

Potential Therapy from *Punica granatum* Peel Extract for the Treatment of Recurrent Aphthous Stomatitis. Design, Formulation and Characterisation of a Mucoadhesive Patch

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Abstract

Recurrent aphthous stomatitis (RAS) is an inflammation that occurs in oral mucosa. Etiology of RAS may be caused by several factors involving systemic conditions, local, microbes (*Candida albicans*), moniliasis, hygiene and genetics. Several studies has shown that pomegranate (*Punica granatum* L.) peel serves to inhibit *Candida albicans*, antibacterial *Staphylococcus aureus*, *Staphylococcus mutans*, *Escherichia coli* and antifungal *Aspergillus niger*, which is common microorganisms involved in dental and oral problems. This study aims to formulate a pomegranate peel extracts (PPE) mucoadhesive patch and test its effectiveness in healing sores. PPE obtained from the maceration method. Polyvinylpyrrolidone patch formulations, chitosan and Hydroxypropyl Methyl Cellulose (HPMC) using a ratio of 1:1, 2:1, and 3:1 weight/volume. Patch tested his physical characteristics, including weight uniformity, uniformity of dimension, thickness, surface pH, swelling and adhesion test, and in vivo test. The F8 formulation of PPE 10% with the addition of HPMC, tween, and glycerin have a fairly elastic properties. Even though experiment of physical evaluation produce uniformity of same weights and dimensions, pH 6.63, an average of swelling percentage $40.69\% \pm 16.37\%$ and an average of stickness 13.50 ± 11.6 seconds. In vivo test show that formulation of PPE 10% has the same effectivity in reducing the diameter of the mouth ulcers with the positive control group.

Keywords: *Recurrent aphthous stomatitis, Pomegranate peel, Mucoadhesive Patch, Antioxidants, In vivo.*

Introduction

Recurrent aphthous stomatitis (RAS) is a disease that is often encountered in society. This disease is characterized by the presence of white round lesions or sores on the oral mucosa [1]. Based on research, RAS is caused by several factors, including local trauma, bacteria, systemic, nutrition, genetics, allergies and immunology [2]. The lesions that form in sprue can actually heal on their own without medication therapy. However, the formation of RAS lesions can interfere

with the patient's physical activity due to the pain they experience. Therefore, medical therapy is needed to overcome this.

In the medical world, topical disinfectant and anti-inflammatory agents have been developed to treat RAS. The preparation for RAS can be in the form of mouthwash or ointment. However, the form of mouthwash or ointment is still ineffective in overcoming RAS because of its use which covers the entire oral cavity and the short period of contact between the active substance and the RAS in the mouth. Meanwhile, in the last few years, there have been many herbal medicines such as black cummin [3], okra seeds [4,5] and pomegranate [6,7]. One plant that has been widely researched is pomegranate peel.

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Research shows that pomegranate peel extract contains compounds that function as antioxidants^[8,9,10], anti-bacterial^[11,12], anti-fungal^[13], and as a potent analgesic and anti-inflammatory^[14]. Its content is very necessary in dealing with RAS. However, a modification is needed, so that the extract can become a preparation that can work optimally to treat RAS. One alternative that is considered suitable for topical application to mucosal tissue is mucoadhesive patches. Mucoadhesive preparations are designed to adhere to the mucosal layer which can prolong the residence and contact time of the drug on the site of application or absorption as to increase drug bioavailability^[15]. This can increase the therapeutic effect of the drug and is also effective, practical, comfortable, and easy to apply by simply attaching it to the affected area to accelerate healing.

The mucoadhesive characteristics of the patch need to be supported by the use of suitable materials. Several previous studies made use of various synthetic, semisynthetic, and natural polymers. Chitosan is a cationic polymer produced by the synthesis of natural compounds which has high adhesion as a mucoadhesive polymer^[16]. Polyvinylpyrrolidone (PVP) is used as an expansion agent so that it is useful for increasing drug release, increasing elasticity and forming a film layer on the patch^[17]. This combination will later function to improve the local drug delivery system. So, it is hoped that later it will help provide a quick (effective) and potential effect.

