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Effectiveness Test of Methanol, Ethyl Acetate, and Chloroform Fractions of Bidara Leaf Extract (*Zizyphus mauritiana* L.) on Wound Healing in Rabbits (*Oryctolagus cuniculus*)

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Abstract

Bidara (*Zizyphus mauritiana* L.) contains alkaloids, glycosides, saponins, flavonoids, terpenoids, phenols, and the best antioxidant properties of its leaves. In bidara leaf, they are antibacterial, antiviral, and antiseptic, play a role in cell regeneration and repair, so they are effective in wound healing. This study aimed to determine the effectiveness of bidara leaf extract fractions on incision wounds and determine which fraction was more effective in narrowing the incision wound. Making wounds on rabbits (*Oryctolagus cuniculus*) using the *Morton* method, by making an incision on the back with a diameter of 2 cm. Testing the effectiveness of fractions in wound healing with rabbits divided into 5 groups, namely positive control, negative control, methanol fraction (MeOH), ethyl acetate fraction (Ethac), and chloroform fraction ($CHCl_3$) with a dose of 100 mg/KgBB rabbit in 1 ml of solvent. The diameter of the wound area was measured using the *Macbiophotonic Image J* program, and the data were analyzed using *One Way Anova* (ANOVA) followed by the *Tukey* test. The results of data analysis using the ANOVA method with a significant value of 0,004 ($p<0,05$). The conclusion of the results of the research conducted for 8 days shows that the MeOH, Ethac, and $CHCl_3$ fractions of bidara leaf extract have the effectiveness of wound healing in rabbits with differences in healing shown by the Ethac fraction with a better effect than the MeOH and $CHCl_3$ fractions.

Keywords: bidara leaf; fractions; wound healing; rabbits.

INTRODUCTION

Living things always do activities and movements that do not rule out accidents and injuries. Injuries can be caused by punctures or scratches with sharp objects, blunt objects, accidents, gunshots, animal bites, chemicals, hot water, fire, electricity, and lightning which cause minor to severe injuries (Karliana & Wikanta, 2019). Based on Basic Health Research (RISKESDAS) data in 2018, in Indonesia the prevalence of injuries due to accidents that occurred at home and the environment ranked first at 44.7%, with the most common types of injuries being abrasions, bruises/bruises at 64.1%, followed by dislocated injuries at 32.8% and puncture/slash/tear injuries at 20.1%. The proportion of injuries was almost the same between urban and rural areas, at 9% (Jainurakhma et al., 2022).

A wound is damage to the skin in the form of loss of skin epithelial integrity. One of the most common wounds experienced by humans is a cut. A cut is a loss of body tissue caused by a sharp object. A cut wound is an acute type of wound that can cause bleeding with hemostatsis and eventual inflammation. If a cut wound is left untreated, wound infection may occur and the condition may worsen. To heal wounds, they are usually treated with medicines in liquid form or semi-solid form such as ointments. A commonly used drug to heal wounds and prevent wound infection is povidone iodine (E. Lestari, 2020). In addition to antiseptics, antibiotics and anti-inflammatory drugs, wounds can also be treated with traditional medicines whose ingredients have been clinically tested and can be used as medicine (Karliana & Wikanta, 2019).

Traditional medicine is an effort to overcome health problems passed down from generation to generation. Materials that are easily available and cheap make traditional medicine widely used. One of the medicinal plants that has the potential to treat wounds is the bidara plant (*Zizyphus mauritiana* L.). The content is in the form of alkaloid compounds, glycosides, saponins, flavonoids, terpenoids, and phenols, as well as the best antioxidant effect from leaves (E. Lestari, 2020; Karliana & Wikanta, 2019; G. Lestari et al., 2021; Kusriani et al., 2015). The content of bidara leaf chemical compounds is efficacious for healing cuts. This is because bidara leaves have antibacterial, antiviral, and antiseptic properties and work in cell regeneration and repair. Alkaloid

compounds contained in bidara leaves have an analgesic effect on wounds. While saponins stimulate collagen growth during the wound healing (Karliana & Wikanta, 2019). The benefits of flavonoids are anti-inflammatory and function to inhibit microbial growth by increasing the antioxidant activity of granulomatous tissue (Kemalasari et al., 2018). On the other hand, polyphenols reduce lipid peroxidation, thereby reducing cell necrosis and vascularization. Tannin compounds support wound healing through their astringent and antibacterial properties (Nuralifah et al., 2022).

