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Total Flavonoid Content and Wound-Healing Activity of *Portulaca Grandiflora* Extract Fractions in Rabbit Skin

Maria Fatmadewi Imawati[1], Antonius Budiawan[1*], Levi Puradewa[1], Bida Cincin Kirana[1], and Agus Purwanto[2]

[1]*Department of Pharmacy Diploma-III, Pharmacy Faculty, Widya Mandala Surabaya Catholic University, Madiun City 63131, Indonesia*
[2]*Department of Biology, Agricultural Technology Faculty, Widya Mandala Surabaya Catholic University, Madiun City 63131, Indonesia*
*Corresponding author's Email: antonius.budiawan@ukwms.ac.id

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ABSTRACT

Portulaca grandiflora extract demonstrated wound-healing activity and contains high levels of flavonoids. However, the specific secondary metabolites of *Portulaca grandiflora* herb extract that are responsible for the wound-healing mechanism of action remain unclear. The present study aimed to evaluate the wound-healing effect of the total flavonoid content of purslane (*Portulaca grandiflora*) extract fractions on rabbit skin. The wound-healing activity was assessed in a New Zealand white rabbit model. The total flavonoid content of aqueous, ethyl acetate, and n-hexane fractions of *Portulaca grandiflora* was quantified using an aluminum chloride ($AlCl_3$) colorimetry method, and the effects of these fractions were compared to the positive control group (betadine solution) and the negative control group (distilled water). Among the fractions tested, the ethyl acetate fraction of *Portulaca grandiflora* exhibited the highest total flavonoid content at 4.7153% ± 0.0012% Weight/Weight (W/W) quercetin equivalent (QE), followed by the n-hexane and aqueous fractions at 2.0507% ± 0.0050% W/W (QE) and 1.9693% ± 0.0012% W/W (QE), respectively. All three fractions, including the aqueous, ethyl acetate, and n-hexane, demonstrated wound-healing activity in rabbit skin, which were significantly different from the negative control group on day 11. The aqueous fraction indicated the highest wound-healing effect, similar to the positive control group. The aqueous fraction and the positive control group were significantly different from the negative control group on day 9. The present findings indicated an inverse correlation between the total flavonoid content and the wound healing activity test. The aqueous fraction, which represented the lowest total flavonoid content, revealed the highest wound healing activity.

Keywords: Flavonoid, Fractionation, *Portulaca grandiflora*, Rabbit, Wound-healing

INTRODUCTION

Wounds represent a significant clinical challenge in daily life. Delayed and inadequate wound management can lead to adverse health outcomes, including increased morbidity and mortality, particularly in underdeveloped countries where healthcare infrastructure is limited (Tottoli et al., 2020). Recent epidemiological evidence indicated that chronic wounds are becoming more common in high-income countries, up to 2.5% of the population (Sen, 2021), and particularly in the United States, up to approximately 10.5 million people. Additionally, the financial impact of wound care is significant. For instance, the US Medicare system invested billions of dollars in healthcare resources recently (Sen, 2025).

A wound is a specific type of injury that primarily affects the skin, which is the outermost layer of the human body. Wounds can potentially cause significant discomfort and subsequent skin diseases when complicated by bacterial infection. Skin infection can extend the concurrent phases of wound healing, including homeostasis, inflammation, proliferation, and remodeling (Low et al., 2021). There are two classifications of wounds, such as acute and chronic. Burns, trauma-related injuries, and surgical wounds, which typically heal in 30 days, are classified as acute wounds. Chronic wounds heal in a slow progression, and the delayed healing process is mostly caused by a combination of internal and external factors. The wound itself could be persistent, and the skin inflammation prolonged over three months (Alemu et al., 2020).

As a conventional approach, wound management involves the administration of antibiotics and antiseptics to mitigate the risk of bacterial infection. Antibiotics exert their bactericidal effects through different mechanisms, including inhibiting DNA synthesis, disrupting cellular membrane formation, altering cell membranes, inhibiting protein translation, and inhibiting metabolite degradation. However, the overuse of antibiotics has become a growing concern due to the development of bacterial resistance (Reygaert, 2018). Povidone iodine is the predominant antiseptic therapeutic agent in wound care. Povidone iodine is a compound comprising water and iodine (Lepelletier et al., 2020). Povidone iodine waste itself contaminates water and disturbs the aquatic ecosystem (Cao et al., 2021). Despite its widespread use as a wound antiseptic, povidone-iodine is associated with certain toxicities when applied to large wound areas. The excess iodine can penetrate into the systemic circulation and affect thyroid function (Pozniak et al., 2025).