This study aims to develop a patch preparation for pomegranate peel extract based on chitosan and polyvinylpyrrolidone as new candidates in the treatment of RAS. In addition, the results of the patch formulation will be tested in vivo on male white rats Wistar strain to see the effectiveness of healing RAS. This research is expected to play a role in the implementation of the "back to nature" program recommended by World Health Organization (WHO) to promote the use of natural traditional medicines in maintaining public health. This momentum is perfect for Indonesia to be more serious in developing and increasing the production of indigenous Indonesian medicines by applying the latest science and technology. The results of this study are expected to have good prospects for commercial preparations

through collaboration with the pharmaceutical industry in the future.

Materials and Methods

This research was conducted from February 2013 to June 2013. The research was conducted in Unit 2 Fitomedicine, Gadjah Mada University and, Research Laboratory and in Animal Laboratory, Faculty of Medicine and Health Sciences, Muhammadiyah University of Yogyakarta.

Pomegranate rind (*Punica granatum L.*) was obtained from Pakem Kaliurang Yogyakarta, Indonesia in January and February 2013 and has been determined at the Laboratory of Pharmaceutical Biology Unit II, Faculty of Pharmacy, Gadjah Mada University.

Commonly used glassware (Pyrex, USA) were analytical scales, water baths, vacuum rotary evaporators, ovens, erlenmeyer flasks, electric stoves, freezers, and digital scales. The materials were Chitosan (Pioneerbiotech, China), Polyvinylpyrrolidone (PVP) (Sigma-Aldrich, Germany), Hydroxypropyl methylcellulose (HPMC) (Shijiazhuang Jianxin Cellulose Co., Ltd, UK), Pappermint (Indotrading, Indonesia), acetic acid (Indo Acidatama, Indonesia), aquadest (Indo Daisun Sakti, Indonesia). Male white rat (*Rattus norvegicus L.*) used aged 40-60 days with a body weight of about 150-200 grams, 10% hydrogen peroxide (H₂O₂) and chloroform, Broiler Pellet II, 70% ethanol, Albothyl® (Pharos, Indonesia).

Extraction

The extract was made using the maceration method. First, the dry skin of pomegranate (*Punica granatum L.*) was mashed in a blender so that it became a fine powder. The fine powder was then soaked in 70% ethanol solvent. The maceration process was carried out for 5 days followed by remaceration for 2 days. During maceration, occasionally the powder was being shaken out for a perfect search. After 5 days, the soaking powder was filtered and separated between the filtrate and the dregs formed. The separated filtrate would be evaporated with a rotary evaporator until a thick extract is formed while the dregs formed earlier would be remacerated for 2 days. The process for obtaining a viscous extract from

remacery was the same as in the maceration process.

Patch Formulation

The obtained extract was used as the active substance in the mucoadhesive patch preparation while the polymers used were PVP, HPMC, and Chitosan. The other additives were glycerin, tween 80, peppermint,

96% ethanol and aquadest. The dissolving method would be used in the preparation of this formulation. Chitosan was first dissolved using acetate buffer pH 4. HPMC and PVP were dissolved in 96% ethanol, respectively. After that, all the ingredients were mixed until they are homogeneous and dried until they form a film. In general, the table of variations in the composition of the formula can be seen in Table 1.

Table 1. Variations in the composition of the patch formula

Formula	Active substance (% w/v)	PVP (% w/v)	Chitosan (%w/v)	Peppermint (%w/v)	HPMC (%w/v)	Tween	Glycerin
F1	5	61.5	31	2.5	-	-	-
F2	10	58	29.50	2.5	-	-	-
F3	5	69.4	23.10	2.5	-	-	-
F4	10	65.5	22	2.5	-	-	-
F5	5	48.75	48.75	2.5	-	-	-
F6	10	43.75	43.75	2.5	-	-	-
F7	5	33.04	21.52	5.19	5.38	22.08	7.79
F8	10	30.29	19.72	5.19	4.93	22.08	5.19

Physical Evaluation Test

The physical evaluation tests carried out in this study included weight and dimensional uniformity, swelling test, pH test, adhesion test and adhesion time.

