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Original Article

FORMULATION OF PROPOLIS-BASED RECURRENT APHTHOUS STOMATITIS (RAS) PROTECTIVE PATCH WITH COMBINATION OF PVP AND CELLULOSE MATERIALS

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ABSTRACT

Objective: The objective of this study was to obtain a mucoadhesive patch for recurrent aphthous stomatitis (RAS) with propolis, proven propolis' antibacterial ability against bacteria in the oral cavity, and to obtain the physical characteristics of the produced patch.

Methods: The patch was produced using a solvent-casting method. The antibacterial properties were determined by the disc diffusion method against *S. mutans, S. oralis, S. sanguinis, and S. gingivalis*. The physical characteristics of the patch was determined by examining weight and thickness dimension, swelling, surface pH, and structure observation.

Results: The patch was made from 8 formulations with different propolis concentrations, Polyvinylpyrrolidone (PVP), and the use of cellulose materials (Hydroxypropyl methylcellulose and Carboxymethylcellulose). The concentrations of propolis used were 3%, 5%, 7%, and 10%. While the ratio of PVP and cellulose material in the formulation is 2:1. Results showed that propolis had a zone of inhibition greater than 2 mm against *S. oralis, S. sanguinis, S. mutans, and P. gingivalis* bacteria. Patches produced were clear to brown-colored films with high swelling percentage due to hydrophilic polymers used. The patch thickness that is closest to the requirements of the buccal patch was F8 with 0.36+0.04 mm. The mean values of the patches have matched normal salivary pH of 5.5–7. Physically, PVP/CMC formulations were more sticky, and the PVP/HPMC patches were more solid and stronger.

Conclusion: A mucoadhesive patch was obtained with a combination of PVP/CMC and PVP/HPMC, tween 80 as a surfactant, glycerin as a plasticizer, peppermint oil as a flavor enhancer and preservative, with the active ingredient propolis.

Keywords: Propolis, Patch, Mucoadhesive, Antibacterial, Polymer

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INTRODUCTION

Recurrent aphthous stomatitis (RAS) is a type of oral soft tissue disease that can be triggered by local trauma, emotional or physiological stress, allergies or sensitivities, etc. Bacteria can contribute to the pathogenesis of RAS, acting either as pathogens or as sources of antigens that induce the production of antibodies, which can cross-react with oral mucosal keratinocytes. Streptococcal species such as *Streptococcus sanguinis* [1, 2], *S. mitis* dan *S. oralis* [3] are expected to trigger the development of RAS.

The use of natural ingredients as herbal medicines in Indonesia has been carried out for a long time. One of the natural ingredients that can be used to treat disease is propolis. Propolis is a natural ingredient produced by bees that has been used by humans for thousands of years because it is proven to have many benefits, especially when it is used as a medicine. It is also available in various forms, from raw propolis, cream, ointment, and so forth. Research shows that propolis contains compounds that function as antioxidants [4], antibacterial [5], and antifungal [6]. This function is very necessary in overcoming RAS, but a modification is needed so that propolis can become a preparation that can work optimally to heal the mouth ulcer.

The nature of the recurrence, the form of the lesion in the form of an ulcer will cause pain and discomfort, often interfere with speech function, chewing function and social function of the patient. There are several ways to relieve pain in RAS, such as giving mouthwash containing topical anesthetic ingredients or giving anti-inflammatory drugs to reduce pain. However, for long-term use, of course, it is necessary to consider the side effects caused to the normal flora of the oral cavity. Another approach is the application of a gel containing an anti-inflammatory agent. However, sometimes the material in the gel form is quickly lost from the oral cavity dissolved in saliva.

Isolating RAS with a protective material can be done so that the ulcer can be protected from friction, resulting in the wound healing process to be faster. Preparations in the form of patches/plasters/films are suitable for covering agents and carrying active drug substances. The plaster/patch products for RAS on the market today are not very popular in the community; apart from the lack of comfort when using the product (because it is relatively thick, long dissolves in the mouth and interferes with speech function), the product also comes from outside Indonesia and cannot be directly found in the nearest shops.

MATERIALS AND METHODS

Samples

Tetragonula sp. Propolis samples were collected from Masamba, which is located in the South Sulawesi Province of Indonesia, to the north of the luwu district. Ulceloocin, a commercial patch acting as (positive control) was purchased from Yenssen Biotech Co., ltd. (Jiangsu, China).

