



# Combination effects oral drops of *Holothuria scabra* extract and *Hylocereus polyrhizus* peel on enhancing recovery from post-chemotherapy oral mucositis

[Efectos combinados de las gotas orales de extracto de *Holothuria scabra* y cáscara de *Hylocereus polyrhizus* para mejorar la recuperación de la mucositis oral posterior a la quimioterapia]

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## Abstract

**Context:** One of the therapies in leukemia children is chemotherapy, but it causes side effects of oral mucositis. Oral mucositis is treated in the form of drugs from chemicals or natural ingredients. *Holothuria scabra* comes from natural collagen ingredients that can help regenerate wound tissue. *Hylocereus polyrhizus* peel extract potentially serves as a natural antioxidant source and is considered an anti-cancer medication.

**Aims:** To analyze the effectiveness of combined oral drops of *H. scabra* extract with *H. polyrhizus* peel having concentrations of 20%, 30%, and 40% in accelerating the healing of post-chemotherapy oral mucositis in Wistar rats analyzed pre-clinically and histopathologically.

**Methods:** The research design was a post-test-only control group. The sample size of 45 rats after being given chemotherapy drugs was divided into 5 groups (positive control group, negative control group, drop application group with concentrations of 20%, 30%, and 40%). After mucositis appeared on the third day after chemotherapy drug administration, rats were given the drug twice a day by 2 drops except for the negative control. Pre-clinical and histopathological observations were made for 3, 7, and 14 days. Data were analyzed using Kruskal Wallis and post hoc using Mann-Whitney.

**Results:** The results of pre-clinical and histopathological observations showed that the combined oral drops of *H. scabra* extract and *H. polyrhizus* peel with various concentrations and the positive control had better mucositis healing than the negative control. The results of *post-hoc* observation on day 7 showed that 20% and 40% concentration drops have the same effectiveness in accelerating the healing of oral mucositis and get better results than the positive control.

**Conclusions:** A 20% concentration extract of *H. scabra* and *H. polyrhizus* peel oral drops was chosen as a drug to accelerate the healing of oral mucositis in post-chemotherapy rats.

**Keywords:** *Holothuria scabra*; *Hylocereus polyrhizus* peel; drops; chemotherapy; mucositis healing.

## Resumen

**Contexto:** Una de las terapias en niños con leucemia es la quimioterapia, pero provoca efectos secundarios de mucositis oral. La mucositis oral se trata en forma de medicamentos a base de sustancias químicas o ingredientes naturales. La *Holothuria scabra* proviene de ingredientes de colágeno natural que pueden ayudar a regenerar el tejido de la herida. El extracto de cáscara de *Hylocereus polyrhizus* sirve potencialmente como una fuente natural de antioxidantes y se considera un medicamento contra el cáncer.

**Objetivos:** Analizar la eficacia de gotas orales combinadas de extracto de *H. scabra* con cáscara de *H. polyrhizus* con concentraciones del 20%, 30% y 40% para acelerar la curación de la mucositis oral posterior a la quimioterapia en ratas Wistar analizadas preclínicamente e histopatológicamente.

**Métodos:** El diseño de la investigación fue un grupo de control solo posterior a la prueba. El tamaño de la muestra de 45 ratas después de recibir medicamentos de quimioterapia se dividió en 5 grupos (grupo de control positivo, grupo de control negativo, grupo de aplicación de gotas con concentraciones de 20%, 30% y 40%). Después de que apareció la mucositis el tercer día después de la administración del medicamento de quimioterapia, las ratas recibieron el medicamento dos veces al día en 2 gotas, excepto el control negativo. Se realizaron observaciones preclínicas e histopatológicas durante 3, 7 y 14 días. Los datos se analizaron utilizando Kruskal Wallis y post hoc utilizando Mann-Whitney.

**Resultados:** Los resultados de las observaciones preclínicas e histopatológicas mostraron que las gotas orales combinadas de extracto de *H. scabra* y cáscara de *H. polyrhizus* con varias concentraciones y el control positivo tuvieron una mejor curación de la mucositis que el control negativo. Los resultados de la observación post hoc en el día 7 mostraron que las gotas de concentración del 20% y 40% tienen la misma eficacia para acelerar la curación de la mucositis oral y obtienen mejores resultados que el control positivo.

**Conclusiones:** Se eligió un extracto de una concentración del 20% de gotas orales de cáscara de *H. scabra* y *H. polyrhizus* como fármaco para acelerar la curación de la mucositis oral en ratas post-quimioterapia.

**Palabras Clave:** *Holothuria scabra*; cáscara de *Hylocereus polyrhizus*; gotas; quimioterapia; curación de la mucositis.

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## INTRODUCTION

The number of cancers in children is increasing every year, and this needs to be taken seriously (Arber et al., 2016). The most common type of cancer in children worldwide is leukemia, followed by brain and nerve cancers (Suarantari and Winata, 2019). In 2020, GLOBOCAN data obtained 474,519 leukemia cases with 311,594 deaths, making leukemia the fifteenth most common cancer in the world, and the percentage of leukemia in children is around 26.1% (Liem et al., 2019; Sung et al., 2021). Based on the RISKESDAS in 2018, the prevalence of childhood cancer aged 0-14 years in North Sumatra was 21,882 cases, with the most common type being leukemia (Ministry of Health of the Republic of Indonesia, 2018).

Leukemia is a malignancy of blood cells from the bone marrow where white blood cells experience abnormal proliferation so that white blood cells are formed but are immature with abnormal functions (Mathur et al., 2012). The causes of leukemia are thought to be radiation, chemicals (benzene), viral infections (Epstein-Barr and Lymphotropic viruses), and possibly genetics (Deliverska and Krasteva, 2013). Therapy for leukemia patients includes chemotherapy, targeted therapy, radiation therapy, bone marrow transplantation, and immunotherapy (Dhakal et al., 2020).

