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EFFICACY OF POMEGRANATE PEEL EXTRACT, MANGOSTEEN PEEL EXTRACT, AND COMBINATION OF BOTH EXTRACT ON INCISION WOUND HEALING IN MICE

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ABSTRACT

Objective: The purpose of this research is to learn about the effectiveness difference between pomegranate peel extract, mangosteen peel extract, and combination of both extracts on incision wound healing of mice.

Methods: This research is experimental research with post-test only control group design. The subjects used in this research were 24 male mice divided into group I pomegranate peel extract, group II mangosteen peel extract, group III combination of both peels, and group IV positive control (povidone-iodine). Every group of mice were then given the treatment, and the length of the wounds was measured and recorded daily until the seventh day after the incision was made, followed by data processing.

Results: On the 7th day, the average wound length in group I was 0.45±0.06 cm, in group II was 0.16±0.25 cm, in group III was 0.0±0.0 cm, and in group, IV was 0.48±0.16 cm. By analyzing the data using Friedman and Wilcoxon test, it shows that there are significant differences between every group.

Conclusion: The extract of pomegranate peel, mangosteen peel, and both combined are effective on incision wound healing of mice. The combination of pomegranate and mangosteen peel extract shows the best healing effect, followed by mangosteen peel extract, and pomegranate peel extract only.

Keywords: Pomegranate peel, Mangosteen peel, Wound healing

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INTRODUCTION

A wound is the breakdown of the normal anatomical structure as well as tissue function. Wound healing is a course of processes involving cellular and subcellular responses to tissue damage, beginning with the release of cytokines, growth factors, and cell activation until tissue regeneration. The healing process occurs in three stages, i.e.: inflammatory, proliferation, and remodelling phase [1]. Wound in soft tissue can be categorized into contusion, abrasion, laceration, penetrating wound, and gunshot wounds [2]. Injuries in the oral cavity may arise from trauma, periodontal disease, tooth extraction, and oral surgery. Tooth extraction is part of dentistry that deals with the removal of teeth from the bone socket. According to Jeffrey and Howe, the ideal dental extraction is defined as a procedure for removing tooth or tooth root painlessly and with minimal trauma to the tissues, resulting in proper wound healing and minimal post-retraction complications [3, 4].

In oral surgery treatment, there may be complications. A study conducted by Blondeau and Daniel from 1 January to 31 December 2013, involved 327 patients consisting of 136 male patients and 191 female patients with the result that complications occurred as much as 6.9%, including 20 cases of alveolar osteitis and 12 cases of infection. Complications can be prevented by providing materials that can work as antibacterial and anti-inflammatory that accelerate wound healing [5].

Indonesia is a nation that has more than 30.000 plant species and 940 species known as medicinal ingredients. One plant that can be used as medicine is pomegranate. Since the presence of a free radical plays a role in wound pathogenesis, research has focused on antioxidant activity. The results indicate that pomegranate extract has potent antioxidant activity by inhibiting lipid peroxidation and increasing the potential of free radical scavenging. Pomegranate (*Punica granatum*) is derived from the Latin *ponus* and *granatus*, also called *Punica granatum Linn* (PGL), is bush or shrub. It is considered to originate from Persia and the Himalayan region located in southern India [6, 7]. Pomegranate has a very high antioxidant content, even higher than grapes and blueberries, and contains flavonoid anthocyanins and higher total flavonoid content than other fruits [8].

Pomegranates are often used as pharmacological and phytochemical substances due to the presence of a high polyphenol component found to be most abundant in the pomegranate peel. Clinical applications include the treatment and prevention of other diseases caused by chronic inflammatory reactions. Pomegranate skin composed of 26-30% membrane by weight of the total fruit weight and contains substantial phenol components including flavonoids (anthocyanins, catechin) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic, and ellagic acid) [9]. The ethanolic extract of pomegranate seeds has an antibacterial effect and no acute systemic toxicity, which can be considered safe on the use as an oral topical solution. The ethanolic extract of pomegranate seeds showed antibacterial properties against S. Sanguis [10].