Chemical and reagent

Polysorbate/Tween 80 ($C_{64}H_{124}O_{26}$), Glycerol ($C_3H_8O_3$), and Ethanol were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Polyvinylpyrrolidone, Carboxymethyl cellulose (CMC), Hydroxypropyl methylcellulose (HPMC) were purchased from BASF SE (BASF, ludwigshafen, Germany). Peppermint oil was obtained from FOODCHEM (Shanghai, China). Natrium Chloride 0.9% was purchased from B. Braun Medical Inc. (Jakarta, Indonesia).

The extraction of propolis

Raw Propolis was cut into small pieces then weighed to get 54 g of propolis. Next, propolis was extracted with 80% ethanol (1:5, w/v)

with a homogenizer (280 rpm) at 25 °C for 8 h and continued maceration overnight. The ethanolic extract solution was then filtered using Whatman #1 filter paper and returned to its original volume with 80% ethanol.

Patch production

The different propolis patch formulations in table 1 were carried out by the solvent casting method [7]. Each polymer was first dispersed in distilled water and stirred using a magnetic stirrer on a hot plate until homogeneous. Then Tween 80 and glycerin were added to the polymer mixture. Finally, propolis is added to the solution until evenly distributed. If there are bubbles, the solution is degassing using a vacuum pump. A total of 25 ml of patch formula was poured onto a 9 cm diameter petri dish and then dried in the oven at 50 °C for 8-12 h.

Weight and dimension uniformity

The patches were cut into 2x2 cm in size and then each of them was weighed using a digital scale and the patch thickness was measured using a micrometer screw.

Swelling test

Patch as dry weight (initial weight or W_0) was placed into the test tube and then 1.0 ml of Physiological Natrium Chloride acted as saline was added to each test tube using a micropipette so that the patch would swell and/or erode. Samples were incubated with certain time intervals at 37 °C, where the excess water that was not absorbed by the patch was carefully removed using a tissue. The wet weight (W_t) of the patch was recorded, after which saline was added to continue the analysis. The swelling percentage can be calculated by the formula [8]:

$$\% \text{ Swelling} = \frac{(W_t - W_0)}{W_o} x100 \ldots (1)$$

Surface pH

Each patch was given 1 ml of distilled water and allowed to swell for 1 h at room temperature [9]. Furthermore, the surface pH of the patch was measured using a Benchtop pH meter inoLab pH 7110 (inoLab, Mexico City) and litmus paper.

Table 1: Percentage of ingredients in each formulation

Formula	Propolis (%)	PVP (%)	CMC (%)	HPMC (%)	Tween 80 (%)	Glycerin (%)	Peppermint (%)	Aquadest (%)
F1	3	40	20	-	8	2	2	Ad 100
F2	5	40	20	-	8	2	2	Ad 100
F3	7	40	20	-	8	2	2	Ad 100
F4	10	40	20	-	8	2	2	Ad 100
F5	3	40	-	20	8	2	2	Ad 100
F6	5	40	-	20	8	2	2	Ad 100
F7	7	40	-	20	8	2	2	Ad 100
F8	10	40	-	20	8	2	2	Ad 100

Scanning electron microscopy

The appearance and morphology of the patch structure were observed by Scanning Electron Microscopy (SEM) with micro magnification and was performed by using Field Emission SEM Inspect F50 with EDAX EDS Detector (FEI Company, Hillsboro, United States).

Antibacterial assay

As the initial discovery of the propolis patch as a stomatitis drug containing antibacterial, a bacterial inhibition test was carried out on the extracted propolis. The antibacterial test was carried out by using the disc diffusion method with the Kirby-Bauer protocol [10] on Brain Heart Infusion (BHI) agar obtained from Microbiology lab (Universitas Indonesia Kampus Salemba, Jakarta, Indonesia). Pathogenic organisms were grown on BHI agar in the presence of various antibiotics and antimicrobial-impregnated filter paper discs (antibiotics, propolis, and 70% ethanol). The isolates used were *S. mutans, S. oralis, S. sanguinis,* and *S. gingivalis*, human oral colonizers isolated and collected by oral biology laboratory, Universitas Indonesia Kampus Salemba. Each agar plate was divided into quadrants and given antibiotic discs of Amoxicillin, Tetracycline, Clindamycin, and Erythromycin, and discs of propolis extract in ethanol, and 70% ethanol which had been left overnight. Re-sterilize the forceps on the Bunsen each time changing disc type. The plate was incubated for 18-24 h at 37 °C and after being removed from the incubator, the diameter of the inhibition zone formed was measured using a ruler.