Chemotherapy is the most common therapy administered to children with leukemia; however, it causes side effects in the form of oral mucositis, with an incidence of 62.25% (Hasni et al., 2020; Hastuti et al., 2019). Oral mucositis is an inflammatory response of the mucosal epithelium due to the cytotoxic effects of chemotherapy and causes pain (Pulito et al., 2020); it is characterized by oral pain, erythema, ulceration sometimes accompanied by bleeding, soft white spots on the palate, buccal and labial mucosa, and tongue to the pharynx (Hasibuan et al., 2019). Ulcer size varies, ranging from <1 cm<sup>2</sup> to >3 cm<sup>2</sup> (Singh and Sing, 2020). Oral mucositis can heal within 2-4 weeks of chemotherapy (Alqahtani and Khan, 2022). Treatments that help accelerate the healing of oral mucositis include treatment with drugs derived from chemicals such as N-acetyl-cysteine, benzydamine, and pentoxifylline and treatment with natural ingredients such as aloe vera, chamomile, and honey (Ferreira et al., 2022).

Ferreira et al. (2022) found that honey effectively cures mucositis because it contains many nutrients that can reduce inflammation and has no side effects. Another natural ingredient that has anti-inflammatory properties enhances the immune response, and treats chronic inflammatory diseases is

sea cucumber (*Holothuria*). *Holothuria* contains steroids, saponins, flavonoids, vitamins, peptides, minerals, polysaccharide sulfate, 12-methyltetradecanoic acid, EPA, and DHA (Janakiram et al., 2015).

On the West Coast of Natal city, Mandailing Natal Regency, North Sumatra, there is 42% *Holothuria atra* (black sea cucumber), 36% *Holothuria leucospilota* (black long sea cucumber), and 22% *Holothuria scabra* (sand sea cucumber) (Machrizal and Khairul, 2017). The collagen content in *H. atra*, *H. leucospilota*, and *H. scabra* was 80%, 66.46%, and *H. scabra*-80-86% (Yuniati and Sulariono, 2019). *H. scabra* extract contains flavonoid compounds that can increase endurance, triterpenoids stimulate collagen formation, and collagen helps regenerate wound tissue. 30% concentration extract of *H. scabra* methanol can heal post-incision wounds on the backs of mice with wound closure for 9-10 days (Abdulkadir et al., 2021). In addition, Wargasetia et al. (2023) stated that *H. scabra* also has an anti-cancer effect, where the methanol extract of *H. scabra* reduces the survival of cancer cells in humans.

Currently, the Indonesian government focuses on natural medicines to treat diseases because using drugs derived from chemicals can cause side effects (Chandra et al., 2022). The use of natural medications reduces sufferers' side effects. The government supports government programs under the Minister of Health Regulation No.006 of 2012 concerning the Traditional Medicine Industry and Business (Minister of Health, 2012).

This study utilized marine products in North Sumatra in the form of *H. scabra* in the dosage form of drops because it has the advantage of better drug absorption to increase the drug's effectiveness (Farkouh et al., 2016). In addition to the use of *H. scabra* extract in drops, red dragon fruit peel extract (*Hylocereus polyrhizus*) was also added as a dye for drops, with the intention that patients could detect the perfection of drug administration in mucositis areas.

In addition to being a natural coloring agent, the advantages of *H. polyrhizus* peel extract include antioxidants that can enhance the immune system of children with leukemia (Noor et al., 2016). In addition, *H. polyrhizus* peel extract has potential as a cytotoxic agent against MCF-7 cells (breast cancer cells) with an IC<sub>50</sub> value of 387.49 µg/mL. The cytotoxic potential of *H. polyrhizus* skin is mediated by the ability of betasianin to inhibit the target protein IκB kinase (IKK) with an affinity of -6.15 kcal/mol so that NF-κB is activated, and MCF-7 cell proliferation can be inhibited (Sarasmita and Laksmiani, 2015).

Given the usefulness of *H. scabra* and *H. polyrhizus* peel in accordance with the purpose of this study, this study combined these two ingredients. The purpose of this study was to analyze the effectiveness of combined oral drops of *H. scabra* extract with *H. polyrhizus* peel at concentrations of 20%, 30%, and 40% to accelerate the healing of post-chemotherapy oral mucositis in Wistar rats analyzed pre-clinically and histopathologically.

## MATERIAL AND METHODS

### Chemicals and instruments

The supporting chemicals for research were ethanol 70% and distilled water (Hexacare, PT Greet Med, Indonesia), 5-fluorouracil (Kalbe Farma, Indonesia), acetic acid 50%, glycerin, citrate acid, sodium benzoate, and formalin buffer 10% (Hexacare, Greet Med, Indonesia), and hyaluronic acid 0.2% (Gengigel, Rice Farma, Italy).

The supporting instruments for research were drying cupboard (Laratorium DFL-18, Difotek, Indonesia), blender (Yasaka, National Yasaka, Indonesia), vacuum rotary evaporator (B-one, B-One Corp, Indonesia), water bath (B-one, B-One Corp, Indonesia), orbital shaker-incubator (B-one, B-One Corp Indonesia), centrifugation (Gemmy, Karya Pratama, Indonesia), microwave (Sharp, Sharp Corporation, Japan), brightfield microscope (Olympus, Olympus, Japan), mortar stamper (ONEMED, Jayamas Medica Industri, Indonesia).

### Marine and plant materials

*H. scabra* in this study was taken from the beach at the port of Sagulung, Batu Aji, Batam, Indonesia, with coordinates 1.0259° N, 103.9255° E. The identification test from the Animal Systematics Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of North Sumatra, with 179/UN5.2.1.11/KRK/2023 states that the sample used was true *H. scabra*. The special characteristics of *H. scabra* are as follows: 14 cm long; elongated cylindrical body shape; rough, soft, and wrinkled body surface; mucus; oral-aboral axis; anterior shrinks; posterior part hardens because it is filled with sand, upper body is dark brown with black pustules, and lower body is light brown.