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Purslane (*Portulaca grandiflora*), a highly popular ornamental plant, is characterized by its rose-like flower variation. The *Portulaca grandiflora* cultivar exhibits vibrant blossoms, is highly versatile for planting, and is flexible in different weather conditions. *Portulaca grandiflora* is widespread in tropical areas. In China, *Portulaca grandiflora* is consumed for its believed health benefits; its use as a medicinal herb in Indonesia remains limited. *Portulaca grandiflora* has been studied for its anti-inflammation, antibacterial, and wound healing effects (Purwanto, 2021; Budiawan et al., 2023). *Portulaca grandiflora* has been reported to contain different secondary metabolites, such as flavonoids, terpenoids, tannins, and saponins (Imawati et al., 2023). Flavonoids, in particular, are known for their antioxidant and antibacterial effects, which are important in tissue repair processes. Flavonoids improve the activity of key antioxidant enzymes, specifically superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). The antioxidant enzymes activity improvement helps neutralize reactive oxygen species (ROS) and reduces oxidative damage (Carvalho et al., 2021). Recent evidence supported the therapeutic potential of *Portulaca grandiflora*, which has demonstrated wound healing activity and a high flavonoid content (Budiawan et al., 2023). Therefore, *Portulaca grandiflora* is a promising natural herb for wound treatment. However, the specific secondary metabolites primarily responsible for its wound-healing mechanism of action remain unclear. Different types of flavonoids extracted using suitable solvent fractions may use several mechanisms of action in wound healing (Carvalho et al., 2021). Exploring such plant-derived alternatives may offer insights into complementary agents such as antibiotics and iodine. The present study aimed to investigate the total flavonoid content and the wound-healing properties of *Portulaca grandiflora* extract fractions on rabbit skin.

MATERIALS AND METHODS

Ethical approval

The present study was ethically approved by the Health Research Ethics Committee STRADA Health Institute of Indonesia (000265/EC/KEPK/I/07/2023).

Materials and plant

The present study used separating funnel, oven (Memmert UN 30, Germany), analytical balance (B-One AB-220, China), thin layer chromatograph (TLC) 10 × 10 cm, UV viewing cabinet (UvOC-02, Indonesia), glassware (Pirex, Indonesia), filter holder, disposable syringe 1 cc and 10 cc (OneMed, Indonesia), micropipette, spectrophotometer UV-Vis (Jasco V-730, Japan), anatomical forceps, surgical scissors (OneMed, Indonesia), biopsy punch 8 mm in diameter (HBMR), vernier caliper, vacuum rotary evaporator (Biobase, China), ethanol 96% (MRI, Indonesia), distilled water (Brataco, Indonesia), ethyl acetate (Indo Acidatama, Indonesia), n-hexane (Jayarindo Pratama Laboratory, Indonesia), chloroform (technical grade; Jayarindo Pratama Laboratory, Indonesia), glacial acetic acid (technical grade; Celanese, Singapura), quercetin standard (Sigma-Aldrich, Germany), methanol (Merck, Germany), aluminum chloride ($AlCl_3$; Merck, Germany), sodium acetate 1M (pro analysis grade; Smart-Lab, Indonesia), magnesium powder (technical grade; Merck, Germany), silica gel GF254 (Himedia Lab, India), Whatman no. 42 filter papers (Whatman, China), lidocaine injection (Phapros, Indonesia), betadine solution (MBF, Indonesia), and rabbit food pellets (Vital Rabits, Indonesia). Male New Zealand white rabbits (*Oryctolagus cuniculus*), aged 3-5 months, weighing 1.5-2.5 kg, were obtained from a rabbit farm, Magetan City, East Java, Indonesia.

Purslane herbs (*Portulaca grandiflora*) were obtained from Madiun City, East Java, Indonesia (Figure 1). The aerial parts of *Portulaca grandiflora* herbs were harvested during the full bloom stage in the morning. *Portulaca grandiflora* was authenticated at the Biology Department Widya Mandala Surabaya Catholic University, Indonesia. The sample voucher with reference number BIO025 was deposited in the institutional repository.

