Characterization and Antibacterial Activity of Dayak Onion (Eleutherine palmifolia) Hydrogel in Vitro

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Abstract: Traditionally, the crushed bulbs of Dayak onion (Eleutherine palmifolia) used for wound healing due to naphthoquinone compounds found in the bulbs of Dayak onion can inhibit the growth of Staphylococcus aureus, Staphylococcus epidermis, and Pseudomonas aeruginosa that cause skin infection. To improve the ease of using the bulbs of Dayak onion as drugs for the treatment of wounds that need to be made in the appropriate dosage form is a topical hydrogel, hydrogels contained Dayak onion extract at a level of 2%, 4.5%, and 6.0% were made then determined its characterization which included organoleptic, pH, viscosity, and spreadability, determined of antibacterial activity by a well-diffusion method. The higher levels Dayak onion extract resulted in hydrogels which homogenous, sharp color intensity, more aromatic, decreased viscosity, and increased spreadability while for pH was not different, while the highest antibacterial activity achieved by Dayak onion hydrogels 6.0% which had antibacterial effectiveness to the Staphylococcus aureus, Staphylococcus epidermis, and Pseudomonas aeruginosa were 59.2%, 49.5%, and 63.1%, respectively, and as control was tetracycline HCL 1.0%. Thus, the Dayak onion hydrogels 6.0% is the best.

1 INTRODUCTION

Dayak Onion (Eleuherine palmifolia) is a plant belonging to the Iridaceae family which originated from tropical America and has been widely cultivated in Central Kalimantan (Indonesia), South China, Thailand, and South Africa(Insanu, Kusmardiyani, & Hartati, 2014). Empirically, Dayak onion has been used by local people as a cure for various types of diseases such as breast cancer, hypertension, diabetes mellitus, cholesterol, ulcer, colon cancer and preventive stroke (Galingging, 2009). In another place, these plants are used for wound healing (Agyare et al., 2009).

The phytochemical screening showed the bulb extract of Dayak onion contained flavonoids, polyphenols, alkaloids, quinones, tannins, steroids, monoterpenoids, and sesquiterpenoid (Puspadewi, Adirestuti, & Menawati, 2013) which the presence of antibacterial activity on onions is due to the naphthoquinone(Kuntorini & Nugroho, 2010). It was reported the ethanol extract of Dayak onion can inhibit the growth of gram-negative and gram-positive bacteria strain included Staphylococcus epidermis (Novaryatiin, Pratiwi, & Ardhany, 2018), Staphylococcus aureus, and Pseudomonas aeruginosa (Padhi & Panda, 2015) that are normal microbiota on the skin (Byrd, Belkaid, & Segre, 2018) and have opportunistic property, so, they easily enter the body if there is an open wound and cause skin infections(Cohen et al., 2016) (Niazi et al., 2010) (Wong, Rodrigue, & Kassen, 2012).

Traditionally, Dayak onion has been used for wound healing by applying crushed bulbs of Dayak onion to the wound (Agyare et al., 2009). This is of course not practical, and therefore needs to be developed into an appropriate topical preparation in the form of hydrogel preparations.

The hydrogel is semisolid systems consisting of dispersions of small or large molecules in water rendered jelly-like by the addition of a gelling agent(Allen, Popovich, & Ansel, 2012). Some of the main advantages of hydrogel formulations over other semisolid dosage forms are the hydrogel easily formulated, elegant, non-greasy, easily applied, biodegradable and biocompatible, forms a protective coating, provides a cool sensation, and it is suitable for both polar and non-polar drugs(Kaur & Guleri,

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2013), moreover, hydrogels have a relatively high water absorption that is very useful for absorbing exudates from wet sores, therefore using of hydrogel base for topical preparations, especially for dealing with acne, atopic dermatitis or wounds is very appropriate.

Purpose of this research was to made hydrogels containing Dayak onion extract at various concentrations, then the determined of characterization which includes organoleptic, pH, viscosity, homogeneity, and spreadability, continued the antibacterial assay against Staphylococcus aureus, Staphylococcus epidermis, and Pseudomonas aeruginosa by the wells-diffusion method.

