089/KEP/FK/2018). Each group consisted of four rabbits and had each treatment as seen in Table 2. The fur around the rabbit's back was shaved cleaned with 70% alcohol. Each rabbit was then injected ketamine-xylazine (25mg/kg – 1mg/kg) intramuscularly. The burn wounds were done by contacted 1 cm standard-sized square-shaped copper plate xinto the back skin for 20 s until second degree of superficial wound was formed which characterized by blistered, dry skin and a pale red wound as seen in Figure 2.

Table 2. Treatment groups for burn healing activity

Groups	Treatments
I	Negative control (not given a membrane)
II	Membrane without usnic acid (F0)
III	Membrane with 0.5% usnic acid (F1)
IV	Membrane with 1% usnic acid (F2)
V	Membrane with 2% usnic acid (F3)
VI	Positive control (a marketed gel)

The average diameter of the wound was measured vertically, horizontally, and diagonally for 21 days of observation and the percentage of burn healing was calculated using this equation:

$$\text{Burn healing } (\%) = \frac{d1^2 - d2^2}{d1^2} \text{x } 100\%$$

Note : d1 = the diameter on day after burn wound (mm)
d2 = the diameter on observation day (mm)

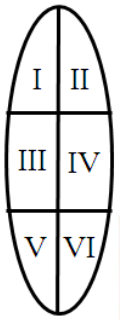

Figure 2. Location for burns on rabbit's back skin

3. RESULTS AND DISCUSSION

3.1. RESULTS

Usnic acid-solid dispersion (UA-SD) membranes were prepared in several concentrations that equivalent to concentration of usnic acid 0.5% (F1), 1% (F2) and 2% (F3). The different concentrations were designed to find out the optimum concentration effect both for physical and mechanical characteristic, and burn healing activity. In general, marketed burn healing preparations contain 1% of active ingredient. Appearance test was done visually and the result is shown in Figure 3. All membranes were transparent, the membrane without usnic acid (F0) has a clear color, while the membrane UA-SD has a yellowish color which became more concentrated in accordance with the increasing concentration of usnic acid in solid dispersion system due to the yellow color of usnic acid. Moreover, all membranes were flexible, had a smooth surface, free of particles and air bubbles.

2.8. Data analysis

The percentage of burn healing was analyzed using two-way ANOVA (Analysis of Variance) method and followed by Duncan's post hoc test to determine the effect of membrane preparation in percentage of burn healing in each group. Results shows significantly different if p values is <0.05.

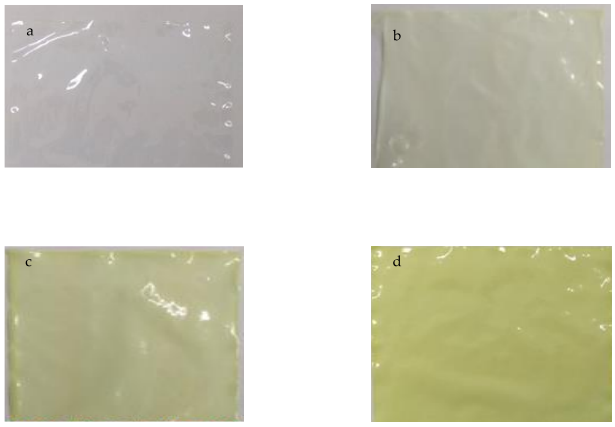

Figure 3. Membrane appearance (a) F0, (b) F1, (c) F2, and (d) F3

The pH of membranes preparation, as shown in Table 3, was in accordance with the pH of skin 4-7 [16]. For topical preparation, the pH should not be too acidic which can cause skin irritation, and also it should not be too alkaline to prevent scaly skin. The highest pH of the membrane preparation was F0 (without usnic acid), and the lowest one is 2% UA-SD membrane (F3). This indicated that pH value was influenced by the amount of usnic acid contained in the membrane formula, where the

greater the concentration usnic acid, the lower the pH value. This result was due to usnic acid as weak acid that has pH 3-5.5 [17]. The average thickness of the membrane were 0.079 - 0.098 mm, as seen in Table 3. At the same membrane volume, the thickness value was greater with increasing concentration of solid dispersion. This result was due to the greater amount of solute in the solid dispersion that incorporated into the membrane.