This research used *H. polyrhizus* from a red dragon fruit plantation at Sei Sijenggi, Perbaungan, Serdang Bedagai Regency, North Sumatera, Indonesia, with coordinates 3.5533° N and 99.0226° E. The plant identification result at Plant Systematics Laboratory (Herbarium Medanense) USU stated that the

specimens for the testing were red dragon fruit (*Hylocereus polyrhizus*).

### Animals

This study was conducted in 2023 and approved by the Animal Research Ethics Committee of the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (AREC) with No. 0580/KEPH-FMIPA/20. This type of research was an *in vivo* laboratory experiment on male Wistar rats (*Rattus norvegicus*). The research design was a post-test control group conducted at the Scholar Research Laboratory and the USU FMIPA laboratory to prepare flavonoids, saponins, triterpenoids, and collagen extractions. Preparation of drops and examination of drops at the USU Pharmaceutics Pharmacy Laboratory and *in vivo* tests at the Scholar Research Laboratory. Phytochemical testing of *H. scabra* extract and dragon fruit peel was carried out at the Aspetri Laboratory, and histopathological examinations were conducted at Adam Malik Hospital Medan.

The sample size for the five test groups was determined using the Federer formula, which results in at least five rats in each group. This study increased the sample size to avoid dropping out, and 45 male Wistar rats (*Rattus norvegicus*) were used. Group 1 included rats that experienced post-chemotherapy mucositis dripped with *H. scabra* extract and *H. polyrhizus* peel at a concentration of 20%, group 2 at a concentration of 30%, group 3 at a concentration of 40%, group 4 was a positive group using hyaluronic acid 0.2% (Gengigel, Rice Farma, Italy), and group 5 was a negative group, namely rats that were not administered drugs. Each test group had nine rats; three were sacrificed for histopathological observations on day 3, three for day 7, and three for day 14.

The sampling technique used in this study was simple random sampling for each test group. The inclusion criteria of this study were male Wistar rats with a body weight of 150-200 g, aged 8-12 weeks and healthy, characterized by active movement. In contrast, the exclusion criteria were rats with a weight loss of >10% or <150 g.

### Extraction of *Holothuria scabra*

#### Preventive parading

*H. scabra* were cleaned by as much as 5 kg, cut into 1 cm cubes, dried at 40°C for 48 h in an oven (Mommert, Mommert, Germany), and mashed with a blender (Yasaka, National Yasaka, Indonesia). Next, the *H. scabra* was macerated using 96% ethanol at a ratio of 1:10 for 3 days and stirred once a day. The

macerate was filtered using Whatman paper no.1 and re-macerated with the same type and amount of solvent. After that, the filtrate was concentrated with a vacuum rotary evaporator (B-one, B-One Corp, Indonesia) at 50°C and the extract was thickened using a waterbath (B-one, B-One Corp, Indonesia) at 100°C (Abdulkadir et al., 2021; Burhan et al., 2019).

The procedure for extracting collagen from *H.scabra*: dried sample of *H. scabra* was macerated as much as 200 g in a 1:10 (w/v) ratio using a 0.1 M NaOH solution for 24 h, then homogenized in an orbital shaker incubator (B-one, B-One Corp Indonesia) at 150 rpm and 4°C for 24 h, centrifuged (Gemmy, Karya Pratama, Indonesia) at 10,000 × g and 4°C for 10 min. The finished pellet was washed with distilled water until the pH was neutral. The pellet was immersed in 0.1 M CH<sub>3</sub>COOH solution at a ratio of 1:10 (w/v), after which it was homogenized with an orbital shaker incubator (B-one, B-One Corp Indonesia) for 24 h at 4°C and 150 rpm. The filtrate was centrifuged (Gemmy, PT Karya Pratama, Indonesia) at 4°C for 10 min at 10,000 × g, and then a viscous collagen liquid was obtained (Gustini et al., 2022).

#### ***Hylocereus polyrhizus* peel extraction**

*H. polyrhizus* peel was cleaned, cut into small pieces, dried at 40°C for 48 h in a Drying cupboard (Laratorium DFL-18, PT Difotek, Indonesia), and mashed with a blender (Yasaka, National Yasaka, Indonesia). Furthermore, it was extracted using the microwave-assisted extraction (MAE) method by dissolving the dried sample in 70% ethanol 1:1, then stirring gently until the solvent and dried sample merged. The material was then placed in a microwave oven (Sharp, Sharp Corporation, Japan) at 450 watts for 8 min, filtered with Whatman paper no.1, and this process was repeated twice. Finally, the extract was thickened in a water bath (B-one, B-One Corp, Indonesia) at 100°C (Yasa et al., 2019).

#### **Phytochemical screening *Holothuria scabra* and *Hylocereus polyrhizus***

a. In the flavonoid test, a 10 g sample was added to 10 mL of hot water and boiled for 5 minutes. The solution was filtered, and 5 mL of filtrate was collected. To the filtrate, 0.1 g of magnesium powder, 1 mL of concentrated HCl, and 2 mL of amyl alcohol were added. The mixture was shaken and allowed to separate. The presence of flavonoids was confirmed by the formation of a red, yellow, or orange color.

b. In the saponin test, 0.5 g of the sample was added to 10 mL of hot water in a test tube and shaken vigorously for 10 seconds. The formation of a stable

foam, measuring 1–10 cm in size and persisting for at least 10 minutes, indicated the presence of saponins. The foam's resistance to the addition of 1 drop of 2 N HCl further confirmed the result.

c. For the triterpenoid/steroid test, 2 mg of the sample was dissolved in acetic anhydride, heated to boiling, and then cooled. Subsequently, 1 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was added to the test tube. The presence of triterpenoids or steroids was indicated by the formation of a red or purple color.