Uniformity of weights and dimensions

Each patch was weighed using a digital scale which then measured its thickness dimensions using a screw micrometer.

Swelling Test

The patch as dry weight (Wd) was placed into the test tube, then 1.0 mL of physiological NaCl was added to each test tube. The samples were then incubated at certain time intervals at 37 °C. After being removed from the incubator, physiological NaCl was removed

and rinsed using aquadest three times. The sample was placed on a tissue to remove the stuck water before weighing the wet weight (Ww). The amount of % swelling was calculated using the following equation:

$$\% \text{ swelling} = \frac{Ww - Wd}{Wd} \times 100$$

pH Test

Each patch was allowed to expand in 1 mL of distilled water for 1 hour at room temperature. Furthermore, the pH of the patch surface was measured using a pH meter.

Adhesion and Sticky Time Test

The patch was affixed to the intestines of the mice which had been attached to the beaker glass. The beaker

glass was put into a container containing 0.9% NaCl physiological fluid. The container was then rotated with a magnetic stirrer. Furthermore, it was observed how long the patch was attached to the rat intestine.

in vivo Test

Patch effectiveness test was carried out in vivo using mice as samples. Rats were divided into 4 groups, namely negative, positive, untreated and treated controls. Each group consisted of 5 mice. In negative control, the mice induced with aphthous stomatitis were simply left alone without any drug administration. As for the positive control, the mice would be treated with albothyl®. Furthermore, for control without treatment, rats were given a patch without containing pomegranate peel extract (*Punica granatum L.*). As for control with treatment, rats were given a patch containing pomegranate peel extract (*Punica granatum L.*). Observations were made after 1, 3 and 5 days after treatment on test animals induced by thrush by measuring the diameter of the lesions on the affected part.

Results and Discussion

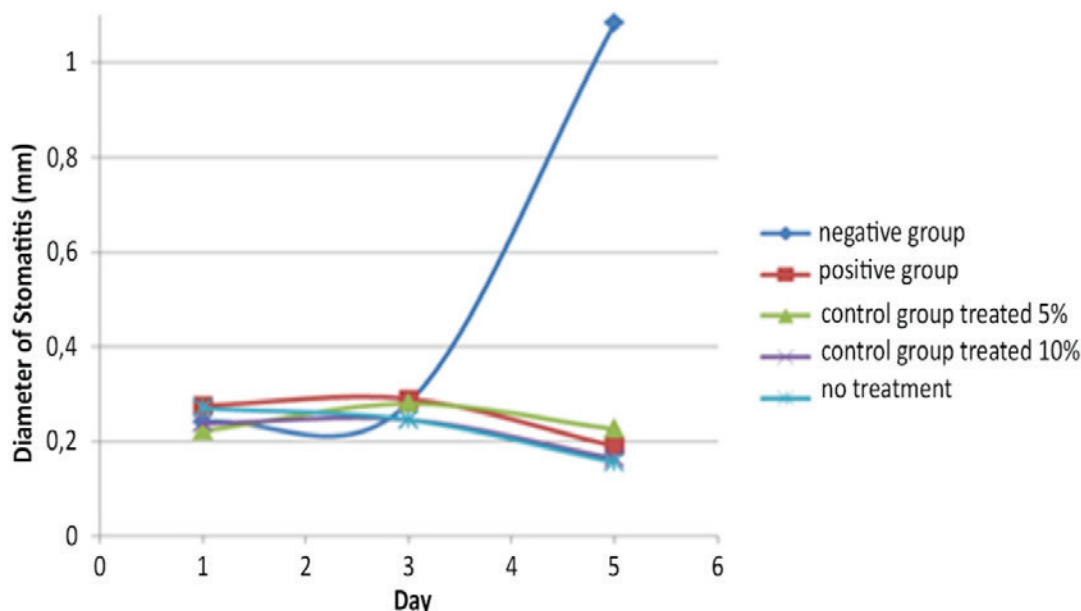
Patch formulation was carried out using the dissolving method. Based on the results of the formulations in table 1, it resulted a patch with quite different characteristics. Formula FI - F6 had properties that were hard, less elastic and easily brittle. Meanwhile, the F7 and F8 formulas produced patches that were not hard and quite elastic, but still fragile. Therefore the patch at F7 and F8 was given an additional layer containing 0.19% PVP and 0.06% HPMC on top. PVP in this case functioned as an adhesive while HPMC functioned as a film forming agent. This layer functioned to maintain consistency, so that the resulting patch was not easily brittle. PVP is water soluble and these characteristics influenced miscibility with the mucoadhesive polymer, the uniformity of the film as well as permeability to water of the film matrix^[18]. HPMC is a derivative of cellulose, obtained by substituting hydroxypropyl and methyl groups to primary and secondary hydroxyl groups, three factors, namely, methyl content, hydroxypropyl content, and molecular weight control the final properties and behavior of HPMC^[19]. The molecular weight determined the viscosity in aqueous solution, with low molecular