Previous research on wound healing was conducted by Karliana & Wikanta (2019), until the administration of bidara leaf extract was effective in healing iris wounds in male mice (*Mus muschulus*). Therefore, this study aims to further determine the effect of compounds in the methanol, ethyl acetate, and chloroform fractions of bidara leaf extract (*Zizyphus mauritiana* L.) on the healing process of incision wounds in rabbits (*Oryctolagus cuniculus*).

MATERIALS AND METHODS

This study is an experimental study conducted in the pharmacy laboratory of the Faculty of Health Sciences, University of Muhammadiyah Pekajangan Pekalongan, where the narrowing of the incision wound was observed by measuring the diameter of the incision wound on the rabbit's back.

Materials

Bidara leaf, rabbit, 96% ethanol, povidone iodine, 70% alcohol, TopSy cream, distilled water, HCl 2N, $FeCl_3$ 5%, *Mayer's* reagent, *Dragendorff's* reagent, H_2SO_4, chloroform, ethyl acetate, methanol, methanol p. a, anhydrous acetic acid, gallic acid p.a, quercetin p.a, Na_2CO_3 15%, *Folin-Ciocalteu* reagent, $AlCl_3$ 10%, CH_3COONa 1 M, and DMSO.

Determination

Determination was conducted on bidara plants (*Zizyphus mauritiana* L.) at Ahmad Dahlan University.

Sampling and Simplisia Preparation

Bidara leaves were obtained from Ayamputih village, Buluspesantren sub-district, Kebumen district. Harvesting was done in the morning before photosynthesis occurred. Wet sorting of leaves is done by separating from impurities such as unnecessary plant parts (stems, twigs, flowers and roots) and other impurities. The leaf washing process is carried out with running water while cleaning from dirt that sticks. Furthermore, the drying process of leaves that are still wet is done by drying them in the sun and covering them with a black cloth until dry. Dry leaf symposia is made into powder by blending it into powder and sieving it with a No. mesh 40 sieve.

Extract Preparation

Extraction was carried out by maceration using 96% ethanol solvent, dried bidara leaves weighed as much as 1.5 kg and were put into a maceration container then 96% ethanol 9 L. Let stand for 5 days at room temperature protected from sunlight while occasionally stirring, then filtered. The maceration treatment was repeated once with 4.5 L of 96% ethanol. The maceration results were collected and then evaporated using a rotary evaporator at 42℃ until thick.

Fractionation

100 g of thick extract was put into a separatory funnel. The sample was fractionated with solvents that have different polarity from low to high polarities (chloroform, ethyl acetate, and methanol). The sample was added 100 mL of solvent and fractionated one by one solvent alternately in a separatory funnel. Each fractionation result was evaporated using a water*bath until a thick extract was obtained.

Phytochemical Screening

Alkaloid Test

Several samples were added 1 mL of 2N HCl and 9 mL of distilled water, heated on a water bath for two minutes, cooled and filtered. The filtrate obtained was used for the alkaloid test. Taken 0.5 ml of filtrate in two test tubes, added 2 drops of *Mayer* reagent and *Dragendorff*. The test results are positive if *Mayer's* reagent forms a yellow-white precipitate and *Dragendorff's* reagent forms a red or orange precipitate (Wirasti, 2019).

Saponins Test

several samples were put into a test tube and added 10 mL of hot water, then cooled and shaken vigorously for 10 seconds. The foam formation on a stable top layer indicates the presence of saponins in the test sample with the addition of 1 drop of HCl 2N (Wirasti, 2019).

Tannins Test

A sample quantity of 10 mL of distilled water is heated, then cooled and filtered. Dilute the filtrate until almost colorless and add 1-2 drops of 5% $FeCl_3$ reagent. A black-blue or black-green color indicates a positive tannin result (Wirasti, 2019).

Flavonoids Test

Several samples are dissolved with a certain amount of 96% ethanol, and added $AlCl_3$ reagent. A positive reaction to flavonoids is characterized by the formation of red, yellow, or orange colors (Ni'ma & Lindawati, 2022).

Phenol Test

Several samples were extracted with 2 mL of 96% ethanol. Take 1 mL of filtrate and add 2 drops of 5%

$FeCl_3$ solution. A positive reaction to phenol shows the formation of a green or blue-green color (Wirasti, 2019).