d. For the alkaloid test, 0.5 g of the dried sample was added to 1 mL of 2 N hydrochloric acid and 9 mL of distilled water, then heated in a water bath for 2 minutes. The solution was cooled and filtered, and the resulting filtrate was used for the test. Three test tubes were prepared, each containing 0.5 mL of the filtrate. To the first, 2 drops of Mayer's reagent were added; to the second, Bouchardat's reagent; and to the third, Dragendorff's reagent. The presence of alkaloids was indicated by the formation of a precipitate. If at least two of the three reagents produced a positive reaction, indicated by a white or yellow precipitate, the sample was considered to contain alkaloids.

e. For the tannin test, 1 gram of the dried sample was boiled for 3 minutes in 10 mL of distilled water, then cooled and filtered. The resulting filtrate was diluted with distilled water until it became clear or colorless. Two milliliters of the solution were taken, and 1–2 drops of 5% ferric chloride (FeCl<sub>3</sub>) were added. The presence of tannins was indicated by a color change to blue or dark green (Fajar et al., 2021)

#### **Oral drop preparation**

To create a 20% concentration of *H. scabra* extract, combined 20 mL of *H. scabra* extract with 0.2 g of citric acid as a buffer, 0.5 g of sodium benzoate as a preservative, 4 g of glycerin as a viscosity enhancer and sweetener, three drops of *H. polyrhizus* and enough distilled water to reach a total volume of 100 mL. The mixture was homogenized using a mortar stamper (ONEMED, Jayamas Medica Industri Tbk, Indonesia) (Fajar et al., 2021). In addition, 30% and 40% concentrations achieved the same result by adding three drops of *H. polyrhizus* peel extract to each concentration.

#### **Evaluation of oral drop preparations**

The drop preparation test used an organoleptic test to determine the physical conditions (clarity, odor, taste, and color). A density determination test was performed using a pycnometer (Iwaki, Indonesia) (Hasanah et al., 2020). The tool was washed with distilled water, rinsed using alcohol, dried in the oven

(Memmert, Memmert, Germany) (100°C), cooled in a desiccator, weighed the empty pycnometer (Iwaki, Indonesia), and weighed the pycnometer (Iwaki, Indonesia) containing the preparation. Next, a viscosity test was carried out with a 12 rpm, 30 rpm, and 60 rpm speed viscometer (NDJ 8s, Shanghai Martests Instrument Co., Ltd, China) (Hasanah et al., 2020). The pH was then measured using a pH meter (ATC, Digital Akurasi, Indonesia) by dipping the electrode into the drip preparation and observing the pH value obtained.

The physical stability test used the cycling test method by storing the preparation at  $4 \pm 2^\circ\text{C}$  for 24 h and then storing it at  $40 \pm 2^\circ\text{C}$  for 24 hours (Hasanah et al., 2020). The storage time at two different temperatures was considered one cycle and carried out for 12 days. The physical conditions of the drops were compared during the experiment with those of the previous preparation.

### Induction of mucositis in mice with chemotherapeutic drugs

Rats were given general anesthesia by intraperitoneal injection of ketamine HCL, using a 1 mL syringe on the lower abdomen of the rat and waiting 3-4 min until the rat was limp. The rats were then injected with 5-fluorouracil chemotherapy drugs at a dose of 60 mg/kg body weight on the first day. On the second day after the injection of the chemotherapy drugs, 50% acetic acid ( $\text{H}_3\text{COOH}$ ) was administered using Whatman paper no. 1 for 30 s to destroy the oral mucosal layer as a chemical stimulus (Thieme et al., 2020). Rats were observed for 3 days until mucositis was observed in their buccal mucosa. The dose of 5-fluorouracil was as much as 60 mg/kg bw in rats and 50% acetic acid because it had passed the previous research experiment, where the rats did not experience death but could cause mucositis.

In 5 groups of rats, twice daily drops of *H. scabra* extract and *H. polyrhizus* peel with a concentration of 20%, 30%, 40%, and as much as 2 drops were given. The positive control group was given hyaluronic acid 0.2% (Gengigel, Rice Farma, Italy) (Kunfiar et al., 2023) by two drops, and the negative group was given no drug.

### Pre-clinical and histopathologic observations

Each test group was observed daily for mucositis healing, but mucositis healing was assessed on days 3, 7, and 14 using a mucositis healing score based on Thieme et al. (2020). The same was performed for histopathological observations on days 3, 7, and 14 using Thieme et al. (2020).

Pre-clinical observations in the form of mucositis severity scores on the buccal mucosa of rats had a score value of 0-3. A score of 0 indicates normal oral mucosa, no or slight erythema and hyperemia, and no areas of bleeding, ulceration, or abscess. A score of 1 indicates mild erythema and hyperemia and no areas of bleeding, ulceration, or abscess. A score of 2 indicated severe erythema, hyperemia, bleeding areas, small ulcers, and no abscesses. A score of 3 describes severe erythema and hyperemia with bleeding, extensive ulcers, and the presence of abscesses.

After observation on days 3, 7, and 14, the rats were sacrificed for histopathological observation. Mucosal tissues were removed using a blade and observed under a 40x, 100x, and 400x magnification microscope (Olympus, Japan) with hematoxylin and eosin (HE) staining. The histopathology score has a score-0-3. A score of 0 indicated normal epithelium and conjunctive tissue, no vasolidation, no or little inflammatory infiltration, and no bleeding, ulceration, or abscess. A Score of 1 indicated mild vascular hyperemia, re-epithelialization, unclear inflammatory infiltrates, and no bleeding, ulceration, or abscess. Score 2 indicated moderate vascular hyperemia, hydropic epithelial degeneration, inflammatory infiltrates with a prevalence of neutrophils, and possible hemorrhage, edema, and sometimes ulceration without abscess. A score of 3 described severe vascular hyperemia with vasodilation, inflammatory infiltrates, and a prevalence of neutrophils, as well as large areas of bleeding, edema, ulcers, and abscesses.

### Statistical analysis

Data analysis for stabilization drops used paired t-test, Wilcoxon test (data not normally distributed), and one-way ANOVA test. Data analysis for comparison of the use of 20%, 30%, and 40% concentration drops, positive control, and negative control, based on pre-clinical observation scores and histopathology, was performed using the Kruskal-Wallis test with the Mann-Whitney post hoc test. The level of significance was set at  $p < 0.05$ .

