

## EFFICACY OF A STANDARDIZED ETHANOL EXTRACT OF ROSELLE CALYX IN THE TREATMENT OF ORAL MUCOSA ULCERATION (*IN VIVO*)

RENI JAYANTINI, DEWI FATMA SUNIARTI, AGOENG T SARWONO\*

Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Depok, Indonesia. Email: agoengtjahajani@yahoo.co.id

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### ABSTRACT

**Objectives:** To analyze the effectiveness of the topical application of a standardized ethanol extract of roselle calyx in the treatment of oral mucous ulceration.

**Methods:** Twelve Sprague Dawley rats were divided into two groups, with oral mucous ulcer being induced in all the rats. The ulcer diameter, reddish color, and the swollen membrane around the ulcer, as well as the inflammation score of the ulcer, were recorded for all animals. The negative control group was treated with aquades, while the positive control group was treated with 0.1% triamcinolone acetonide. The treatment group received the topical application of 7.5% and 15% standardized ethanol extract of roselle calyx twice a day for 3 or 7 days. On the 4<sup>th</sup> day, six rats were sacrificed, while the remaining six rats continued to receive treatment as before. On the 8<sup>th</sup> day, all the rats were sacrificed. Specimens were fixed, and histology slides were made. Further, microscopic slides were observed and scored.

**Result:** Both the 7.5% and 15% standardized ethanol extracts of roselle calyx were found to reduce the ulcer diameter and inflammation score. No swelling or redness was observed.

**Conclusions:** The application of 7.5% standardized ethanol extract of roselle calyx for 7 days is equally as effective as the application of 0.1% triamcinolone acetonides, except in terms of the decrease in the inflammation score.

**Keywords:** Standardized ethanol extract of roselle calyx, Ulcer, Healing.

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### INTRODUCTION

In Indonesia, herbs have traditionally been used to treat numerous conditions, and they are believed to be an effective tool for public health services as well as being suitable for further development [1]. The government also supports the use and development of herbs in the public health context. Indeed, article 47 of Law No. 23 of 1992 stipulates that traditional medicine is recognized as a viable alternative therapy, while Minister of Health Decree No. 1076/SK/VII/2003 concerns the use of traditional medicine involving herbs [2,3].

One of the herbs that are currently being widely studied is the roselle plant (*Hibiscus sabdariffa* Linn.), which belongs to the Malvaceae family [4]. A study conducted by Abdulkadir reported that the ethanol extract of the roselle flower, which can be obtained in concentrations of 3.75%, 5%, and 7.5%, has an anti-inflammatory effect [5].

Other researchers, who have performed the standardization of the roselle flower petal extract, have stated that the results of phytochemical and toxicity tests concerning the extract indicate the presence of chemicals such as phenolic compounds, flavonoids, tannins, and saponins. The standardization of the roselle extract has been achieved using 8:13% ash content, 8:03% water content, a specific gravity of 0.9036 g/ml, and 22.2% drying shrinkage [4]. The Indonesian National Agency of Drug and Food Control has reported that the roselle plant exhibits various beneficial properties, including antihypertensive, antiobesity, anticholesterol, hepatoprotective, antioxidant, antibacterial, and anti-inflammatory properties [6].

At the oral mucosa, ulcers can form that lead to stomatitis, which often occurs as the result of trauma. Ulcer is not a severe condition, although it can seriously impact the patient's quality of life due to the symptoms it causes. The clinical features of acute ulcer

are characterized by the presence of purulent yellowish fibrin membranes, which are often accompanied by pain. In the case of chronic ulcer, the ulcerated area of the membrane is covered with yellowish and slightly protruding edges that indicate hyperkeratosis. The etiology of the emergence of ulcer includes microorganisms and trauma as well as thermal, chemical, and immunological reactions. The traditional theory for an ulcer is symptomatic, and it typically involves reducing pain or preventing secondary infection. In this study, the effectiveness of a standardized ethanol extract of roselle calyx in the treatment of oral mucosa ulceration is tested in Sprague Dawley (SD) rats.

### METHODS

A laboratory experiment was conducted in this study, with each protocol having been tested and approved by the Dental Research Ethics Committee of the University of Indonesia. The study was conducted on 12 SD rats. An ulceration model on the labial mucosa (Figs. 1 and 2) of 2- to 3-month-old male rats (weight 130-160 g) was created. The trial animals were then divided into two groups, namely, the control group and the treatment group. Four SD rats were randomly enrolled into the control group, which was further divided into the negative control and positive control groups. Eight SD rats were randomly enrolled into the treatment group.