Table 3. The pH and thickness of membrane

Formula	pH	Membrane thickness (mm ± SD)
F0	4.8 ± 0.02	0.079 ± 0.004
F1	4.5 ± 0.01	0.084 ± 0.003
F2	4.4 ± 0.01	0.090 ± 0.002
F3	4.2 ± 0.01	0.098 ± 0.004

Generally, good membrane has requirements including convenience, easy to use and has elasticity [12,18]. The tensile strength, the percent elongation, and Modulus Young's are the general parameters to determine the mechanical properties of a membrane. The results of membrane mechanical test can be seen in Table 4. F0 had the greatest tensile strength and percent elongation, while F2 had the highest Modulus Young's. This phenomenon indicated the interaction between PVA and PVP-K30 in membrane likely influenced the mechanical properties of the membrane. PVP is known as an amorphous and rigid polymer which has high glass transition temperature (Tg), while PVA is a semi crystalline polymer that has hydroxyl groups that form hydrogen bonds [19]. These polymer that blends in hydrogel created intermolecular hydrogen bond [20]. Therefore, membranes containing UA-SD had lower percent elongation and tensile strength compared to membrane without solid dispersion (F0). However, the decrease in percent elongation was not influenced by the concentration of UA-SD. As shown in Table 4, F2 membrane had the highest percent elongation and tensile strength compared to F1 and F3. This was likely due to that 1% of usnic acid in solid dispersion generated an interaction between PVP and PVA under optimal conditions.

Table 4. The mechanical properties of membrane

Formula	Tensile strength (N ± D)	% Elongation (% ± SD)	Modulus Young's (N ± D)
F0	22.72 ± 1.51	622.9 ± 23.01	3.65 ± 0.12
F1	16.48 ± 1.76	487.6 ± 21.78	3.38 ± 0.25
F2	20.34 ± 1.88	530.0 ± 17.47	3.82 ± 0.25
F3	17.74 ± 2.55	494.7 ± 34.38	3.57 ± 0.28

The effectiveness of membrane in burn activity was carried out using white male rabbits (*Oryctolagus cuniculus*). Rabbits are benign and non-aggressive experimental animals, and have large back area so that one rabbit would have 1-6 wounds so that it can be easily observed [21]. The membrane, size 2.5 cm x 2.5 cm, was changed every 3 days with consideration on prior test result. The membrane began to dry and difficult to be removed from the wound surface on the 4th day, resulting damage to form new fibroblast tissues. Based on the observation, it was known that the animals suffered superficial degree burns with an average burn diameter 17.65 mm. As positive control, a marketed gel was chosen and given once a day. Burn area observation for all test groups on days 0 to 21 showed changes in wound size and shape, as can be seen in Figure 4.

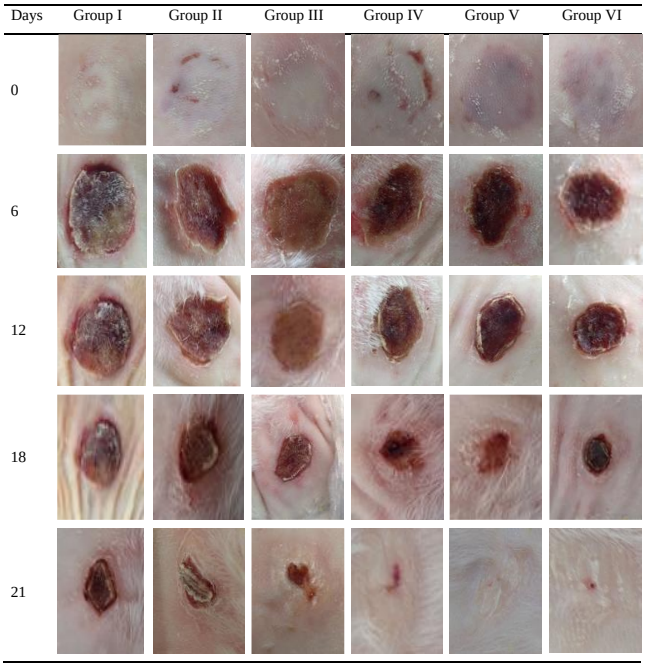

Figure 4. The burns healing observation in each treatment group

The calculation of the average percentage of wounds from day 0 to day 21 is shown in Table 5. The results of two-way ANOVA test of all test groups on the percentage of burn healing showed a significant difference between the treatment groups and healing time on the percentage of burns healing. Moreover, the ANOVA result using Duncan's post-hoc showed between groups had significant differences, except F3 and positive control groups. According to this result, F3 was the most effective of all test groups.