RESULTS AND DISCUSSION

Propolis extraction

The extraction process produced the final result in the form of a brownish liquid extract. Propolis in ethanol obtained after filtration using filter paper was 238 ml.

Antibacterial assay

The presence or absence of growth around the disc was an indirect measure of the compound's ability to inhibit the organism. The results of the inhibition zone measurements can be seen in table 2.

Table 2: Inhibition zone diameter

Antibacterial drug/agent	*Zone Diameter (mm)*			
	S. oralis	***S. sanguinis***	***S. mutans***	***P. gingivalis***
Amoxicillin	12	17	19	17
Clindamycin 10µg	12	0	6	8
Erythromycin	20	18	17	20
Clindamycin 2µg	10	0	0	0
Tetracycline	30	30	26	25
Propolis	9	6	11	8
Ethanol 70%	0	0	0	0

The data was presented in mean, n=3

Interpretation of the results of disc diffusion using propolis is done to categorize the resulting data into sensitive, intermediate, and resistant [11]. Amoxicillin and ethanol were used as positive and negative control, respectively.

In this study, Propolis showed a varied inhibition zone, 6 mm in *S. sanguinis*; 8 mm in *P. gingivalis*; 9 mm in *S. oralis*; and 11 mm in *S. mutans.* Although the inhibition zone of propolis was lower than the commercial antibacterials Amoxicillin, Tetracycline, and Erythromycin, the qualitative analysis concluded that the sensitivity to propolis was intermediate, which means that it has good inhibitory power against bacteria in the oral cavity that have the potential to cause canker sores. Previously in a study by Asawahame *et al.* [12], *S. mutans* was exposed to 37.5, 75, 150, 300, and 600 mg/ml of propolis extract that was completely dissolved in DMSO, showed that the antibacterial activity against *S. mutans* was dose-dependent and increased with increasing concentrations of propolis; the inhibition zone was ranging from 12 to 16 mm. Further research needs to be done to determine the best percentage of propolis in inhibiting bacterias, so that the correct and safe dose for the RAS protective patch can be obtained.

Patch formulation

The patch was made from 8 formulas with different concentrations of the active substance (propolis) and the use of cellulose material (HPMC or CMC).

The complete formulation design can be seen in table 1. From the formula, the following results are obtained in fig. 1 and 2.

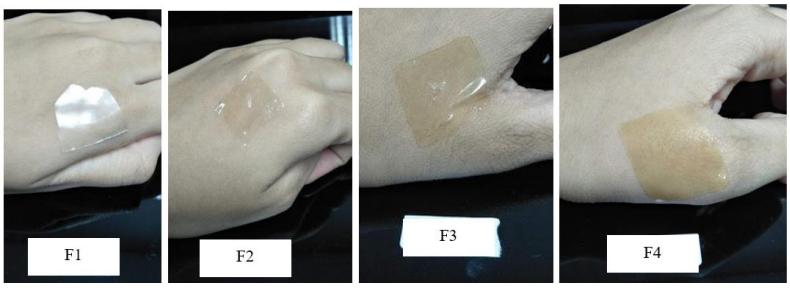

Fig. 1: PVP/CMC patch formulation (F1-F4) with propolis variation

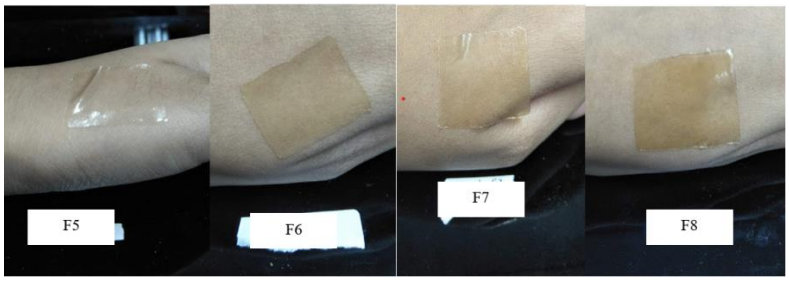

Fig. 2: PVP/HPMC patch formulation (F5-F8)