Figure 1. Purslane herbs (*Portulaca grandiflora*)

Extraction and fractionation

Dried, ground aerial herb parts from *Portulaca grandiflora* were used for maceration. The plant powder, weighing 200 g, was soaked in 500 mL of 96% ethanol in a container kept protected from light at 25˚C. After an initial five-day period, the mixture was filtered through filter paper. The second maceration was performed on the remaining solid residue with an additional 500 mL of 96% ethanol for another five days under similar conditions. Both macerates were

mixed and concentrated using a vacuum rotary evaporator at 45˚C until the volume was reduced to 290 mL. The concentrated extract was then oven-dried at 50˚C for 24 hours, resulting in a thick extract with a yield of 12.99%. The maceration method was in accordance with Kinasih and Indriasari (2024), with modifications in the maceration period.

Two grams of the concentrated extract were dissolved in distilled water at 40°C. The solution was transferred to a separating funnel and partitioned with 100 mL of n-hexane. The mixture was stirred manually for 10 minutes and then allowed to stand for complete phase separation for 30 minutes. The n-hexane phase layer was collected into a beaker. One hundred mL of ethyl acetate was added to the remaining distilled water phase, stirred manually for 10 minutes, and allowed to separate into two phases for 30 minutes. The ethyl acetate phase layer was then collected in a beaker. All resulting fractions were evaporated in an oven for 24 hours at 50˚C (Rashmi et al., 2023). For the wound-healing activity test, all fractions were dissolved in distilled water at a 10% of concentration.

Qualitative flavonoids test

Each fraction of *Portulaca grandiflora* with a concentration of 2000 ppm was applied at 1 µL onto a silica gel GF_{254} plate and allowed to dry. The plate was then placed in a chromatography chamber previously saturated with the mobile phase solvent system for 30 minutes. The mobile phase used in the present study was methanol, chloroform, and glacial acetic acid (9:1:0.5 ratio). After development of the mobile phase migration to the front distance of 8 cm, the plate was sprayed with a 10% $AlCl_3$ in ethanol reagent and dried using a hair dryer to visualize flavonoid compound spots. Flavonoid compounds were identified by bright yellow spots under UV light at 366 nm (Zych and Pyka-Pająk, 2025).

Quantitative total flavonoids content test

The total flavonoid content of the aqueous, ethyl acetate, and n-hexane fractions of *Portulaca grandiflora* was quantified using the $AlCl_3$ colorimetric method based on Sulastri et al. (2018) with minor modifications (1M sodium acetate was used in the present experiment instead of potassium acetate).

Blank solution preparation

A blank control was prepared by combining 1.5 mL of methanol, 0.1 mL of 1M sodium acetate, 0.1 mL of 10% $AlCl_3$, and 2.8 mL of distilled water. The mixture was allowed to react at room temperature for 30 minutes. The baseline absorbance was determined using a UV-Vis spectrophotometer at a wavelength of 424.6 nm.

Quercetin standard solution preparation

The quercetin standard solution was prepared by dissolving 50 mg of quercetin in 25 mL of ethanol (p.a.). The initial solution was subsequently diluted to prepare standard solutions with concentrations of 10, 15, 20, 25, 30, and 35 ppm. Two mL of each standard solution was pipetted into a test tube, followed by adding 0.1 mL of 1 M sodium acetate, 0.1 mL of 10% $AlCl_3$, and 2.8 mL of distilled water. The mixtures were vortexed, followed by a 30-minute incubation period at room temperature. The absorbance was quantified using a UV-Vis spectrophotometer at a wavelength of 424.6 nm. The method was validated, yielding a regression of $y=0.0257x-0.0084$ ($R^2=0.9982$), with a limit of detection (LOD) of 1.55 ppm and a limit of quantitation (LOQ) of 4.71 ppm.

Sample solution preparation

Each fraction sample (aqueous, ethyl acetate, and n-hexane) was weighed at 50 mg and dissolved in 5 mL of 96% ethanol. The solution was transferred into a 10 mL volumetric flask and diluted with 96% ethanol. The resulting solution underwent filtration using Whatman No. 42 filter paper. From the filtrate, 1 mL was pipetted and transferred into another 10 mL volumetric flask. The volumetric flask was then filled with 96% ethanol to obtain a volume of 10 mL.