2 METHODS

Materials.
Active Ingredients: Dayak onion bulbs obtained from Tabalong, South Kalimantan, Indonesia which had been determined by the Materia Medica Technical Services Unit, Batu, East Java Province Health Office, Indonesia.
Excipient Ingredients: Carbopol (Fagron Hallas, Greece), glycerin, propylene glycol (Dow Chemical Co.), triethanolamine, methylparaben (Ueno Fine Chemical, Japan); pure water, Oxytetracycline HCl., Mueller Hinton Agar (MHA) Media, Na Cl 0.9%.

Research Procedure.
Prior making of ethanol extract of Dayak onions uses the maceration method. The formula of hydrogel contained active ingredients extracts of Dayak onions at a concentration of 2%, 4%, and 6 %. The determined of physical-chemical characteristics carried out which includes organoleptic, homogeneity, pH, viscosity, and spreadability, while the antibacterial activity of the Dayak onion hydrogel tested against Staphylococcus aureus, Staphylococcus epidermis, and Pseudomonas aeruginosa using agar well diffusion method and as a positive control, is Oxytetracycline HCl.

Preparation of Ethanol Extract of Dayak Onion.
A Dayak onion extract made by maceration using 96% ethanol. A total of 1.5 kg dry of Dayak onions made powder, the powder soaked in 5.0 liters of ethanol 96% for 24 hours, it separated between the filtrate and the pulp then the filtrate that contains ethanol, it evaporated with a vacuum rotary evaporator at a temperature of 55°C to obtain a thick extract of Dayak onions.

Preparation of Dayak Onion Hydrogel.
Prepared carbopol hydrated, added methylparaben which has been dissolved in propylene glycol then glycerin. This mixture dropped with triethanolamine until a neutral pH. Furthermore, added Dayak onion extract which has been dissolved in propylene glycol and then remaining water. The composition of the Dayak onion hydrogel formulation as shown in Table 1.

Determined Ethanolic Extract of Dayak Onion.
Determined ethanolic extract of Dayak onion included the characteristic (consistency, smell, and color) and pH.

Determined of physical-chemical characteristic
Evaluation of the Dayak onion hydrogel is a physical and chemical characteristic. Determined physical characteristics include organoleptic, homogeneity, viscosity, and spreadability, whereas chemical characteristic test is pH.

Physical Characteristic.
Organoleptic. Organoleptic determination of gel products carried out by visually including color, odor, and consistency.
Homogeneity. Weighed of 0.1 gram of hydrogel spread evenly and thinly on the object-glass. The gel must show a homogeneous arrangement and there are no spots.

Determined of Viscosity.
Measurement of gel viscosity carried out using a Brookfield Viscometer. A total of 150 grams of the hydrogel remained into the beaker glass. The spindle no.4 installed, then it lowered until immersed into a gel product. The speed on the device is the start from 0.3 rpm to 60 rpm. On each measurement with a difference in speed, the scale reads until the moving red needle has stabilized.

Spreadability.
The spread test is performed to determine the ease of preparation when applied to the skin. Weighed 0.5 g hydrogel which is placed in the middle of a large round glass. On top of the hydrogel is placed a transparent round glass that has been weighed beforehand and left for 1 minute. The dispersing hydrogel is measured in diameter by taking the average length of the diameter from several sides.

Table 1: The Composition of Dayak onion hydrogel.

Materials	Composition (%)			
	F0	F1	F2	F3
Dayak Onion Extract	-	2	4	6
Carbopol	1	1	1	1
Tri ethanolamine	*	*	*	*
Propylene glycol	5	5	5	5
Glycerin	5	5	5	5
Methylparaben	0.1	0.1	0.1	0.1
Pure water to	100	100	100	100

*) is sufficient to neutral.

Above it given a load of 50 grams, left for 1 minute and recorded the distribution diameter. Continue to increase the load each time by 50 grams, after 1 minute the distribution diameter recorded until a sufficient diameter obtained to see the effect of the load on the change in the diameter of the gel spread.

Chemical Characteristic: pH.

The determination of pH is done using a pH meter. Previous calibrating the device with a buffer solution of pH 4.0 and pH 7.0. The pH determination carried out by dipping the pH meter electrode into the hydrogel, observing the number printed on the monitor. A good hydrogel must have a pH that matches the skin's pH which is 4-6.5(Ali & Yosipovitch, 2013).

Determined of Antibacterial Activity.