Organoleptically, the patch was in the form of a film, clear to brown in color as the propolis content in the preparation increases. The patches gave off a slight peppermint scent. For the F1 formulation, the patch was very easy to fold and crumble, while the F8 was very sturdy and has good adhesion. The F7 formulation was difficult to remove from the dish and folded easily. Physically, the F3, F4, and F8 patches looked sturdy and did not tear easily. The F1-F4 formulation which has PVP/CMC as the base material, produced a sticky film when compared to the PVP/HPMC formulation.

Weight and dimension uniformity

Uniformity of weight and uniformity of dimensions can be a benchmark whether the patch-making method is good enough and feasible to be reproduced. The data obtained from the study cannot be concluded from the ANOVA test because the data are not normally distributed ($p<0.05$, considered statistically significant). So that the data was calculated by the average, standard deviation, and coefficient of variation and then analyzed for uniformity with Indonesian Food and Drug Authority (BPOM) parameters. Based on the Regulation of the BPOM number 32 of 2019 [13] concerning the Safety and Quality Requirements of Traditional Medicines on the point of weight uniformity, from 3 sheets of Film Strips that were weighed, the maximum percentage of weight variation was not more than 5%. Patch weight measurement results can be seen in fig. 3.

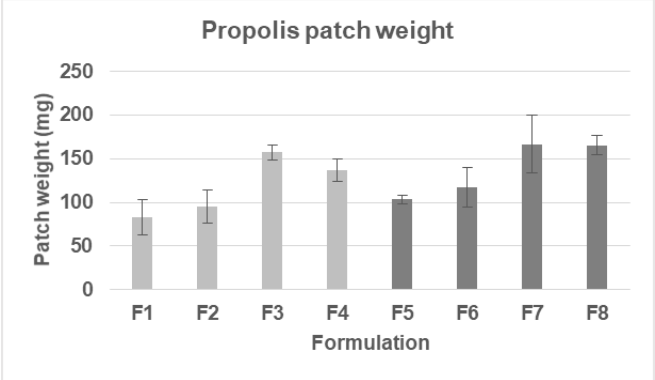

Fig. 3: Propolis patch weight, data was presented in mean ± SD, n=3

From the graph above, it can be seen that from the results of this study, the weight uniformity test still has a large standard deviation, so that only F3 and F5 meet the requirements of BPOM. The large difference in patch weight in the same formulation can be caused by human error during sample preparation, the manufacture was done manually and not machinery, so the patches produced per batch are not standardized.

There are no BPOM or Pharmacopeia regulations that regulate the thickness or dimensions of the patch. According to Mathiowitz, *et al.* [14] the thickness of the buccal patch is 0.5 – 1 mm, if it is less than 0.5 it will complicate the application process and if it is too thick it will cause discomfort when applied. The results of patch thickness measurements can be seen in fig. 4.

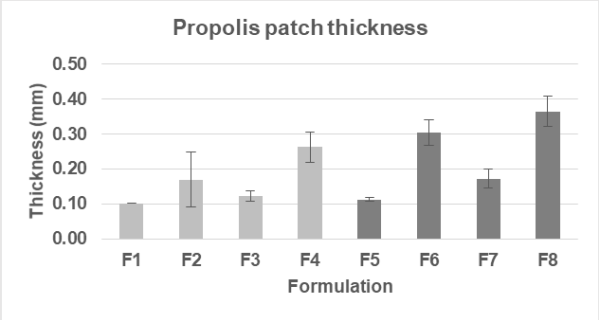

Fig. 4: Propolis patch thickness data was presented in mean ±SD, n=3

The results of this study indicate that the resulting patches were very thin, ranging from 0.1 to 0.4 mm. The patch thickness has not yet reached the minimum value of 0.5 mm, so it can be further modified by adding a backing layer on top of the active substance and its bioadhesive. In addition to controlling thickness, a good-binding backing layer can protect the active ingredients area in patches which are directly attached to the target, and also provide unidirectional drug release [15].

