi, E. . Ramadhan, N. Ibrahim, and G. Wibisono, "Penutup luka hydrogel berbasis polivinil alkohol (pva), kitosan, pati dengan penambahan asap cair dan vitamin k," *UMS*, vol. 002, pp. 1–10, 2020.

[11] N. Wathoni, N. Yuniarsih, A. Cahyanto, and M. Muhctaridi, "applied sciences α -Mangostin Hydrogel Film Based Chitosan – Alginate for Recurrent Aphthous Stomatitis," *Appl. Sci.*, vol. 9, no. 5235, pp. 1–14, 2019.

[12] D. I. Rudyardjo, "Karakteristik Hidrogel Kitosan-Glutaraldehid Untuk The Effect Of Addition Glycerol As Plasticier To The Characteristics Of Chitosan-Glutaraldehyde Hidrogel For Wound Dressing Application," *J. Ilm. Sains*, vol. 14, no. 1, 2014.

[13] R. A. . Wardhani, "Sintesis dan Karakterisasi Bioselulosa–Kitosan Dengan Penambahan Gliserol Sebagai Plasticizer." Program Studi Fisika FSAINTEK, Surabaya, 2012.

[14] E. . Baran, J. . Mano, and R. . Reis, "Starch-Chitosan Hydrogels Prepared by Reductive Alkylation Cross-linking.," *J. Mater. Sci. Mater. Med.*, vol. 15, pp. 759–765, 2004.

[15] A. Singh, S. S. Narvi, Dutta, and Pandey, "External Stimuli Response on a Novel Chitosan Hydrogel Crosslinked with Formaldehyde," *Indian Acad. Sci.*, vol. 29, no. 3, pp. 233–23, 2005.

[16] Erizal, "The Effect Of Hydrogel Dressing Copolymer Poli(Vinylpirrolidone) (Pvp) - K- Carrageenan Prepared By Radiation and Healing Times On The Radius Reductions Burn Injuried Of Wistar White Rat.," *Indones. J. Chem.*, vol. 8, no. 2, pp. 271–278, 2008.

[17] G. Mutiara PI, Nurdiana, and Y. W. Utami, "Efektifitas Hidrogel Binahong (*Anredera cordifolia* (Ten.) Steenis) terhadap Penurunan Jumlah Makrofag pada Penyembuhan Luka Fase Proliferasi Tikus Putih (Rattus norvegicus) Galur Wistar Kondisi Hiperglikemia," *Maj. Kesehat. FKUB*, vol. 2, no. 1, pp. 29–40, 2015.

[18] S. C. Rohma, E. Umayah, and D. Holidah, "Pengaruh Gel Binahong (*Anredera cordifolia (Ten* .) Steenis) terhadap

Penyembuhan Luka Tikus Diabetes yang Diinduksi Aloksan (The Effect of Binahong (Anredera cordifolia (Ten .) Steenis) Gel on Wound Healing Process of Diabetic Rats Induced by Allox," *e-Jurnal Pustaka Kesehat.*, vol. 3, no. 3, pp. 414–418, 2015.

[19] N. Thomas, A. Rusdin, M. Tulsyahra, N. Wathoni, and A. Kuswandi, "Accelerated wound healing ability of Jatropha sap by iota carrageenan-poly (vinyl alcohol) hydrogel film," *J. Technol. Res.*, vol. 11, no. 4, 2020, doi: 10.4103/japtr.JAPTR.

[20] Z. Ghiasi, F. Esmaeli, M. Aghajani, G. Faramarzi, and A. Amani, "Enhancing analgesic and anti-inflammatory effects of capsaicin when loaded into olive oil nanoemulsion: An in vivo study," *Int J Pharm*, vol. 559, 2019.

[21] Dewantara and Arsana, "Uji Eritema dan Edema Secara In Vivo Pada Natrium Lauril Sulfat 10%," *J. Farm. Udayana*, vol. 4, no. 2, 2015.

[22] E. Kemalasari, *Karakteristik Mikrobiologi dan Biodegradasi Edible Film Berbasis Pati Ubi Kayu : Tesis*. Medan: Program Studi Kimia FMIPA, 2010.

[23] O. . Prameswari and S. B. Widjanarko, "Uji Efek Air Daun Pandan Wangi Terhadap Penurunan Kadar Glukosa Darah dan Histopatologi Tikus Diabetes Melitus," *J. Pangan dan Agroindustri*, vol. 2, no. 2, pp. 16–27, 2014.