Total flavonoid content analysis

The $AlCl_3$ colorimetric method was used to determine the total flavonoid content of the aqueous, ethyl acetate, and n-hexane fractions of *Portulaca grandiflora*. The absorbance was measured using UV-Vis spectrophotometry. A half mL of each *Portulaca grandiflora* fraction sample solution was mixed with 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M sodium acetate, 1.5 mL of 96% ethanol, and 2.8 mL of distilled water to reach a total volume of 5 mL. The mixture was incubated at room temperature for 30 minutes, and the absorbance was recorded at a wavelength of 424.6 nm. The measurements were performed in triplicate, and the total flavonoid content of all *Portulaca grandiflora* fractions was calculated using the following formula.

$$F = (c \times V \times f \times 10^{-6})/m \times 100\% \quad \text{(Formula 1)}$$

F is the total flavonoid content (Weight/Weight [W/W]; quercetin equivalent [QE]), c stands for Quercetin equality (g/mL), V means the sample solution volume (0.5 mL), f is the dilution factor, and m is the fraction weight (0.05 g)

Wound healing activity test

Three New Zealand rabbits were used in the wound-healing activity assessment. The rabbits' dorsal hair was removed, and local anesthesia of 2% lidocaine injection was administered subcutaneously at a dose of 1 mg/kg of body

weight (Flecknell, 2016). Wound excision was performed using an 8 mm diameter biopsy punch. A total of six wounds were created on the dorsal region of each rabbit. The wounds were assigned to the treatments, and one wound remained untreated as a visual baseline to the natural wound healing process (Budiawan et al., 2023). The wounds were assigned to five treatment groups with three wounds in each group using a randomized block design. The treatment groups included the positive control (Betadine solution), the negative control (distilled water), the aqueous fraction of *Portulaca grandiflora*, the ethyl acetate fraction of *Portulaca grandiflora*, and the n-hexane fraction of *Portulaca grandiflora*. The treatments were applied twice a day (every 12 hours) for 14 consecutive days. The wounds were cleaned with distilled water and dried before the application. Wound contractions of each group were measured and documented visually on days 0, 3, 7, 9, 11, and 14 to monitor the healing process. The wound contraction percentage was calculated using the following formula (Mekonnen et al., 2013; Hammeso et al., 2025).

Percentage of wound contraction (%) = (Wound diameter on day 0 – Wound diameter on day n) / Wound diameter on day 0 × 100% (Formula 2)

n is the number of days when the wound was observed.

Statistical analysis

The total flavonoid content of distilled water, ethyl acetate, and n-hexane fractions of *Portulaca grandiflora* extract and wound-healing activity measurement data were subjected to statistical analyses using IBM SPSS Statistics version 22. Data normality was analyzed using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) followed by the Tukey post-hoc test was used for statistical evaluation for parametric data. The confidence level of the data analysis was 95% ($p < 0.05$). For non-parametric data, the Kruskal-Wallis test was used.

RESULTS AND DISCUSSION

Extraction and fractionation

The extraction of *Portulaca grandiflora* was 12.99% W/W. Fractionation of the herb extract aimed to separate phytochemicals based on their polarity. The polarity index of the distilled water, ethyl acetate, and n-hexane was 10.2 points, 4.4 points, and 0.1 points, respectively. The highest fraction yield was obtained from the ethyl acetate fraction with 3.615% W/W (Table 1). The ethyl acetate fraction yield could be attributed to its intermediate polarity index, which aligns with the solubility of the *Portulaca grandiflora* extract phytochemical content (Rodríguez De Luna et al., 2020).

Table 1. *Portulaca grandiflora* extract fraction yield through fractionation for a 14-day wound-healing activity test in New Zealand rabbits

Extract weight (g)	Fraction solvent	Fraction weight (g)	Yield (% W/W)
2.00	Aqueous	0.0547	2.735
	Ethyl acetate	0.0723	3.615
	N-hexane	0.0501	2.505

% Weight/Weight (W/W): Calculated as fraction weight (g) / extract weight (g) × 100. All fractions were evaluated for *in vivo* wound healing activity.

Qualitative flavonoids test

Based on the qualitative TLC flavonoids analysis, the ethyl acetate fraction of *Portulaca grandiflora* indicated equivalent spot and Rf point with quercetin standard of 0.775. The aqueous and n-hexane fractions did not indicate the same result. The qualitative TLC flavonoids result of the ethyl acetate fraction aligned with the findings of Anghel et al. (2013), who indicated that *Portulaca grandiflora* contains quercetin and isoquercetin. Quercetin and isoquercetin are flavonols, which are dissolved in semipolar solvents such as ethyl acetate (Rodríguez De Luna et al., 2020). *Portulaca grandiflora* has been found to contain rutin, a flavonoid glycoside that exhibits solubility in a polar solvent (Anghel et al., 2013; Frutos et al., 2019). The TLC flavonoids analysis explained the differences in the aqueous fraction resulting from the quercetin standard spot and Rf.