Mangosteen (*Garcinia mangostana Linn*) is a green Malar tropical plant and one of Clusiaceae genus. The origin of the mangosteen is thought to be from Southeast Asia. Communities in Southeast Asia have been using dried mangosteen peel as an antibacterial, antifungal, anti-tumor, anti-inflammatory, anti-parasitic, antipyretic, analgesic, and treatment of wounded skin [11-13]. Mangosteen peel has very high anti-free radical properties compared to other fruit. Mangosteen peel is shown to reduce the infiltration of inflammatory cells and increase the formation of collagen significantly in burns cases. The xanton derivatives of α-mangostin contained in the mangosteen peel have the greatest antioxidant and antimicrobial activity [14, 15].

MATERIALS AND METHODS

Materials

Pomegranate peel extract, mangosteen peel extract, and combination of both extracts were produced in a phytochemical laboratory, Faculty of Pharmacy of Hasanuddin University. The extracts were prepared using the maceration method with 95% ethanol solvent. Furthermore, the selection of mice, including male mice aged 2-4 mo and weighing 20-30 grams. Razor, scalpel and blade, calliper.

Methods

This research was conducted in biopharmaceutical laboratory at the Faculty of Pharmacy, Hasanuddin University of Makassar. After receiving approval from the faculty ethics committee, we took 24 male mice (*Mus musculus*) in the pharmaceutical laboratory. The mice were divided into 4 groups. Each group consists of 6 mice. Group I was pomegranate extracts, group II was mangosteen peel extracts, group III was combinations of pomegranate and mangosteen peel extracts and group IV as positive controls (povidone-iodine). This was experimental laboratory research with post-test only control group design.

The study begins with anaesthetizing mice with diethyl ether, shaving the hair of the mice's back using a razor, and then making an incision 2 cm long and 1 mm deep with a scalpel and blade. In treatment groups I, II, and III, mice were given an application of pomegranate peel extract, mangosteen peel extract, and combination of pomegranate peel and mangosteen peel extract, respectively, topically on the incision wound. The positive control group IV were treated with povidone-iodine before bandaging the wound. Clinical observation was done by measuring the wound length on 1st to 7th day in each treatment group using a calliper.

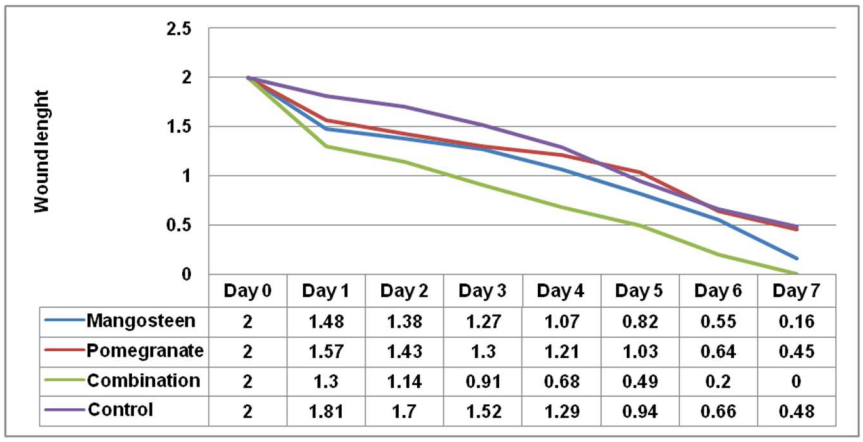
Data analysis

The mean and standard deviation were calculated for each group. Data analysis was performed using SPSS version 24. Comparison between each group was analyzed using Wilcoxon and Mann Whitney test.

RESULTS AND DISCUSSION

RESULTS

The results showed that mean length of the wound on the 7th day in pomegranate treatment group I was 0.45±0.06 cm, the mangosteen treatment group II was 0.16±0.25 cm, the combination treatment group III 0.0±0.0 cm, and control group IV 0.48±0.16 cm fig. 1.



	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Mangosteen	2	1.48	1.38	1.27	1.07	0.82	0.55	0.16
Pomegranate	2	1.57	1.43	1.3	1.21	1.03	0.64	0.45
Combination	2	1.3	1.14	0.91	0.68	0.49	0.2	0
Control	2	1.81	1.7	1.52	1.29	0.94	0.66	0.48

Fig. 1: Graph of mean wound length (cm)

Friedman test aims to determine the difference of wound length of each treatment group on every observation day because the value p = 0.000<0.05, then Ho rejected. This means that there is a significant difference in every observation day in every group.