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## RESULTS

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### Phytochemical screening

A qualitative phytochemical screening test assessed the bioactive compounds in the *H. scabra* and *H. polyrhizus* peel. Bioactive compounds were identified by examining the chemical reactions, such as color changes and the formation of precipitates in the extract after reacting with specific reagents (Table 1).

**Table 1.** Phytochemical screening test results of sand sea cucumber and red dragon fruit peel extracts.

| Type of extract           | Secondary metabolites   | Reagent                                     | Results |
|---------------------------|-------------------------|---------------------------------------------|---------|
| <i>H. scabra</i>          | Steroids- Triterpenoids | Liebermann-Bouchard                         | +       |
|                           | Alkaloids               | Dragendroff                                 | -       |
|                           |                         | Bouchardat                                  | -       |
|                           |                         | Mayer                                       | +       |
|                           |                         | Wagner                                      | +       |
|                           | Flavonoids              | Mg (s) + HCl (p)+                           | +       |
|                           | Saponin                 | distilled water                             | +       |
|                           | Tannin                  | FeCl <sub>3</sub> 1%                        | +       |
|                           | Glycosides              | H <sub>2</sub> SO <sub>4</sub> (p) + Molish | +       |
|                           | Collagen                | NaOH and CH <sub>3</sub> COOH               | +       |
| <i>H. polyrhizus</i> peel | Steroids- Triterpenoids | Liebermann-Bouchard                         | +       |
|                           | Alkaloids               | Dragendroff                                 | +       |
|                           |                         | Bouchardat                                  | -       |
|                           |                         | Mayer                                       | +       |
|                           |                         | Wagner                                      | -       |
|                           | Flavonoids              | Mg (s) + HCl (p)+                           | +       |
|                           | Saponin                 | distilled water                             | +       |
|                           | Tannin                  | FeCl <sub>3</sub> 1%                        | +       |
|                           | Glycosides              | H <sub>2</sub> SO <sub>4</sub> (p) + Molish | +       |

+ : Compound present; - : No compound.

### Organoleptic and stability test results and characteristics of oral drop preparation

The material stability test based on the organoleptic test using the 6-cycle cycling test revealed that the color, aroma, shape, and homogen before the cycling test and after the completion of the 6-cycle obtained the same results, without any visible changes in the drops. Observation of the characteristics of the drops based on pH, spreadability, viscosity, and adhesiveness resulted in significant differences before and after cycling test cycle 6 was completed in several examinations, which means that the stability of the drug needs to be improved (Table 2).

### Pre-clinical observation results

Pre-clinical observations on day 3 showed no difference in recovery from *H. scabra* and *H. polyrhizus* peel drops, positive, or negative controls, but the most severe mucositis score was found in the negative control (Table 3). On the 7th day of observation, all rats in the group given *H. scabra* and *H. polyrhizus* peel drops at a concentration of 20% had experienced healing. In contrast, the negative group had the worst

mucositis score (score 3). On day 14, all rats were treated with *H. scabra* drops, and the positive control group healed from mucositis, whereas the negative control group had not yet healed.

Significant differences existed between the test groups on the 7<sup>th</sup> and 14<sup>th</sup> days of pre-clinical observation, so the post hoc test with Mann Whitney was continued. Post hoc results obtained that differed significantly on the 7<sup>th</sup> day of observation were in the 20% vs. positive control group = 0.025\*, 20% vs. negative control = 0.025\*, 30% vs. negative = 0.034\*, 40% vs. positive = 0.025\*, 40% vs. negative = 0.025\*, positive vs. negative control = 0.025\*; for other groups, no significant differences were found. Post hoc observations on day 14 significantly differed in groups 20% vs. negative control=0.034\*, 30% vs. negative=0.034\*, 40% vs. negative=0.034\*, and positive control vs. negative=0.034\*.

The results of pre-clinical observations for 14 days on the healing of mucositis obtained the average days of mucositis healing in rats given the combination of *H. scabra* and *H. polyrhizus* peel drops at 20% concentration for 7.33 days, 30% concentration for 7.6 days, 40% concentration for 6.83 days, positive control for

**Table 2.** Results of organoleptic observations and drug characteristic tests before and after the drops stability test using the 6-cycle cycling test method.

| Observation                        | Concentration | Before            | After             | p-value             | Average | p-value             |
|------------------------------------|---------------|-------------------|-------------------|---------------------|---------|---------------------|
| <b>A. Organoleptic</b>             |               |                   |                   |                     |         |                     |
| Color                              | 20%           | Clear yellowish   | Clear yellowish   | -                   | -       | -                   |
| Aroma                              | 30%           | Distinctive smell | Distinctive smell | -                   | -       | -                   |
| Form                               | 40%           | Solution          | Solution          | -                   | -       | -                   |
| Homogeneity                        | 20%           | Homogeneous       | Homogeneous       | -                   | -       | -                   |
| <b>B. Characteristics of Drops</b> |               |                   |                   |                     |         |                     |
| <b>pH</b>                          | 20%           | 7.33              | 7.29              | 0.109 <sup>a</sup>  | 7.31    | 0.000* <sup>b</sup> |
|                                    | 30%           | 7.36              | 7.33              | 0.102 <sup>a</sup>  | 7.345   |                     |
|                                    | 40%           | 7.43              | 7.40              | 0.102 <sup>a</sup>  | 7.415   |                     |
| <b>Spreadability (cm)</b>          | 20%           | 8.4               | 7.7               | 0.094 <sup>c</sup>  | 8.05    | 0.097 <sup>b</sup>  |
|                                    | 30%           | 7.73              | 7.33              | 0.020* <sup>c</sup> | 7.53    |                     |
|                                    | 40%           | 7.23              | 6.9               | 0.010* <sup>c</sup> | 7.06    |                     |
| <b>Viscosity (cP)</b>              | 20%           | 0.82              | 0.84              | 0.001* <sup>c</sup> | 0.832   | 0.000* <sup>b</sup> |
|                                    | 30%           | 1.33              | 1.66              | 0.038* <sup>c</sup> | 1.466   |                     |
|                                    | 40%           | 1.44              | 1.63              | 0.446 <sup>c</sup>  | 1.535   |                     |
| <b>Adhesion (seconds)</b>          | 20%           | 1.176             | 1.373             | 0.001* <sup>c</sup> | 1.275   | 0.000* <sup>b</sup> |
|                                    | 30%           | 1.956             | 2.296             | 0.009* <sup>c</sup> | 2.126   |                     |
|                                    | 40%           | 2.196             | 2.713             | 0.004* <sup>c</sup> | 2.455   |                     |