Quantitative total flavonoids content test

The standard absorbance measurement had the regression equation of $y = 0.0257x - 0.0084$ with a correlation of $R^2 = 0.9982$. The highest total flavonoid content estimation of the ethyl acetate fraction was 4.7153% ± 0.0012% W/W (QE). The n-hexane and aqueous fractions indicated 2.0507% ± 0.0050% W/W (QE) and 1.9693% ± 0.0012% W/W (QE), respectively (Figure 2).

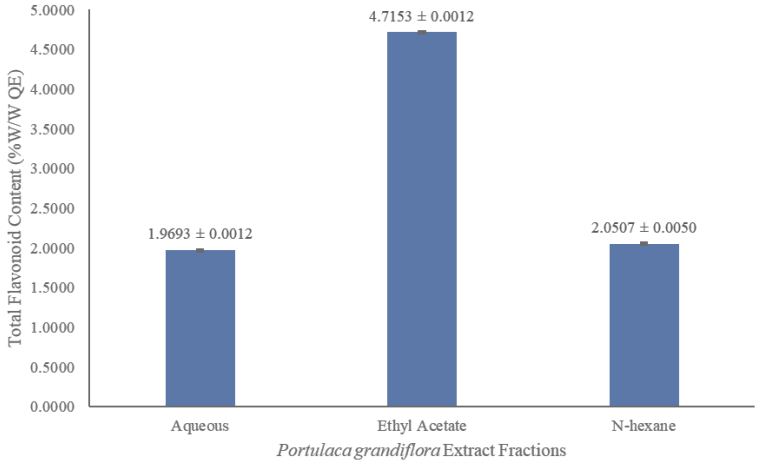

Figure 2. Total flavonoid content of different *Portulaca grandiflora* extract fractions used in a 14-day wound healing activity test on New Zealand rabbits. Data is presented as mean ± SD from triplicate measurements (n = 3).

Wound healing activity

The width of the wound exhibited a decreasing trend as time progressed. The wound visualization was different among groups on day 9 (Figure 3). The wounds treated with distilled water indicated the lowest wound contraction with 62.78 ± 10.20% on that day. Meanwhile, the wounds treated with betadine solution and *Portulaca grandiflora* fractions indicated a higher wound-healing percentage. The highest wound contraction at 97.02 ± 4.21% was indicated by wounds treated with the aqueous fraction of *Portulaca grandiflora* (Table 2).