Terpenoids/Steroids Test
Several samples were added to chloroform solvent and separated. 2 drops of acetic acid anhydride and 1 drop of H_2SO_4 were added to the resulting filtrate, with positive results for the presence of steroids forming a blue to green color, and the presence of terpenoids forming a red to purple color (Anisa & Najib, 2022).

Total Flavonoids Test
10 mg of sample was weighed and dissolved in 10 mL of methanol p.a to obtain a 1 mg/mL concentration. A total of 0.5 mL test sample was added to 1.5 mL of methanol p.a, followed by 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M CH_3COONa, and 2.8 mL of distilled water. After 20 minutes of incubation, the absorbance was measured at maximum λ with 3 times replication using UV-Vis spectrophotometer (Wirasti, 2019). Flavonoid content can be calculated with the following formula:

$$Total\ flavonoids = \frac{C\ x\ v\ x\ fp}{g}$$

Note:
C : flavonoids concentration (x-value)
V : volume of sample used (mL)
fp : dilution factor
g : weight of sample used (g)

Total Phenol Test
A total of 10 mg of sample was dissolved in distilled water p.a up to 10 mL and a concentration of 1 mg/mL was obtained. Pipette 0.1 mL, add 7.9 mL of distilled water and 0.5 mL of *Folin-Ciocalteu* reagent, vortex for 1 minute and let stand for 8 minutes. Then, added 1.5 mL of 15% Na_2CO_3 into the solution and let stand at room temperature for 30 minutes. Absorbance was measured at maximum λ using a UV-Vis spectrophotometer. The measurement was repeated 3 times, and the results of the phenol content obtained were expressed in mg gallic acid equivalent (GAE)/g sample (Wirasti, 2019). Calculation of total phenolic content using the following formula:

$$Total\ phenolics = \frac{C\ x\ v\ x\ fp}{g}$$

Note:
C : phenolic concentrations (x-value)
V : volume of sample used (mL)
fp : dilution factor
g : weight of sample used (g)

Preparation and Treatment of Test Animals
Test animals were used as local male rabbits weighing 1.5-3 kg as many as 15 heads. The initial treatment of the test animals was wounding. The rabbit's back hair was shaved and cleaned using 70% ethanol, then anesthetized with TopSy cream. An incision was made using a sterile scalpel ±2 cm long with a depth of 0.2 cm. The second stage is the administration of samples to the incision wound which is divided into group 1 (MeOH 100 mg/kg BW in DMSO), group 2 (Ethac 100 mg/kg BW in DMSO), group 3 ($CHCl_3$ 100 mg/kg BW in DMSO), positive control (povidone iodine) and negative control (DMSO). Rabbits were put into cages, treatment was carried out once a day in the morning for a maximum of 14 days. The measurement of the effect of wound healing is seen from the wound closure time and the decrease in wound diameter on the rabbit's back.

Data Analysis
ANOVA analysis was used to observe wound closure characterized by a reduction in wound diameter, absence of erythema, and swelling.

RESULTS AND DISCUSSION

Determination
The plant determined to be *Zizyphus mauritiana* Lam. and belongs to the *Rhamnaceae* family. The purpose of determination is to ensure the authenticity of the identity of the plant under study so that there is no error when taking bidara plant material (*Zizyphus mauritiana* L.).

Extracts and Fractions
From the sample powder, an extract with a dense form, blackish green color, and characteristic odor of the extract was obtained. In concentrating the extract at 42°C, it is intended that bioactive components such as flavonoids that cannot tolerate high temperatures above 50°C do not experience structural changes due to temperature increase (Yuliantari et al., 2017). The resulting extract weighs 183 g with a yield of 12.2% and a moisture content of 0.49%. Moisture content testing aims to determine the moisture content in the extract to minimize the growth of fungi, bacteria, and enzymes that can damage the extract during storage. The resulting extract is fractionated to separate and purify the active ingredients from the original extract (Abubakar & Haque, 2020).

Table 1. Fraction yield.

Sample	Weight of extract (g)	weight of fraction (g)	Yield (%)	Moisture content (%)
MeOH fraction	100 g	20,55 g	20,55%	0,74%
Ethac fraction	100 g	6 g	6%	0,47%
$CHCl_3$ fraction	100 g	7,3 g	7,3%	1,49%

In **Table 1**, the methanol fraction data gives the highest yield, which indicates that most of the compounds in the extract dissolve in methanol which is a polar solvent. According to the principle of like dissolve like, polar compounds will dissolve in polar compounds and non-polar compounds will dissolve in non-polar solvents. The amount of yield dissolved in methanol indicates the amount of polar compounds contained in the extract.