Swelling test

The swelling ability of a patch or the percentage of swelling is related to the ability of the matrix to release drugs and the effectiveness of the patch to adhere to the mucosa. From the results of this study, the triplicate test did not show a pattern and the difference in the percentage of each batch was also high (table 3). Although the data cannot be averaged and concluded statistically, it is possible to analyze the physical phenomena of this swelling patch process. The swelling process can occur in three specific steps: (a) diffusion of water molecules through the matrix, (b) relaxation of the polymer chains through hydration, and (c) expansion of the polymer network after relaxation [16].

The longer the incubation time, the higher the swelling percentage, up to 120 min in some samples the swelling percentage decreased. The higher the swelling percentage, the higher the ability of the patch to absorb fluids in its environment, and the easier it is for the drug to be released or released from the drug dosage form (patch).

All of the polymers used PVP, CMC, and HPMC are categorized hydrophilic groups which provide the possibility of hydrogen bond formation, and thereby high water absorption [17]. The nature of PVP which is easily soluble in water is able to attract the surrounding liquid, so that it becomes loose and causes the patch to swell [18]. CMC is an anionic hydrophilic polymer and is suitable for use in antifungal patch preparations, it can expand in the intestinal mucosa at a pH of around 7. The combination of CMC/PVP can increase drug release by increasing elasticity and film formation on the patch [19].

From the study, the swelling percentage of PVP/CMC formulation (F1-F4) were higher than PVP/HPMC (F5-F8) formulation, and while the numbers for CMC always increased over time, HPMC showed some inconsistency in F6 and F7 where the ratio after 120 min were recorded lower than 60 min. When compared in the same amounts, carboxymethyl cellulose has a lower water retention rate and hydroxypropyl methylcellulose a higher water retention rate. Water can affect how soluble HPMC is, impacted with other variables like pH and temperature [20].

The results of observations and three times repetitions did not show a pattern, so further research was needed. This can happen because the duration and speed of stirring of each formula is not controlled so as to allow bond interactions between polymers to occur randomly.

Table 3: Swelling percentage of patch

Time (min)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
5	115.63+30.5	52.76+5.8	23.89+7.28	61.07+11.16	85.54+37.75	130.51+63.14	45.40+46.78	80.53+31.99
10	180.31+33.6	139.72+20.7	50.80+24.42	146.16+30.38	104.01+14.71	197.16+101.8	64.77+74.35	133.08+58
60	270.33+109.6	217.13+8.08	77.20+30.72	221.26+24.13	141.19+12.49	216.20+57.06	73.66+56.1	154.09+39
120	324.21+220.6	244.07+99.2	122.42+60.66	346.05+160	144.27+28.61	208.75+96.85	63.96+23.66	160.91+29.65

Data was presented in mean ± SD, n=3

Surface pH

Under normal conditions, the pH of saliva ranges from 5.6–7.0 with an average of 6.7. Several factors that cause changes in salivary pH include the average salivary flow rate, oral microorganisms and the buffering capacity of saliva. Bacteria can live in saliva at a pH of 6.5–7.5 and if the oral cavity has a low pH between 4.5–5.5, it will facilitate the growth of acidogenic bacteria such as *Streptococcus mutans* and l*actobacillus* [21]. Polymers with pK equivalent to the extract's helped create films with a narrow range of neutral surface pH (7–7.4) that are appropriate for oral ulcers that are susceptible to extremely acidic or basic conditions and that suitable for oral application without irritating the mucosa [22].

The data obtained from the pH test also could not be concluded with ANOVA ($p<0.05$) so that statistical calculations of the mean, standard deviation, and coefficient of variation were carried out, as well as analysis based on the pH requirements mentioned above. Based on the results of measurements with a pH meter, the resulting patch already has a pH that is suitable for oral cavity conditions, except for some F5 and F6 samples; also, the F8 sample, which was still too low, the average F4 exceeds pH 7. This

showed that the F1-F3 formulation based on PVP/CMC polymer has a good pH value. As for PVP/HPMC, the pH value was more acidic. An increase in pH was found to induce swelling of the polymer layer [23]. Compared to CMC which has a neutral pH, HPMC is stable in the pH range of 3-11. The commercial *Ulceloocin* patch was also measured for its pH value and showed a pH of 6.53.