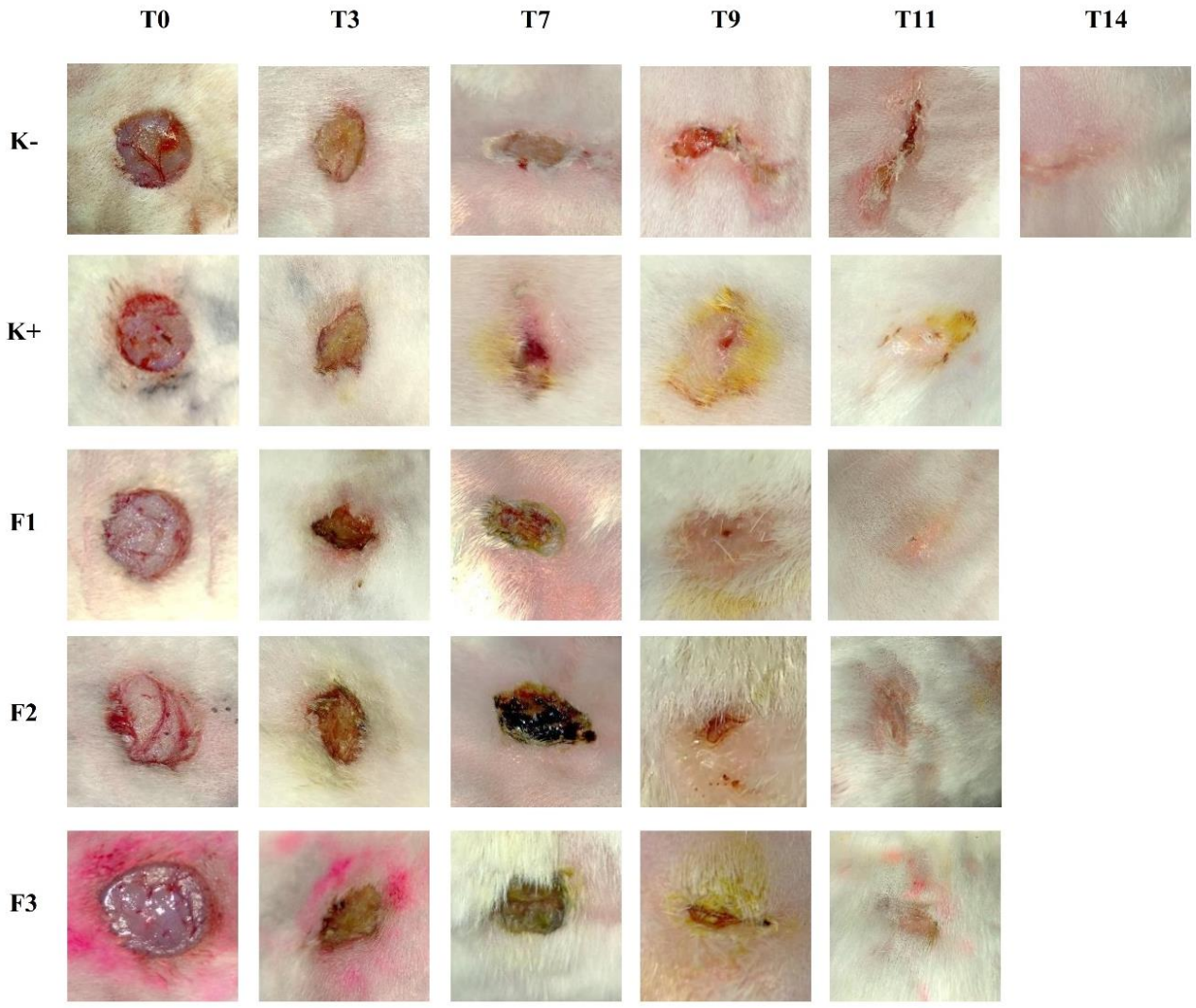

Figure 3. Wound healing progress in New Zealand rabbit skin over a 14-day treatment period. **K-**: Negative control group, **K+**: Positive control group, **F1**: Aqueous fraction of purslane herbs, **F2**: Ethyl acetate fraction of purslane herbs, **F3**: The n-hexane fraction of purslane herbs on days 0 (**T0**), 3 (**T3**), 7 (**T7**), 9 (**T9**), 11 (**T11**), and 14 (**T14**).

Table 2. Wound contraction percentage in New Zealand rabbits treated with *Portulaca grandiflora* fractions over 14-days

Groups	T0	T3	T7	T9	T11	T14
K-	0.00 ± 0.00	35.33 ± 14.00	55.16 ± 9.08	62.78 ± 10.20	81.10 ± 26.73	100.00 ± 0.00
K+	0.00 ± 0.00	38.23 ± 13.18	37.96 ± 26.80	96.08 ± 5.54*	100.00 ± 0.00*	-
F1	0.00 ± 0.00	41.09 ± 6.11	47.85 ± 6.06	97.02 ± 4.21*	100.00 ± 0.00*	-
F2	0.00 ± 0.00	35.39 ± 1.02	51.53 ± 15.13	83.45 ± 13.95	100.00 ± 0.00*	-
F3	0.00 ± 0.00	28.94 ± 5.07	42.31 ± 18.12	82.39 ± 2.80	100.00 ± 0.00*	-

K-: Negative control group (distilled water), K+: Positive control group (betadine solution), F1: Aqueous fraction of *Portulaca grandiflora*, F2: Ethyl acetate fraction of *Portulaca grandiflora*, F3: The n-hexane fraction of *Portulaca grandiflora*. *Significantly different ($p < 0.05$) in comparison of treatments and the negative control group.

The positive control and the aqueous fraction groups demonstrated significantly greater wound contraction compared to the negative control group by day 9 ($p < 0.05$). The aqueous fraction performed wound healing activity similar to the positive control group. Consistent with findings of Budiawan et al. (2023), who studied male New Zealand rabbit skin, all wounds in groups besides the negative control group were fully closed by day 11. Wounds in the negative control group completely healed on day 14.

The present study employed rabbits as experimental subjects to induce wounds. Naturally, wound-healing in rabbits involves four overlapping stages, including hemostasis, inflammation, proliferation, and remodeling, typically within 13-16 days (Grada et al., 2018). The wound-healing process in rats is similar to the human skin process, where blood flows to the wound site in the homeostasis phase. The vascular flow brings peripheral blood cells and released plasma to the injured skin tissue. Bacteria can enter the wound site in the homeostasis phase through water contamination, direct contact, and from the physiological flora of the skin. Initially, bacteria may enter the wound site without eliciting a response from the defense system of the skin. Bacterial proliferation can lead to critical colonization, delaying wound healing, particularly when bacterial loads reach infection levels (Zegadło et al., 2023). Antiseptics, antibiotics, or proper dressing are some methods to treat wound infection, each with its specific mechanism of action (Deng et al., 2022). Distilled water has no pharmacological mechanism to treat wounds; it cannot prevent wound infection, which prolongs the wound healing process (Velovexia et al., 2025).