From day 1 until day 3, the diameter of the ulcer, any changes in the color of the ulcer, any inflammation of the ulcerative area, weight, body temperature, and attitude were all observed. During this period, 0.1% triamcinolone acetonide was applied to the positive control group once daily, while a standardized ethanol extract of roselle calyx (*H. sabdariffa* Linn.) of either 7.5% or 15% was applied to the treatment group twice daily for 15 seconds. On day four, six rats were sacrificed, and specimens were obtained and fixed with 10% formalin solution.



**Fig. 1: Ulceration model was created**



**Fig. 2: Ulceration after rats were sacrificed**

From day 4 until day 7, the diameter of the ulcer, any changes in the color of the ulcer, any inflammation of the ulcerative area, weight, body temperature, and attitude were again all observed. During this period, 0.1% triamcinolone acetonide was applied to the positive control group once daily, while a standardized ethanol extract of roselle calyx of either 7.5% or 15% was applied to the treatment group twice daily for 15 seconds. On the 8<sup>th</sup> day, the remaining six rats were sacrificed, and specimens were obtained and fixed with 10% formalin solution.

All the specimens were sent to the Histology Laboratory Faculty of Medicine of the University of Indonesia for histological preparation. Hematoxylin and eosin staining was performed on pieces of 30, 40, and 70.

A light microscopic analysis was conducted on every specimen, with the scoring performed by three researchers using the Eda and Fukuyama score, whereby: (1) score 0 = tissue looks normal, (2) score 1 = widening of blood vessels, (3) score 2 = widening of blood vessels along with the erosion of the epithelial layer and lymphocytes, (4) score 3 = widening of blood vessels and moderate amounts of erosion of the lining epithelial lymphocytes, and (5) score 4 = dilation of the blood vessels, erosion of the epithelial layer, and a dense lymphocyte group accompanied by lymphocyte cell breakthrough from the epithelial tissue. A descriptive analysis was used for all data analyses in this study.

## RESULTS AND DISCUSSION

Based on the macroscopic observations (Table 1), a reddish color discoloration and changes in the swollen membrane around the ulcer were seen in the treatment group following topical application of 7.5% ethanol extract of roselle calyx for 3 days. There was no difference observed between the treatment group and the control group in this

regard. After 7 days, redness and swelling were seen in the treatment group following the application of 7.5% ethanol extract of roselle calyx; however, on the 7<sup>th</sup> day, before the rats were sacrificed, no redness or swelling was seen in the tissues surrounding the ulcer, which was also the case in the positive control group. There were no differences between the treatment group and the control group for 3-day following the topical application of 15% ethanol extract of roselle calyx in the treatment group. In early stages of the study, one rat from the treatment group showed redness and swelling following 7 days' application of 15% ethanol extract of roselle calyx although, during the next 7 days, no redness or swelling around the ulcer was seen in the rat before it was sacrificed.

Redness and swelling were still visible on day 3, most likely due to the ongoing inflammatory process. Yet, on day 7, no visible redness or swollen tissue was seen around the ulcer following the application of standardized ethanol extract of roselle calyx since the inflammatory phase had ended, and the fibroblastic stage had begun. This shows that roselle is an effective anti-inflammatory agent. These results are consistent with those of a previous study, which found that the ethanol extract of roselle can reduce the volume of edema in a mouse's legs when induced using albumin [5]. Other studies have reported the efficacy of the anti-inflammatory property of roselle calyx in inhibiting the formation of ear edema in rats induced using xylene [7].