SEM

The surface and morphology of the films were visualized using Scanning Electron Microscopy (SEM), with micro magnification. Fig. 6 and 7 use a magnification of 200 micrometers with a magnification of 500 times.

Observation of the patch surface structure at micro magnification showed different results. The results on F4 showed a smooth surface, which means that the agitation and mixture of ingredients are well distributed. F4 is the formulation with the most propolis, which is 10%. Compared to the F1-F4 formulation, the SEM results shown in F5-F8 were more random. F8, which also contains 10% propolis showed a more homogeneous result, although not like the PVP/CMC formula. This can happen because during the formulation, mixing PVP/HPMC polymer tends to be more difficult, and the resulting solution was also more viscous, close to gel.

The results shown are the top view of SEM observations, to be able to observe the structure of the polymer matrix more clearly and in detail, cross-sections can be carried out for further analysis.

From a series of experiments and tests that have been carried out throughout the study, propolis-based patches with PVP and cellulose as basic ingredients (CMC and HPMC) have become a promising drug delivery system as a covering agent for oral lesions. One cause of thrush. The propolis patch showed good swelling, not far from the swelling ratio of the commercial *Ulceloocin* patch. The pH test showed that the PVP/CMC formulation was in accordance with normal salivary pH, while HPMC tended to be more acidic. Characteristically, the resulting patch is still too thin and heavy and more sticky than *Ulceloocin*. The measurement results showed that the deviation was still very large because the patch production was not standardized, affecting the diversity of test results. It is necessary to make variations and optimizations for the propolis patch in order to meet the standard.

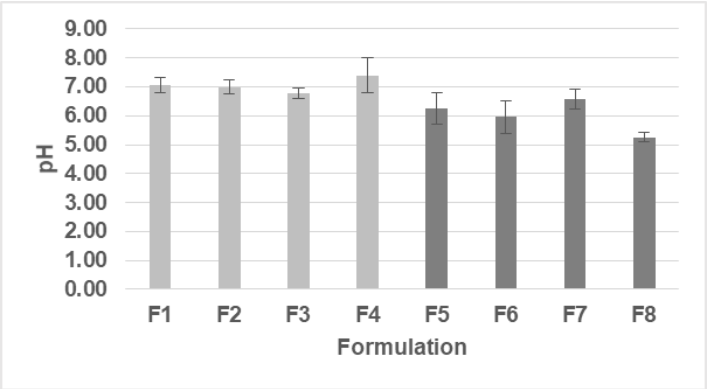

Fig. 5: Surface pH of propolis patch, data was presented in mean±SD, n=3

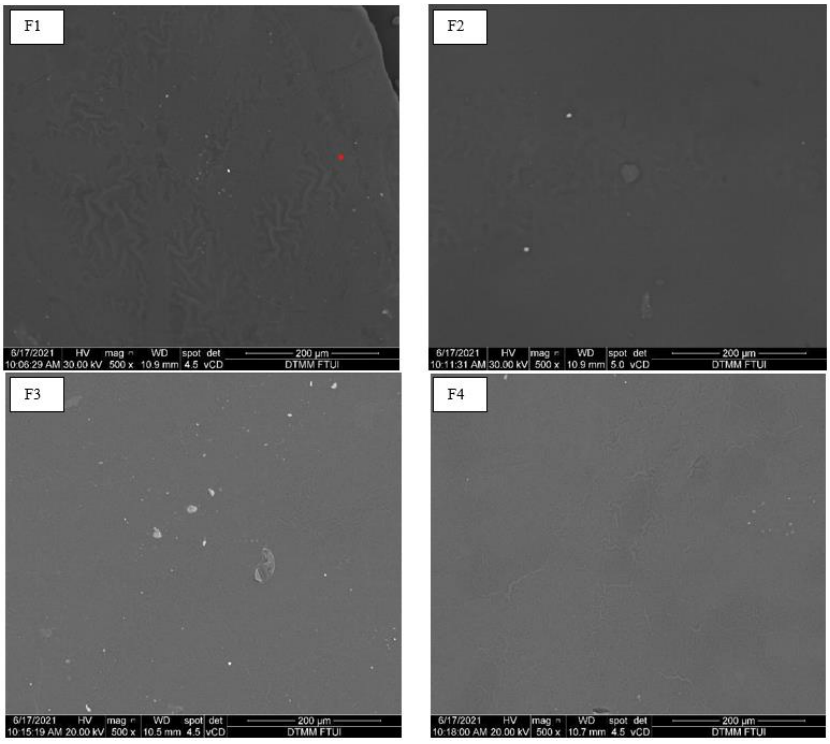

Fig. 6: SEM for PVP/CMC formulation (F1-F4)