<sup>a</sup>Wilcoxon; <sup>b</sup>Anova one way; <sup>c</sup>Paired t-test; \*p<0.05 statistically significant.**Table 3.** Pre-clinical observation of mucositis healing on days 3, 7, and 14 based on Thieme et al. (2020).

| Observation | Score mucositis | Concentration drops |         |         | Control      |              | p-value             |
|-------------|-----------------|---------------------|---------|---------|--------------|--------------|---------------------|
|             |                 | 20% (n)             | 30% (n) | 40% (n) | Positive (n) | Negative (n) |                     |
| Day 3       | 0               | 0                   | 0       | 0       | 0            | 0            | 0.964 <sup>a</sup>  |
|             | 1               | 0                   | 0       | 1       | 1            | 0            |                     |
|             | 2               | 2                   | 2       | 0       | 0            | 1            |                     |
|             | 3               | 1                   | 1       | 2       | 2            | 2            |                     |
| Day 7       | 0               | 3                   | 2       | 3       | 0            | 0            | 0.014*              |
|             | 1               | 0                   | 1       | 0       | 3            | 0            |                     |
|             | 2               | 0                   | 0       | 0       | 0            | 0            |                     |
|             | 3               | 0                   | 0       | 0       | 0            | 3            |                     |
| Day 14      | 0               | 3                   | 3       | 3       | 3            | 0            | 0.008* <sup>a</sup> |
|             | 1               | 0                   | 0       | 0       | 0            | 0            |                     |
|             | 2               | 0                   | 0       | 0       | 0            | 1            |                     |
|             | 3               | 0                   | 0       | 0       | 0            | 2            |                     |

<sup>a</sup>Kruskall Wallis test; \*p<0.05 statistically significant.

**Table 4.** Histopathological observation of mucositis healing on days 3, 7, and 14 based on Thieme et al. (2020).

| Observation | Mucositis score | Concentration drug drops |            |            | Control         |                 | p-value             |
|-------------|-----------------|--------------------------|------------|------------|-----------------|-----------------|---------------------|
|             |                 | 20%<br>(n)               | 30%<br>(n) | 40%<br>(n) | Positive<br>(n) | Negative<br>(n) |                     |
| Day 3       | 0               | 0                        | 0          | 0          | 0               | 0               | 0.964 <sup>a</sup>  |
|             | 1               | 0                        | 0          | 1          | 1               | 0               |                     |
|             | 2               | 2                        | 2          | 0          | 0               | 1               |                     |
|             | 3               | 1                        | 1          | 2          | 2               | 2               |                     |
| Day 7       | 0               | 3                        | 1          | 3          | 0               | 0               | 0.013* <sup>a</sup> |
|             | 1               | 0                        | 2          | 0          | 3               | 0               |                     |
|             | 2               | 0                        | 0          | 0          | 0               | 0               |                     |
|             | 3               | 0                        | 0          | 0          | 0               | 3               |                     |
| Day 14      | 0               | 3                        | 3          | 3          | 3               | 0               | 0.008* <sup>a</sup> |
|             | 1               | 0                        | 0          | 0          | 0               | 0               |                     |
|             | 2               | 0                        | 0          | 0          | 0               | 1               |                     |
|             | 3               | 0                        | 0          | 0          | 0               | 2               |                     |

\*Kruskall Wallis test; <sup>a</sup>p<0.05 statistically significant.

9.0 days and negative control found no mucositis healing until day 14 (p=0.007). Here, it can be seen that administering drops of a combination of *H. scabra* and *H. polyrhizus* peel can accelerate the healing of mucositis.