Antibacterial activity of each formula of Dayak onion hydrogel carries out by the agar well diffusion method. The pathogenic bacteria used were Staphylococcus aureus and Staphylococcus epidermis obtained from the Laboratory of Biomedical, University of Muhammadiyah Malang, Indonesia, and Pseudomonas aeruginosa obtained from Laboratory of Microbiology, Brawijaya University, Malang, Indonesia. Each antibacterial assay was carried out in three replicated. As a negative control was a gel base and as a positive control was Oxytetracycline ointment 1.0%.

All test bacteria cultured in Mueller Hinton Agar (MHA) Media for overnight at 37^0 C before used for the antibacterial assay. Antibacterial assay was carried out as follows. A 6 mm diameter well was made on the Mueller Hinton Agar medium which had been inoculated of the bacterium. Dayak onion hydrogel 2.0%, 4.0%, and 6.0%, as well as positive control and negative control, added as much as 0.10gram in the hole. These incubated for 1x24 hours at 37ºC then the diameter of inhibition zone observed after the incubation period. The inhibitory activity calculated by equation (1):

$$\text{Inhibitory activity } (\%) = \frac{(d2-d1)}{d1}\ x\ 100\% \qquad (1)$$

Where d1 is the diameter of well (6mm) and d2 is the diameter of the inhibition zone(mm).

Then, the result of the measurement of inhibition zone of each gel formula was calculated its antibacterial effectiveness by comparing the diameter of inhibition zone each gel formula with that of positive control, oxytetracycline ointment 1.0%.

The antibacterial effectiveness of hydrogel formulation to the positive control was calculated by equation (2) (Oroh, Kandou, Pelealu, & Pandiangan, 2015):

$$\text{Antibacterial effectiveness} = \frac{D}{Da}\ x\ 100\% \qquad (2)$$

Where D is a diameter of inhibition zone of hydrogel (mm) and Da is a diameter of inhibition zone of positive control (mm).

Data Analysis.

The organoleptic examination is done one day after making by visual means to observe the preparation directly including texture, odor, color, and taste. Meanwhile, physical-chemical characteristics including pH, viscosity, spreadability as well as inhibitory activity test result were analyzed using a one-way ANOVA test at a degree of confidence $\alpha = 0.05$ using the SPSS 16.0 program. If the results of $P < \alpha$, the analysis is continued by the Least Significant Difference (LSD) or Honestly Significant Difference (HSD) test to find out where the different data are.

3 RESULTS AND DISCUSSION

Characteristic of Ethanol Extract of Dayak Onions.

Ethanol extract of Dayak onions obtained from the extraction process by maceration method using 96% ethanol is a thick liquid that has a strong brownish-red and aromatic, and the result of pH determined hows it has a pH of 5. So it is acidic property.

Table 2: Physical-chemical characteristics of Dayak onion hydrogels.

Formula	Organoleptic	Homogeneity	pH	Viscosity (P·as) speed			Spreadability (g/cm)
				0.3rpm	0.6rpm	1.5rpm	
F1	Semisolid, soft, aromatic, brownish-red color	homogen	6.31±0.10	753±110	460±65	300±24	0.0040±0.0000
F2	Semisolid, soft, aromatic, brownish-red color	homogen	6.12±0.21	633±23	437±15	281±38	0.0043±0.0001
F3	Semisolid, soft, aromatic, brownish-red color	homogen	5.97±0.28	520±2	386±3	217±8	0.0050±0.0000

Physical-chemical Characteristics of Dayak Onion Hydrogels.

All are hydrogels that have a flat texture, the same color and no coarse particles in it. They had a soft, aromatic, and brownish-red color whose color intensity according to the levels of extract in each formula, the higher the extracted content, the sharper the color (Figure 1).

The physical-chemical characteristic of hydrogels as shown in Table 2. The pH of hydrogels containing Dayak onion 2.0%, 4.0%, and 6.0% each have a pH of 6.31, 6.12, and 5.97.

Viscosity determination by a Brookfield Viscometer uses a spindle number of 64 at speeds of 0.3 rpm., 0.6 rpm., and 1.5 rpm. The results of determining the viscosity of hydrogel 2.0%, 4.0%, and 6.0% at each speed as shown in Table 2. The viscosity of hydrogel 2.0%, 4.0%, and 6.0% at 0.3 is 753P·as, 633P·as, and 520P·as, respectively; at 0.6rprpm is 460P·as, 437P·as, and 386P·as, respectively; at 1.5rpm is 300P·as, 281P·as, respectively, while the spreadability each has of 0.0040g/cm, 0.0043g/cm, and 0.0050g/cm.