Betadine solution, as the positive control, contains 10% povidone-iodine as an active compound. As an iodophor, povidone-iodine can penetrate bacterial cells and eliminate them by oxidizing nucleotides, fatty acids, and key proteins (Lepelletier et al., 2020). The physiological mechanism of povidone-iodine regulates and diminishes bacterial presence at the wound site, thereby reducing the extended inflammatory phase of wound healing. The wounds treated with the aqueous fraction of *Portulaca grandiflora* indicated greater wound healing activity than ethyl acetate and n-hexane fractions. On day 9, the average wound contraction in rabbits treated with the aqueous fraction of *Portulaca grandiflora* was not considerably different from the wound contraction in rabbits treated with betadine solution. The wound contraction in the positive control and *Portulaca grandiflora* aqueous fraction groups notably differed from the distilled water-treated group. On day 11, the wounds were closed in rabbits treated with the *Portulaca grandiflora* three fractions and the positive control group, indicating remarkable differences with the negative control group. The wound healing result might be associated with the compounds in each *Portulaca grandiflora* herb fraction.

Based on the wound healing activity of *Portulaca grandiflora* fractions, the present findings revealed an inverse relationship between total flavonoid content and pharmacological effect. Notably, the aqueous fraction, which contained the lowest flavonoid content, exhibited the highest wound healing activity. The wound healing observation posed a notable challenge to the initial hypothesis that flavonoids are the primary bioactive compounds of *Portulaca grandiflora*, responsible for the healing process. *Portulaca grandiflora* contains different flavonoids such as rutin, quercetin, and isoquercetin (Husein et al., 2021). Rutin is a flavonoid glycoside known for its partial solubility in water (Pedriali et al., 2008). The inverse relationship between the total flavonoid content and the wound healing findings indicated that polar non-flavonoid metabolites or flavonoid glycosides, such as rutin, may play an important role in wound healing. Rutin has been previously studied for its role in wound healing. It has been indicated that rutin mainly promotes cell migration, collagen fiber proliferation, and inactivates inflammatory responses, thereby shortening the wound healing process (Chen et al., 2020). Aqueous fractionation can extract polar compounds such as flavonoids, tannins, and saponins. The polar compounds (flavonoids, tannins, and saponins) are widely recognized for their antibacterial, antioxidant, and anti-inflammatory activities (Irfan et al., 2022), which are relevant to wound healing acceleration. Tannin is widely known as an antibacterial that can penetrate the bacterial cell membrane wall and interfere with cell metabolism, leading to

bacterial death (Kaczmarek, 2020). Similar to flavonoids, tannin may scavenge free radicals in oxidative stress conditions that disturb cell formation in the wound healing process (Asumang et al., 2021; González et al., 2022). Furthermore, tannins contribute to wound healing through their ability to facilitate the adhesion of injured tissue (Santana et al., 2017; Sharma et al., 2022). Samavati et al. (2025) reported that *in vivo* pre-treatment of tannin-rich Jaft extract increased wound closure and tissue regeneration. Saponins participate in the wound healing process by enhancing endothelial cell proliferation and increasing collagen production (Lei et al., 2022; MacKay and Miller, 2003). Collagen production strengthens the new skin tissue binding in the wound healing process (Mathew-Steiner et al., 2021). Saponin has been observed to enhance the permeability of bacterial cell membranes, leading to bacterial eradication (Wei et al., 2021). Based on the findings of Xu et al. (2026), total saponins of the *Rhizoma Panacis majoris* enhanced the wound healing process in diabetic rats within 14 days.