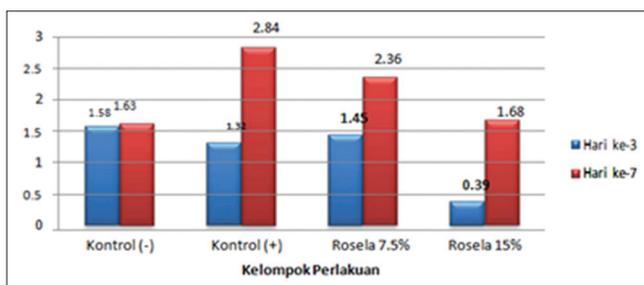
The microscopic observation (Fig. 3) revealed the difference between the diameters of the ulcers in the group treated with the topical application of 7.5% ethanol extract of roselle calyx for 3 days, with one of the mice showing a difference in the ulcers of 1.45 mm. The group that was treated using the application of 7.5% ethanol extract of roselle calyx for 7 days showed an average difference of 2.36 mm in the diameter of the ulcers. Further, the treatment group that received 15% ethanol extract of roselle calyx for 3 days showed an average difference of 0.39 mm in the diameter of the ulcers, while the group that received 15% ethanol extract of roselle calyx for 7 days showed an average difference in the diameter of the ulcers of 1.68 mm. A greater reduction in the diameter of the ulcers was achieved in the treatment group than in the negative control group, most likely because a standardized ethanol extract of roselle calyx is much more efficient in terms of its antioxidant and anti-inflammatory properties. This corresponds with the findings of previous studies, which found that the antioxidants property of roselle calyx is effective to inhibit the formation of lipopolysaccharide in inflammation [8,9].

The microscopic observation (Fig. 4) concerning the inflammation score at day 3 in the treatment group following the application of 7.5% ethanol extract of roselle calyx showed a decrease in the inflammation score (score 1). Similarly, one of the mice in the treatment group that received 15% ethanol extract of roselle calyx showed a decrease in its inflammation score (score 3). The microscopic observations (Fig. 5) conducted on day 7 showed a decrease in the inflammation score of the treatment group that received 7.5% ethanol extract of roselle calyx (score 2). Further, the treatment group that received 15% ethanol extract of roselle calyx also showed a decrease in the inflammation score (score 3). The decreases in the inflammation scores in the treatment group were more significant than those seen in the negative control group, most likely due to the difference in the anti-inflammatory properties of the different treatment options. This finding corresponds with the results of previous studies, which found the anti-inflammatory action to be effective in limiting the endothelial wall attached to the leukocyte, which further served to reduce cell necrosis and increase vascularization [10].

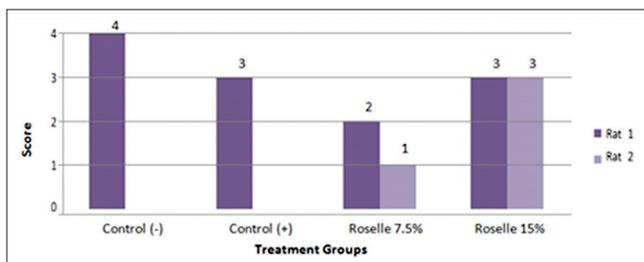
The reduction in the ulcer diameters and the reduction in the inflammation scores, as well as the disappearance of signs redness and swelling in the tissue around the ulcer, are believed to be due to the content of flavonoids and saponins, which are both efficient in terms of their antioxidant and anti-inflammatory properties. Previous studies have suggested that flavonoids and saponins are responsible for the

**Table 1: Redness and inflammation of tissue surrounding the ulcer in the control group, and 7.5% and 15% standardized ethanol extract of roselle calyx applied to the treatment group for 3 or 7 days**

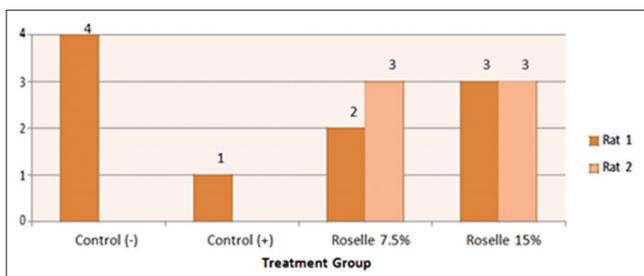
Group	Description	Rat no.	Days	Redness of tissue around ulceration		Inflammation of tissue around the ulceration	
				Early	Final	Early	Final
Control	Negative (-)	1	3 days	+	+	-	+
		2	7 days	+	+	+	+
	Positive (+)	3	3 days	+	+	+	+
		4	7 days	+	-	+	-
Treatment	Roselle 7.5%	5	3 days	+	+	+	+
		6	7 days	+	-	-	-
		7	3 days	+	+	+	+
		8	7 days	+	-	+	-
	Roselle 15%	9	3 days	+	+	+	+
		10	7 days	+	+	+	-
		11	3 days	+	+	+	+
		12	7 days	+	-	+	-



**Fig. 3: Differences in diameter of ulcers in the control group, and in the treatment group following the application of 7.5% or 15% standardized ethanol extract of roselle calyx for 3 or 7 days**