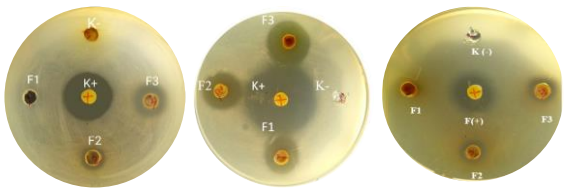
F1 F2 F3

Figure 1: Hydrogel of Dayak Onion at a Concentration of 2.0% (F1), 4.0% (F3), and 6.0% (F3).

Antibacterial Activity.

Anti-bacterial test results showed that the hydrogel of Dayak onion can inhibit the growth of Staphylococcus aureus, Staphylococcus epidermis, and Pseudomonas aeruginosa as shown in Figure 2, it appears that the largest inhibition zone occurs in Staphylococcus epidermis while to the Staphylococcus aureus and Pseudomonas aeruginosa are not different.

The result calculation of inhibitory activity and antibacterial effectiveness can be seen in Table 3. The inhibitory activity to the growth of Staphylococcus aureus of hydrogel 2.0%, 4.0%, and 6.0% is 0.0%, 63.3%, and 96.7%; to the growth of Staphylococcus epidermis is 140.7%, 144.4%, and 196.3%; and to the growth of Pseudomonas aeruginosa is 0.0%, 66.7%, and 107.4%. While using oxytetracycline 1.0% as a standart the result of antibacterial effectiveness hydrogel 2.0%, 4.0%, and 6.0% to the Staphylococcus aureus is 0.0%, 38.6%, and 59.2%; to the Staphylococcus epidermis is 35.5%, 36.5%, and 49.5; to the Pseudomas aeruginosa is 0.0%, 39.3%, and 63.1%.



(A) (B) (C)

Figure 2: Inhibition Zone Antibacterial Activity of Dayak Onion Hydrogel 2.0% (F1), 4.0% (F2), and 6.0% (F3) against S. aureus (A), S. epidermis (B), P. aeruginosa (C).

Table 3: Antibacterial activity of Dayak onion hydrogel.

Bacteria	Inhibitory Activity %		
	F1	F2	F3
S. aureus	0.0	63.3	96.7
S. epidermis	140.7	144.4	196.3
P.aeruginosa	0.0	66.7	107.4
	Antibacterial effectiveness(%)*		
	F1	F2	F3
S. aureus	0.0	38.6	59.2
S. epidermis	35.5	36.5	49.5
P. aeruginosa	0.0	39.3	63.1

*oxytetracycline 1.0% as a positive control

Physical-chemical Characteristics of Dayak Onion Hydrogels.

Hydrogel preparations that made containing the bulb of Dayak onion extracts at a concentration of 2.0%, 4.0%, and 6.0%. It found that all hydrogels are homogenous, not greasy, aromatic red-brown that is sharpener according to the level of the bulbs of the Dayak used (Figure1). If the terms of organoleptic, then all is good hydrogel formula.

The degree of acidity or pH of topical preparations must be safe for the skin in the range of pH 4.1-5.8 (Proksch, 2018), so it does not cause skin irritation. The results of the determined pH of Dayak onion extract gel as shown in Table 2. The pH of the hydrogel is more than 5.8, but this does not harm the skin because the skin has a tolerance for exposure to materials that have a pH of up to pH 8 (Kim et al., 2009). The result of analysis using ANOVA One-way at α=0.05 was obtained p (0,270) > 0.05, it is means all of these formulas have no different pH and are safe for the skin. Thus the different levels of extract used do not affect the pH of the gel produced even though the extract is acidic (pH 5).

That the viscosity of the Dayak onion hydrogel 2.0% and 4.0% are not significantly different, but these have a higher viscosity than Dayak onion hydrogel 6.0%. This difference in viscosity may be due to the acidic nature of the Dayak onion extract that has a pH of 5. The gelling agent used in this formulation is carbopol. The viscosity of carbopol depends on pH, carbopol will have a good viscosity at a neutral pH and will decrease at acidic pH (Gibson, 2016).