**Histopathologic observation results**

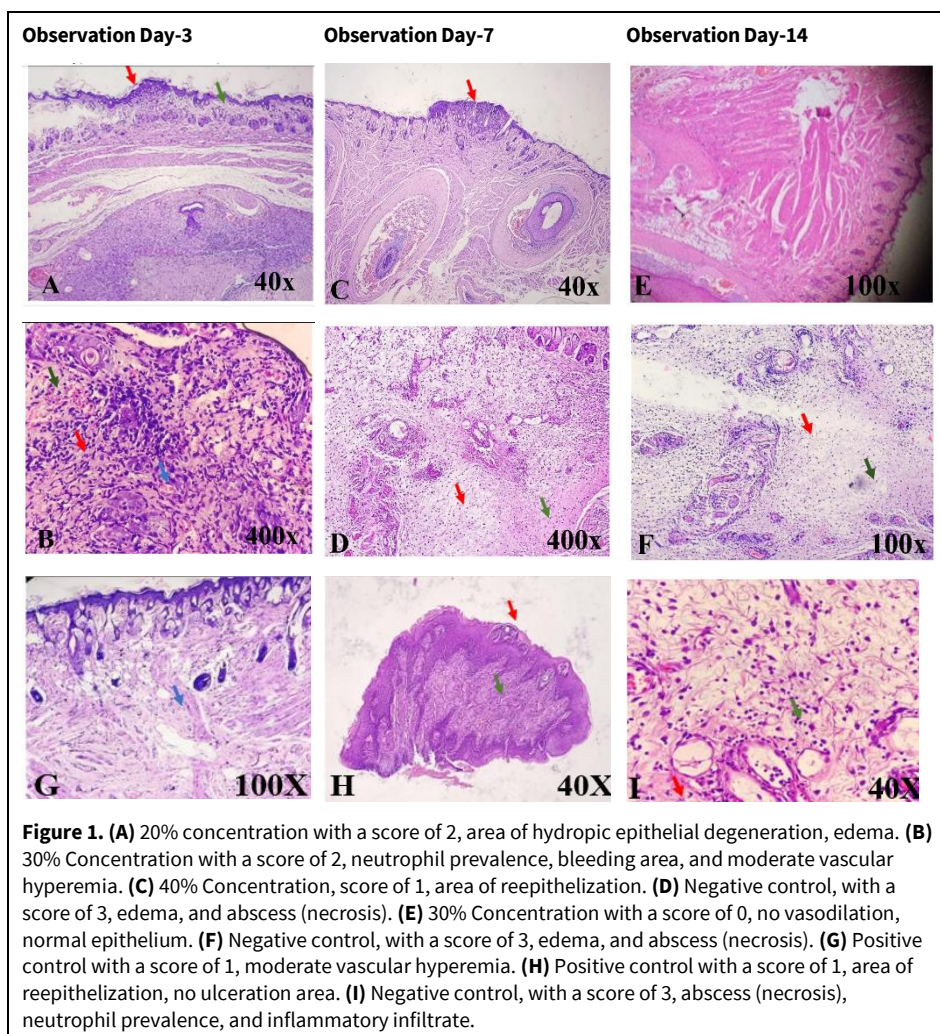
Histopathology results based on mucositis scores showed significant differences between days 7 and 14 (Table 4). All groups, except the negative group on day 14, showed healing of mucositis characterized by normal epithelium and conjunctive tissue, no vasodilation, no or little inflammatory infiltration, and no bleeding, ulceration, or abscess. In the negative group, two rats scored 3, with histopathological features of severe vascular hyperemia with validation, inflammatory infiltrates, and prevalence of neutrophils, as well as large areas of hemorrhage, edema, ulcers, and abscesses. *Post hoc* Mann-Whitney tests on histopathologic observations obtained the same results as *post hoc* pre-clinical observations. Fig. 1 shows the histopathology images obtained on days 3, 7, and 14.

**DISCUSSION**

This study also found the optimal dose of 5-fluorouracil (5-Fu) to induce mucositis in rats. The optimal dose of 5-Fu to trigger oral mucositis was 60 mg/kg and 50% acetic acid. According to various sources, the exact dose of 5-Fu to induce mucositis

remains unclear. Research conducted by Takeuchi et al. (2020) found that the optimal doses to induce mucositis were 40 mg/kg and 50 mg/kg, which caused 40% mortality in all experimental mice. Kim et al. (2023) found that the optimal dose to induce oral mucositis was 200 mg/kg body weight. The variation in the optimal dose of 5-Fu across different studies may be due to stress in the rat and their physical condition, where 5-Fu is known to trigger mucositis in the intestinal organs with severe diarrhea, leading to anorexia, dehydration, and various systemic toxicities that cause the rat to drop. This study used 50% acetic acid, according to Takeuchi et al. (2020), in which acetic acid is necessary to destroy the surface layer of the oral mucosa as a chemical stimulus, which then forms oral mucositis with 5-Fu as the inducing agent, where ulcers will form with a large area and sufficient duration to conduct further research.

The therapeutic effect of combining *H. scabra* extract and *H. polyrhizus* peel drops in healing oral mucositis is closely related to their active compounds. Flavonoids and saponins in *H. scabra* can increase growth factor levels, which help increase fibroblast proliferation and migration, thereby accelerating the wound-healing process (Abdulkadir et al., 2021). Flavonoids are antioxidants that boost immunity and protect cells from oxidative damage, which can lead to disease. Triterpenoids in *H. scabra* can stimulate collagen formation, which aids in the regeneration of wound tissue (Yuniati and Sulardiono, 2019).



Alkaloids act as antibacterial agents, and terpenoids serve as antimicrobials and antioxidants with antiangiogenic, anti-inflammatory, immunomodulatory, and antitumor properties (Janakiram et al., 2015).

*H. scabra* also contains flavonoids, saponins, triterpenoids, and tannins that act as antibacterial agents. Flavonoids can form complex bonds with the extracellular proteins of bacteria, disrupting the integrity of bacterial cell membranes. Saponins can lyse bacterial cell walls. Triterpenoids cause damage to purines, leaving bacterial cells nutrient-deficient and eventually killing the bacteria (Octiara et al., 2023). This antibacterial property is crucial in the healing process, as bacteria can cause infections that may hinder the healing of mucositis.

The findings of this study also showed that *H. polyrhizus* peel contains alkaloids, flavonoids, terpenoids, saponins, and tannins. *H. polyrhizus* peel extract produced an orange color, used as a dye in the *H. scabra* extract mouth drop medication. *H. polyrhizus* peel is a natural source of anthocyanins, grouped as flavonoid compounds (Noor et al., 2016).

Red dragon fruit (*H. polyrhizus*) has a higher antioxidant activity than white dragon fruit (*H. undatus*) (Sutisna and Humaedi, 2016). *H. polyrhizus* peel extract has higher antioxidants than its flesh, thus potentially serving as a natural antioxidant source and is considered an anti-cancer medication (Nizori et al., 2020). Several studies have reported that flavonoids, polyphenols, and betacyanins in the *Hylocereus* species have anti-cancer properties (Jayakumar and Kanthimathi, 2011; Li et al., 2013). Flavonoids bound to N-heterocyclic compounds are a group of compounds used as antioxidants, and their activity is responsible for their anti-cancer effects (Nishikito et al., 2023). Polyphenols act by binding to nitric oxide (NO) free radicals that trigger vascularization and metastasis of tumors (Padmavathy et al., 2021). Betacyanins have shown anti-proliferative effects on cancer cells, with reports indicating that they can inhibit the growth of MCF-7 breast cancer (Verrira et al., 2023).