The statistical test is then continued with Wilcoxon test. Wilcoxon test aims to see whether there was any significant change of wound length between each day of observation. Based on the Wilcoxon test, all treatment groups had significant changes of wound length, except in the combination group III on the 6th day until the 7thday was not significant because p = 0.68 indicating the wound had already closed on that day. Furthermore, to determine whether treatment groups had the same or different effects with one another, a Mann Whitney test was used.

Based on the Mann Whitney test, it was seen that the combination group III was significantly different from the pomegranate group I and the control group IV (all observations day p<0.05), means that combination group III had different effects compared to the pomegranate group I and the control group IV. The control group IV also differed significantly with mangosteen group II, but on the 7th day observation, p>0.05 means the control group had almost the same effect as the mangosteen group II. The mangosteen and pomegranate groups almost have the same effect because there were many p>0,05 values and on days 5-7 p-value>0.05.

This study used mice as experimental animals because having advantages such ability to reproduce quickly and it's well adaption to the new environment [16, 17]. The mice used were male mice considering more stable biological conditions compared with female mice, which are affected by uterine periods. In addition, mice were used because they had some growth alignment with human conditions [18].

The parameters observed in this trial were the wound length on the 7th day. Period of wound healing was observed to see the effect of test materials on wound healing time. Wound length in the positive control group using povidone-iodine on 7th day showed the largest wound length compared to the other treatment groups but had a similar size to the pomegranate peel group. This suggests that the positive control group IV had a similar ability to pomegranate peel group I in wound healing. This is because the pomegranate peel has 2.8 times higher antioxidant activity than pomegranate seed or leaves extract.

Anti-inflammatory components of pomegranate peel such as punicalagin, punicalin, strictinin A, and granatin B reduce the production of nitric oxide and PGE_2 by inhibiting the expression of pro-inflammatory proteins. Aqueous extract of pomegranate peel is able to inhibit myeloperoxidase activity and production of hypochloric acid enzyme from hydrogen peroxide, thus inhibiting inflammatory stimulator [8]. In addition, the content of gallic acid, ellagic acid, and punicalagin has the ability to prevent free radicals that can accelerate wound healing significantly and protect tissue from oxidative damage, also acts as an antibacterial against certain pathogenic bacteria such as *Escherichia coli*, *Salmonella spp*., *Shigella spp*., etc [19]. The healing ability is also shown by povidone-iodine because it has the antimicrobial ability by oxidizing the respiratory enzyme from bacteria to prevent infection [20].

The mean wound length measurement in the mangosteen group II on the 7th day was 0.16±0.25 cm. This length indicates faster wound healing occurring in mangosteen group than in pomegranate and control groups. This is due to the presence of xanthones capable of inhibiting the secretion of pro-inflammatory mediators by altering primary human cells. Research shows that α-mangostine present in xanthones can inhibit IL-8 or TNF-α secretion by forming cells from

other tissues. α-Mangostine and γ-mangostine also inhibit the production of nitric oxide (NO) and prostaglandin (PGE2)[21].

The results showed that the average length of wounded animals in the combination of mangosteen and pomegranate on the 7th day was 0.0±0.0 cm. This indicates that the combination group of mangosteen and pomegranate have the best wound healing ability among other groups. This is due to the combination of pomegranate and mangosteen compounds. The presence of pomegranate-containing phenol components in the form of anthocyanins, gallotannins, ellagitannins, and derivatives collaborating with xanthone components in mangosteen containing α-mangostine, β-mangostine, γ-mangostine, garcinone B, and garcinone E, and mangostineone, tannins, etc. along to assist healing process of wounds in mice. The presence of compounds from pomegranate and mangosteen peel extracts causes the wound healing ability to become faster because each compound is complementary in the wound healing process in the experimental animals [9, 12].

CONCLUSION

The combination of pomegranate and mangosteen peel extract has better effect on wound healing in mice, followed by mangosteen peel extract, and lastly the pomegranate peel extract.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

The authors report no conflict of interest

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