Thus the higher the extracted content, the more acidic the pH of the hydrogel preparation so that the viscosity decreases. The curve profile in Figure 3 shows the viscosity of Dayak onion hydrogels is different at each speedy, the higher the stirring speed, or shear rate the lower the viscosity, which means the flowing of Dayak onion hydrogel is pseudo-plastic behavior (Kaur & Guleri, 2013).

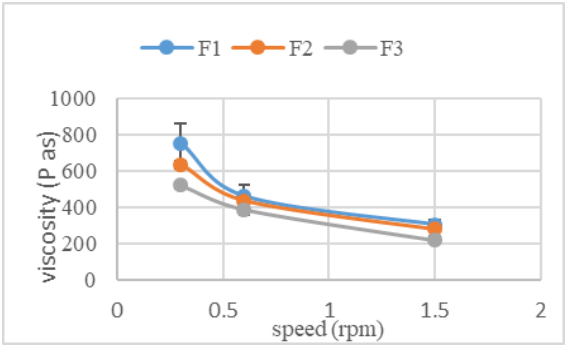


Figure 3: The Viscosity of Dayak onion hydrogel at speed variation.

The ease of spread of Dayak onion hydrogels, when applied to the skin surface, is called spreadability. The ability to spread is an important characteristic in topical preparations because it influences the transfer of active ingredients to the target area in the right dosage and ease of use. It has seen the spreadability of Dayak onion hydrogel 2.0% and 4.0% were not significantly different, respectively 0.040g/cm and 0.043g/cm, and both of these were lower than Dayak onion hydrogel 6.0% which had a spreadability of 0.05 g/cm. This result is according to the viscosity of each gel formula that an increase in viscosity will reduce the spreadability. So, the Dayak onion hydrogel 6.0% is the best.

Antibacterial Activity.

Determination of antibacterial activity of Dayak onion hydrogel against Staphylococcus aureus, Staphylococcus epidermis, and Pseudomonas aeruginosa by the agar well diffusion method, antibacterial activity indicated by inhibition zone formed around the well and the results of the antibacterial activity test indicated that Dayak onion hydrogel positively inhibit the growth of Staphylococcus aureus, Staphylococcus epidermis, and Pseudomonas aeruginosa as shown in Figure 2,

Dayak onion hydrogel 2.0% only have antibacterial activity toward Staphylococcus epidermis, it's inhibitory activity is 140.7%, while inhibitory activity Dayak onion hydrogel 4.0% toward Staphylococcus aureus, Staphylococcus epidermis, and Pseudomonas aeruginosa each is 63.3%, 144.4%, and 66.7%. And the inhibitory activity of Dayak onion hydrogel 6.0% is 96.7%, 196.3%, and 107.4%, respectively. That increasing the concentration of the extract correlated with an increase in inhibitory activity except for Staphylococcus epidermis where Dayak onion hydrogel 2.0% and 4.0% have no different antibacterial activity. Thus, the highest inhibitory

activity of Dayak onion hydrogel is toward the Staphylococcus epidermis.

Antibacterial effectiveness was calculated using oxytetracycline ointment 1.0% as a positive control. The results showed the effectiveness of antibacterial Dayak onion hydrogel 2.0%, 4.0%, and 6.0% to the growth of Staphylococcus aureus, Staphylococcus epidermis, and Pseudomonas aeruginosa was lower than the positive control, the effectiveness of antibacterial Dayak onion hydrogel 6.0% to the Staphylococcus aureus, Staphylococcus epidermis, and Pseudomonas aeruginosa were 59.2%, 49.5%, and 63.1%, respectively, Dayak onion hydrogel 4.0% even lower, each is 38.6%, 36.5%, and 39.3% while Dayak onion hydrogel 2.0% just to Staphylococcus epidermis which its antibacterial activity is 35.5%. The Dayak onion hydrogels 6.0% have antibacterial effectiveness more than about 50%, so they were categorized effectively.

4 CONCLUSIONS

Physical-chemical characteristics of Dayak onion hydrogels at all concentrations are good while Dayak onion hydrogel 6.0% is effective as an antibacterial against Staphylococcus aureus, Staphylococcus epidermis, and Pseudomonas aeruginosa.

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