weight also correlating to good water solubility and good film-forming properties. Furthermore, a physical evaluation test was carried out on the patches with the F7 and F8 formulas which had better physical properties, so it increased the comfort of the formulations and possibly affected mucoadhesion through better interactions with the mucosa.

The uniformity of weight and dimensions obtained from the physical evaluation test of patches was uniform, because nothing deviated from 5%-10% of the average weight^[20]. The pH test results that met the requirements of mucosal pH were patches with 5% extract resulting 6.63 because the pH range of human mucosa was 5.6 -7^[21]. Yet, from the results of this pH test, pH optimization still needed to be done because after replicating pH measurements, the pH drop was unstable. Meanwhile, for the mean \pm SD of extract 5% was 35.76% \pm 15.87, and the mean \pm SD of 10% extract was 40.69% \pm 16.37%. The higher the % sweating, the higher the ability of the patch to absorb fluids in the environment, and the easier for the drug to be released or released from the drug dosage form (patch). Power test and patch adhesion time with 5% and 10% extract obtained mean \pm SD of 31.01 \pm 9.72 and 13.50 \pm 11.6, respectively. The ideal adhesion test and patch adhesion time was above 180 minutes^[22]. This test was part of the physical characteristics of the patch which was very important if the patch was to be used in biomedical applications, because it was a major factor in the mucoadhesive system^[16].

The results of the in vivo test showed that there was no significant difference between the positive control group, 5% treatment, 10% treatment, and no treatment in reducing the diameter of RAS. However, the reduction in RAS diameter in the control group treated 10% was greater than the control group 5%. Meanwhile, the negative control group showed an increase in the development of RAS diameter. The graph of the effectiveness of the patch can be seen in Figure 1. The results showed that the patch containing 10% pomegranate peel extract had the same effectiveness as the positive control group containing albothyl®.

Patch Effectiveness Chart



Conclusion

Pomegranate peel extract can be formulated with chitosan and polyvinylpyrrolidone (PVP) polymers into mucoadhesive patches with the addition of HPMC, tween and glycerin. Meanwhile, the characteristics of the patch (weight and dimensional uniformity, swelling, pH, adhesion and adhesion time) resulted from the formulation are sufficient. The *in vivo* test showed that the 10% treatment control group had the same effectiveness in reducing the diameter of thrush with the positive control group containing albothyl®.

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Ethical Approval: The procedures were carried out in accordance with the recommendations of the Research

Ethics Committee of the Faculty of Medicine and Health Science, Muhammadiyah University of Yogyakarta. This procedure were examined and approved by the Committee.

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