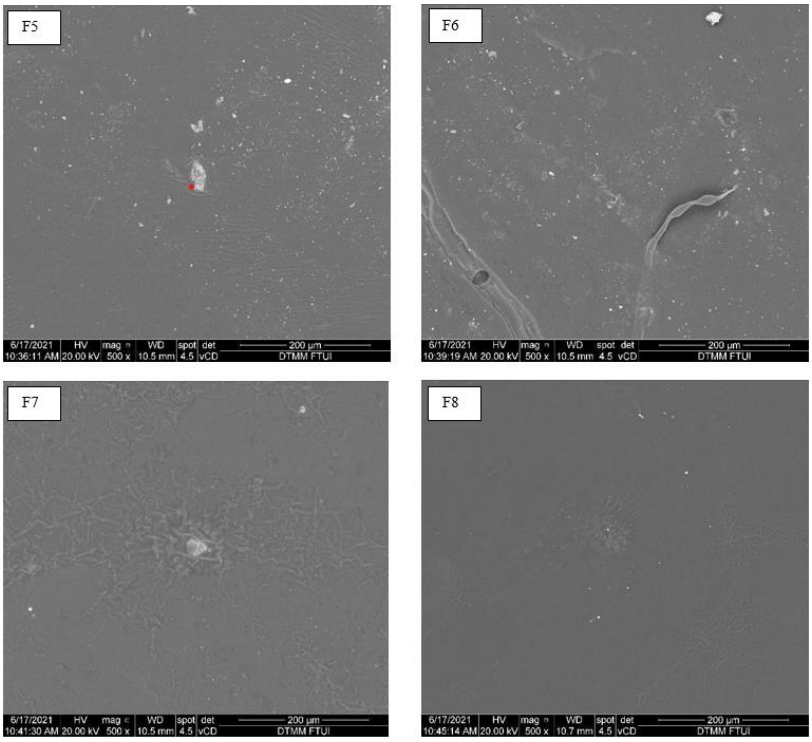

Fig. 7: SEM for PVP/HPMC formulation (F5-F8)

CONCLUSION

A mucoadhesive patch was obtained with a combination of PVP/CMC and PVP/HPMC, tween 80 as a surfactant, glycerin as a plasticizer, peppermint oil as a flavor enhancer and preservative, with the active ingredient propolis. Propolis has intermediate inhibition, indicated by the zone of inhibition >2 mm, against *S. oralis, S. mutans, S. sanguinis,* and *P. gingivalis*. Patches produced were clear to brown colored films. Physically, PVP/CMC formulations were more sticky, and the PVP/HPMC patches were more solid and stronger. The characteristics of the obtained propolis patch with PVP/CMC met the pH surface requirement (5.6 – 7) and high swelling percentage. HPMC formulation has lower pH than CMC, and a rise in pH causes the polymer layer to swell. The thickness of the patch based on BPOM can be obtained with formula modifications such as adding a backing layer material so that the patch is not too thin. With further development, the formulation of this patch, especially with the combination of PVP/CMC, can become more feasible as an alternative to protect and relieve pain in RAS. Other characteristic tests can be performed such as folding endurance, strength and time of attachment, as well as uniformity content of the active ingredient (propolis) in the patch preparation.

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AUTHORS CONTRIBUTIONS

Muhamad Sahlan: conceived and planned the experiments support the financial research; Nadiah Husna Shofwatalloh: carried out the experiments and analysis the data; Yuniardini Septorini Wimardhani: contributed to the interpretation of the results; Risqa Rina Darwita: supervised and verified the result; Diah Kartika Pratami: contributed to sample preparation and administration the research. All authors provided critical feedback and helped shape the research, analysis and manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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