[24] Zakiatun Azma Amani and R. Mustarichie, "Review Artikel : Aktivitas Antihiperglikemia Beberapa Tanaman Di Indonesia," *Farmaka*, vol. 18, no. 1, pp. 53–59, 2021.

[25] Y. P. Utami, A. H. Umar, R. Syahruni, and I. Kadullah, "Standardisasi Simplisia dan Ekstrak Etanol Daun Leilem *(Clerodendrum minahassae Teisjm. & Binn.)*," *J. Pharm. Med. Sci.*, vol. 2, no. 1, pp. 32–39, 2017.

[26] S. U. Hasnaeni, Wisdawati, "Pengaruh Metode Ekstraksi Terhadap Rendemen Dan Kadar Fenolik Ekstrak Tanaman Kayu Beta-Beta (Lunasia amara Blanco)," *J. Farm. Galen. (Galenika J. Pharmacy)*, vol. 5, no. 2, pp. 166–174, 2019, doi: 10.22487/j24428744.2019.v5.i2.13149.

[27] Departemen Kesehatan RI, *Farmakope Herbal Indonesia*. Jakarta: Departemen Kesehatan Republik Indonesia, 2008.

[28] S. Susanty and F. Bachmid, "Perbandingan Metode Ekstraksi Maserasi Dan Refluks Terhadap Kadar Fenolik Dari Ekstrak Tongkol Jagung (*Zea mays L.*)," *J. Konversi*, vol. 5, no. 2, p. 87, 2016, doi: 10.24853/konversi.5.2.87-92.

[29] Mulchandani, Shah, and T. Mehta, "Synthesis of ChitosanPolyvinyl Alcohol Copolymers for Smart Drug Delivery Application," *Polym. Polym. Compos.*, vol. 25, pp. 241–246, 2017.

[30] D. A. Samsuri, S. Samsuri, and A. A. S. Kendran, "Kadar Glukosa Darah Tikus Putih (*Rattus norvegicus*) yang Diberikan Ragi Tape," *Indones. Med. Veterinus*, vol. 9, no. 4, pp. 531–539, 2020.

[31] A. Dharmayudha and M. Anthara, "Identifikasi Golongan Senyawa Kimia Estrak Etanol Buah Pare (*Momordica charantia*) dan Pengaruhnya Terhadap Penurunan Kadar Glukosa Darah Tikus Putih Jantan (*Rattus novergicus*) yang Diinduksi Aloksan," *Bul.Vet. Udayana*, vol. 5, no. 2, pp. 87–95, 2013.

[32] Hestiana, "Faktor-Faktor yang Berhubungan Dengan Kepatuhan Dalam Pengelolaan Diet Pada Pasien Rawat Jalan Diabetes Mellitus Tipe 2 Di Kota Semarang," *Jhe*, vol. 2, no. 2, 2017.

[33] Abdullah, Mikrajuddin, N. Virgus, Y, Irmin, and Hairurrijal, "Review: Sintesis Nanomaterial.," *J. Nanosains dan Nanoteknologi*, vol. 1, no. 2, pp. 33–57, 2008.

[34] A. A. Nugroho, C. Adianto, and Y. Patria, "Inovasi Wound Healing Gel Dari Nanopartikel Daun Binahong dan Kayu Manis Pada Luka Kronis," *Bimfi,* vol. 7, no. 1, pp. 26–42, 2020.

[35] S. Kumar Singh, A. Dhyani Assistant Professor, A. Shailesh Kumar Singh, C Dhyani, and D. Juyal, "Hydrogel: Preparation, Characterizationand Applications," *Journal*, vol. 6, no. 6, pp. 25–32, 2017.

[36] I. D. K. Irianto, P. Purwanto, and M. T. Mardan, "Aktivitas Antibakteri Dan Uji Sifat Fisik Sediaan Gel Dekokta Sirih Hijau (*Piper betle L.*) sebagai Alternatif Pengobatan Mastitis Sapi," *Maj. Farm.*, vol. 16, no. 2, p. 202, 2020.

[37] S. Siregar, "Hubungan Kadar Gula Darah Sewaktu Dengan Proses Penyembuhan Luka Pada Pasien Post Sectio Caesarea Di Rsu Ipi Medan," *J. Ilm. Keperawatan Imelda*, vol. 6, no. 1, 2020.