Ethyl acetate and n-hexane fractions of *Portulaca grandiflora* indicated lower wound healing activity than the distilled water fraction and the positive control group. The lower wound healing activity of ethyl acetate and n-hexane fractions might be attributed to the differences in secondary metabolites within each fraction. The aqueous fraction of *Portulaca grandiflora* contains rutin, tannins, and saponins, which synergistically effect on wound healing process. Quercetin and isoquercetin, which are found in purslane herbs, belong to the class of flavonol compounds that exhibit solubility in a semipolar solvent, such as ethyl acetate (Rodríguez De Luna et al., 2020). The total flavonoid content assay using a quercetin standard supported the flavonol solubility in a semipolar solvent assertion. The quantitative analysis results revealed that the ethyl acetate fraction attained the highest total flavonoid concentration among the aqueous and n-hexane fractions. Although rutin has partial solubility in water, the concentration of flavonoids in the ethyl acetate fraction was likely due to the presence of semi-polar flavonoids such as quercetin (Rodríguez De Luna et al., 2020). Quercetin acts as an antioxidant and can suppress the ROS overproduction by scavenging them (Lesjak et al., 2018). The ROS are metabolic byproducts, which are essential for cellular defense against bacterial invasion, inflammation mediation, and cell signaling during the wound healing process. However, ROS overproduction in oxidative stress would be followed by excessive production of hydrogen peroxide (Collin, 2019), leading to cellular damage through protein, DNA modification, and lipid peroxidation (Fitzmaurice et al., 2011). Furthermore, quercetin caused a reduction in wound healing duration by stimulating epithelial cell proliferation, activating extracellular remodeling, and facilitating collagen cross-linking (Irfan et al., 2022).

Terpenoids contained in *Portulaca grandiflora* have high solubility in n-hexane, a nonpolar solvent (Jiang et al., 2016). The total flavonoid content analysis indicated that the n-hexane fraction had a higher content than the distilled water fraction. The higher flavonoid content level may be caused by quercetin, which is insoluble in water, semipolar, and partially dissolved in nonpolar solvents. Wounds treated with the n-hexane fraction indicated the lowest activity, which might be due to the secondary metabolites, where only terpenoids and quercetin play wound-healing roles. Terpenoids have an antibacterial effect that facilitates wound healing and optimizes the re-epithelialization rate (Setyowati, 2017). Terpenoids, along with flavonoids, possess astringent properties that promote wound contraction (Asumang et al., 2021).

CONCLUSION

The total flavonoid content estimation of the ethyl acetate fraction of *Portulaca grandiflora* was 4.7153% ± 0.0012% W/W (QE), representing the highest total flavonoid content. The n-hexane and aqueous fractions of *Portulaca grandiflora* indicated 2.0507% ± 0.0050% W/W (QE) and 1.9693% ± 0.0012% W/W (QE), respectively. The aqueous fraction, which was represented the lowest total flavonoid content, indicated the highest wound-healing activity, similar to the positive control group (betadine solution) on day 9. The discrepancy between the highest flavonoid concentration of the ethyl acetate fraction and the highest wound contraction of the aqueous fraction revealed an inverse correlation between the total flavonoid content and the wound healing activity. The inverse relationship between flavonoid levels and wound-healing activity suggested that polar non-flavonoid metabolites or flavonoid glycosides, such as rutin, play important roles in the wound-healing process. The sample size was limited to three rabbits, which affects the generalizability of the findings to a larger population. Furthermore, it is crucial to note that while the wound-healing activity of the *Portulaca grandiflora* extract fractions might be related to metabolites such as tannins, saponins, and terpenoids alongside flavonoids, the present study did not qualitatively and quantitatively analyze these compounds within each fraction. The phytochemical analysis was limited to the total flavonoid content. A study with a larger sample size and different secondary metabolite content analyses is needed. Formulation studies of *Portulaca grandiflora* extract fractions with different medicine delivery systems are also necessary to find a suitable form to be applied in the clinical field.

DECLARATIONS

Acknowledgments

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Authors' contributions

Maria Fatmadewi Imawati, Antonius Budiawan, Levi Puradewa, Bida Cincin Kirana, and Agus Purwanto conducted the experiments. Maria Fatmadewi Imawati performed the fractionation and the total flavonoid content analysis. Antonius Budiawan conducted the wound-healing activity test. Levi Puradewa carried out the TLC flavonoid test. Bida Cincin Kirana performed the statistical data analysis. Agus Purwanto conducted the identification and determination of *Portulaca grandiflora*. All authors were involved in the drafting, revising, and approving of the final edition of the manuscript.

Competing interests

The authors declared that they have no competing interests.

Ethical considerations

The authors declared that this manuscript is an original article and has already been checked for plagiarism. The original article has not been submitted or published elsewhere. The authors confirm that no AI tools or generative models were used to prepare the present study.

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Availability of data

The data that support the findings of this study are available from the corresponding author upon reasonable requests.

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