**Fig. 4: Inflammation score in the control group and the treatment group following the application of 7.5% or 15% standardized ethanol extract of roselle calyx for 3 days**



**Fig. 5: Inflammation score in the control group and the treatment group following the application of 7.5% or 15% standardized ethanol extract of roselle calyx for 3 days.**

binding process of free radicals, which works to limit the leukocytes attached to the endothelial cell wall, and in turn, to further reduce necrosis and increase vascularization. In normal circumstances,

leukocytes circulate in the blood vascularization. However, when inflammation is present, the leukocytes will attach to the endothelial cell wall and stimulate neutrophil degranulation. This may cause the formation of free radicals, which can damage tissue and inhibit healing. Their active substances on roselle allegedly serve to reduce the inflammation score and thus accelerate healing [7,8].

Previous studies have suggested that inflammation causes the membrane potential difference that serves to increase the number of free radicals and then oxidize compounds and phospholipids to the lipid peroxides. The bond between the free radicals and the phospholipid compounds causes the formation of openings in the cell membrane so that extracellular fluid will be able to enter and thereby increase the osmotic pressure. This results in the enlargement of cells and cell lysis over time. In addition, an increase in free radicals causes antioxidants in the body such as the enzymes superoxide dismutase, catalase, and glutathione peroxidase to decrease in number. Flavonoids are antioxidants thought to help in curing ulcers since flavonoids can prevent the free radical activity that slows down the process of inflammation by stabilizing the component of the free radicals. The high reactivity of the flavonoids results in the inactivity of the free radicals, so that the activation of the inflammatory mediators by the free radicals can be inhibited and lipid peroxides are not formed. In addition, flavonoids are thought to be able to increase the antioxidant activity in the body that originally decreases when there are many free radicals present so that the healing process will be even faster [10].

Flavonoids work efficiently as anti-inflammatory agents by limiting the release of inflammatory mediators. Inflammatory mediators cause the inflammation cells in the ulcer. The flavonoid anti-inflammatory activity is achieved through the inhibition of cyclooxygenase and lipoxygenase, which results in restrictions on the number of inflammatory cells that migrate to the damaged tissue. Furthermore, the inflammatory reaction will shorten and not serve to block the proliferation ability of TGF-β; thus, the process of proliferation may occur. In addition, antioxidant property of flavonoids also promotes the healing of ulcers by inhibiting free radicals [9,10].

Other studies have indicated that the proliferation of fibroblasts is stimulated by IL-1b. The fibrin clot formed by the increased activity of fibronectin will serve as the framework for reepithelialization and fibroblast proliferation. Thus, when blood clots are rapidly formed, fibroblasts will soon proliferate to the wound area and stimulate tissue healing. Saponins play an antioxidant and antimicrobial role in wound contraction, and they work to improve epithelialization. The formation of new epithelial cells as an ulcer-healing mechanism can be seen through the reduction in the ulcers' diameter. The microscopic observations showed the formation of new epithelial ulcers in areas with a thicker layer than the surrounding epithelium, whereas the macroscopic observations indicated a decrease in the diameter of the ulcers.

Previous studies have suggested that another advantage of roselle is achieved through its antibacterial effect. Therefore, the healing of the ulcers may have been indirectly affected by the antibacterial content. This notion is consistent with a previous study that found roselle to have an efficient antibacterial effect due to inhibiting the growth of *Streptococcus sanguinis* [4].

In this study, the topical application of 7.5% standardized ethanol extract of roselle calyx for 7 days demonstrated an effectiveness equivalent to that of 0.1% triamcinolone acetonide in terms of eliminating inflammation and lowering the diameter of the ulcer but not in relation to the inflammation score. These results indicate that a standardized ethanol extract of roselle calyx still experiences difficulty in penetrating into the target tissue, which necessitates the development of new formulations and forms of preparation. An effective dosage and duration of application of roselle for the healing of oral mucosa ulcers have not yet been determined due to the time interval and limitations of concentration.

#### CONCLUSION

From this study, it can be concluded that the topical application of 7.5% standardized ethanol extract of roselle calyx is effective in healing oral mucosa ulcers SD rats. Indeed, the effectiveness of 7.5% standardized ethanol extract of roselle calyx is equivalent to that of 0.1% triamcinolone acetonide in terms of removing signs of inflammation and rescuing the ulcer's diameter but not with regard to the inflammation score.

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