The stability of the drops was tested using a 6-cycle cycling test. In the spreading test, all three concentrations of the combined extract drops of *H. scabra*

and *H. polyrhizus* peel tended to decrease. The spreading power of concentrations 20%, 30%, and 40% before the cycling test was 8.44, 7.73, and 7.23 cm<sup>2</sup> and after the cycling test, it was 7.7, 7.33, 6.9, respectively, indicating that the lower the concentration of the drops, the wider the spreading power and the better the coverage of the medication distribution. This may be due to the decrease in spreading power being inversely proportional to the viscosity value of each formula, where the viscosity of each formula also increased after the cycling test (Zam and Musdalifah, 2022).

In the adhesion test, an increase in the value was found in all formulas of the drops after the cycling test, where the average of each formula was 1.27-2.45 seconds. However, the adhesion power of these drops was less than 4 min, where a good adhesion power of the medication should not be less than 4 s; the higher the adhesion power of the medication, the higher the possible absorption (Yati et al., 2018). Further research is required to obtain stable drops with good medication characteristics.

The healing of mucositis in rats after administration of the combined extract drops of *H. scabra* and *H. polyrhizus* peel was observed based on the decrease in the severity score of mucositis. Pre-clinical and histopathological observations in all test groups showed no significant difference in mucositis scores among all test groups on day 3. However, the mucositis scores of *H. scabra* and *H. polyrhizus* peel drops were better than those of the negative control, especially at 20% and 30% concentrations. This was evident from the decrease in mucositis score with clinical signs of reduced inflammation in mucositis. This is because *H. scabra* contains anti-inflammatory components such as saponins, flavonoids, and steroids (Burhan et al., 2019). The mechanism of action of saponins as anti-inflammatory agents is to decrease cyclooxygenase-2 activity, which plays a role in stimulating inflammatory mediators. The ability of these compounds to act as anti-inflammatory agents inhibits the activity of enzymes, such as cyclooxygenase, in converting arachidonic acid into prostaglandins as inflammatory mediators. Flavonoids act as anti-inflammatory agents by inhibiting the activity of phospholipase A2, cyclooxygenases, and lipoxygenases (Alqahtani and Khan, 2022; Burhan et al., 2019).

On day 7, a significant difference in mucositis scores was observed between *H. scabra* and *H. polyrhizus* peel drop groups and the control group compared to the negative control group. Based on pre-clinical and histopathological observations, even *H. scabra* drops at concentrations of 20% and 40% had better mucositis healing than the positive control group. All rats administered *H. scabra* drops at con-

centrations of 20% and 40% had mucositis scores of 0, indicating that they had healed.

On day 14, all concentrations of *H. scabra* and *H. polyrhizus* peel drops, and the positive control showed mucositis healing in all rats. In contrast, the negative control still had a mucositis score of 3. Pre-clinical observation results for each mouse group showed that, on average, the days for mucositis healing in the 20% concentration group were 7.33 days, 30% for 7.6 days, 40% for 6.83 days, and the positive control group for an average of 9 days, with no mucositis healing in the negative control group until day 14. Here, it was observed that *H. scabra* and *H. polyrhizus* peel drops could accelerate mucositis healing in rat post-chemotherapy, compared to the negative control group without medication, which might heal on its own within 2-4 weeks after chemotherapy (Alqahtani and Khan, 2022).

The components in *H. scabra* that help accelerate the mucositis healing process include collagen, the most abundant protein in *H. scabra*. Collagen is synthesized by cells such as fibroblasts, and the most abundant type of collagen found in *H. scabra* is type I collagen (predominantly found in the skin). Fibrillar collagen and glycosaminoglycans (GAG) in *H. scabra* release hyaluronic acid, which plays a role in accelerating the re-epithelialization of cells, thus aiding in skin regeneration and wound healing (Alpayer et al., 2023; Senadheera et al., 2020; Wargasetia et al., 2023). Additionally, collagen in *H. scabra* is an antioxidant associated with the capacity to clear free radicals (Wargasetia et al., 2023).

The formulation of combined extract drops of *H. scabra* and *H. polyrhizus* peel can be an effective alternative for treating oral mucositis, especially post-chemotherapy, but further clinical research is necessary. Children with leukemia post-chemotherapy can be administered these drops to address mucositis quickly. Mucositis can cause pain in children, difficulty speaking, dysphagia, loss of taste sensation, changes in nutritional status, and risk of infection, all of which can decrease the quality of life of the child (Novrianda and Arif, 2017).

Some future studies should be performed to determine the quantitative detection of secondary metabolites and collagen in these species and formulations.

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## CONCLUSION

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The combination drops of *H. scabra* extract and *H. polyrhizus* peel at concentrations of 20%, 30%, and 40% accelerated the healing of oral mucositis induced by chemotherapy drugs. However, observations on the seventh day showed that the drops at 20% and 40%

concentrations had the same effectiveness in accelerating the healing of oral mucositis and achieved better results than the positive control. Drops at a 20% concentration are recommended to use raw materials efficiently.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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## AUTHOR CONTRIBUTION:

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|------------------------------------|-----------|-----------|-------------|-----------|---------|---------|-------------|
| Concepts or ideas                  | x         |           |             |           |         |         |             |
| Design                             | x         |           |             |           |         |         |             |
| Definition of intellectual content | x         | x         |             |           |         |         |             |
| Literature search                  | x         |           | x           | x         | x       | x       | x           |
| Experimental studies               | x         | x         | x           | x         | x       | x       | x           |
| Data acquisition                   | x         |           | x           |           | x       | x       | x           |
| Data analysis                      | x         |           |             | x         |         |         |             |
| Statistical analysis               |           | x         |             |           |         |         |             |
| Manuscript preparation             | x         | x         | x           | x         | x       | x       | x           |
| Manuscript editing                 | x         |           | x           |           |         |         |             |
| Manuscript review                  | x         | x         | x           | x         | x       | x       | x           |

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