Table 5. The average percentage of burn healing

Day	% of burn healing					
	Group I	Group II	Group III	Group IV	Group V	Group VI
3	5.24 ± 1.24	11.66 ± 3.63	11.97 ± 0.98	19.55 ± 1.47	18.61 ± 3.21	16.78 ± 3.3(
6	19.24 ± 1.90	25.17± 3.06	31.70 ± 1.97	36.08 ± 0.81	36.09 ± 5.18	32.43 ± 4.9]
9	32.27 ± 2.00	40.37 ± 2.36	49.35 ± 1.60	48.88 ± 4.56	50.16 ± 5.31	47.62 ± 1.3(
12	48.65 ± 0.93	52.33 ± 6.48	57.95 ± 1.84	63.01 ± 3.89	64.54 ± 5.66	63.32 ± 1.7(
15	58.12 ± 0.97	64.08 ± 1.77	71.25 ± 1.56	74.41 ± 4.10	76.92 ± 5.45	74.57 ± 3.5-
18	71.05 ± 1.76	73.78 ± 1.73	83.67 ± 1.86	87.60 ± 3,97	92.20 ± 2.25	90.54 ± 2.1]
21	80.27 ± 1.47	81.94 ± 1.45	91.69 ± 1.90	96.47 ± 4.20	100.00 ± 0.00	100.00 ± 0.0
$\overline{X}$	44.98^{a} ± 1.47	49.94^{b} ± 2.93	56.80^{c} ± 3.28	60.86^{d} ± 3.28	62.65^{e} ± 3.87	60.75^{d} ± 2.-

Means within column with different letter are significantly different (P<0.05)

3.2. DISCUSSION

Research on usnic acid has been extensively conducted due to its considerable pharmacological activities. However, the low solubility of usnic acid in water has been a challenge to prepare into pharmaceutical dosage forms. The preparation of usnic acid in solid dispersion is one of strategies to improve saturated concentration of usnic acid that likely increase the activities [5].

The F3 membrane and marketed gel showed complete healing process on the 21st day. The process of healing burns is a series including inflammatory phase, proliferation and maturation phase [22]. From day 0 to 6, there is an initial inflammatory phase that characterized by releasing various inflammatory mediators such as leukotrienes, prostaglandins and histamine as a response and reaction from the body. As the results, vasoconstriction of large blood vessels in the injured area, retraction of blood vessels, fibrin deposits, and formation of blood clots in the wound area occurred in order to keep hemostasis and prevent contamination from microorganisms [23]. The observations show changes in color and extent of the wound in the test animal. When the proliferation phase occurs on 6th day until the 21st day, the cells produce Fibroblast Growth Factor (FGF) and angiogenic factors to repair injured tissue. In addition, collagen and proteoglycans are synthesized which will form new polymeric tissue into wound area [24]. Thus, the wound is filled with inflammatory cells, fibroplasia and forms a reddish tissue with a smooth surface. The experiment result showed that there was the formation of fine collagen fibers which thicken align with length of day. This indicated that the process of collagen and tissue formation continue to strengthen new strong tissues. The final phase of the wound healing process is the maturation phase which starts from the 21st day and ends 1-2 years [22,23,25,26].

The amount of usnic acid influenced on the acceleration of burn healing. The presence of anti-inflammatory and antibacterial activities from usnic acid is known to play role in burn healing. Usnic acid has anti-inflammatory activity by inhibiting the secretion of pro-inflammatory cytokines and mediators such as TNF-α, IL-6, IL-1b, iNOS and COX-2, as well as increasing the release of anti-inflammatory molecules such as IL-10 and HO-1, and minimizing the excessive inflammatory reaction that can damage the tissue around the wound [27]. In addition, usnic acid shows antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faceum* through inhibition of RNA synthesis and direct mechanisms including disrupting DNA replication [28–30].

4. CONCLUSION

In conclusion, usnic acid solid dispersion membrane had proper physical and mechanical properties. The burn healing experiments have shown that application of usnic acid solid dispersion membrane with 2% concentration of usnic acid was the most effective among all test groups.

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