Phytochemical Screening

Bidara leaves are known to be helpful in treating wounds and are proven to contain secondary metabolites. Therefore, to maximize the function of bidara leaves as medicinal compounds, it is necessary to know the secondary metabolite compounds through phytochemical screening **(Table 2)**.

Table 2. Phytochemical screening of bidara leaf fraction.

Metabolite compounds	Sample		
	MeOH fraction	Ethac fraction	$CHCl_3$ fraction
Alkaloids			
▪ *Mayer*	(-) No precipitates	(-) No precipitates	(-) No precipitates
▪ *Dragendorff*	(-) No precipitates	(-) No precipitates	(-) No precipitates
Saponins	(++) Stable foam 2 cm	(+) Stable foam 0,7 cm	(+) Stable foam 0,5 cm
Tannins	(+++) Greenish-black	(++) Greenish-black	(++) Greenish-black
Flavonoids	(++) Yellowish-green	(++) Yellowish-green	(+) Yellowish-green
Phenol	(++) Blackish-green	(+++) Blackish-green	(+) Blackish-green
Steroids	(++) Greenish-black	(++) Greenish-black	(++) Greenish-black

Note:
- : no compound content
+ : there is little compound content
++ : there is a compound content
+++ : there are many compound contents

Several studies showed that ethanol extract from bidara leaves positively contained alkaloid compounds (Khoirunnisak, 2020), while ethyl acetate extract from bidara leaves negatively contained alkaloid compounds (Bialangi et al., 2023). Alkaloids are usually found in various parts of plants, such as flowers, seeds, leaves, twigs, roots, and bark. In testing metabolite compounds, the addition of certain materials has its purpose. The addition of *Dragendorff* and *Mayer* reagents to the alkaloid test can produce orange to reddish brown precipitates and white to yellow precipitates. In this reaction, a ligand exchange occurs in which nitrogen with a free electron pair in the alkaloid forms a covalent bond with the K^+ ion of potassium tetraiodobismutat, resulting in the formation of an alkaloid potassium complex that precipitates (Habibi et al., 2018). The addition of HCl to the saponin test will increase the polarity of the saponin compound and change the position of the forming group. Under these conditions, polar (hydrophilic) groups face outward and nonpolar (hydrophobic) groups face inward, forming a structure called a micelle structure (Putri & Lubis, 2020). The addition of $FeCl_3$ in the tannin test which can form Fe^{3+} and Cl^-. The addition of $AlCl_3$ to flavonoids to form complexes between neighboring hydroxyl and ketone groups or with adjacent hydroxyl groups to form stable yellow complex compounds. While the addition of $FeCl_3$ to the phenol test will form a colored complex that is believed to be iron (III) hexaphenolate (Habibi et al., 2018). Then steroid testing is carried out using the *Lieberman-Bouchard* method (acetic acid anhydride-H_2SO_4) which produces a green color based on the ability of steroid compounds to form colors through H_2SO_4 in acetic anhydride solvents (Habibi et al., 2018).

Total Flavonoids Test

The examination of the total flavonoid compound content was carried out because flavonoid compounds are known to play a role in promoting the hemostasis process through a vasoconstriction mechanism (Nikola et al., 2021). The method used to determine the total flavonoid content is the colorimetric method using 1 M acetic acid

reagent and 10% $AlCl_3$. In this method, a reaction occurs and a stable yellow color is formed due to the reaction of $AlCl_3$ and OH groups on flavonoids (Wahyudi & Minarsih, 2023). $AlCl_3$ reacts with ketone groups at C4 and OH groups at C3 or C5 of flavone or flavonol compounds to form stable yellow complex compounds. The compound used as a standard to determine flavonoid levels is quercetin. Because quercetin is a flavonoid of the flavonol group with a ketone group at the C4 atom and a hydroxyl group at the adjacent C3 and C5 atoms (Sari & Ayuchecaria, 2017). Quercetin was also chosen as a standard also because it is the most widespread compound in plants (Wirasti, 2019).

Total Phenolic Test

The examination of the total content of phenolic compounds was carried out because phenolic compounds are known to have antiseptic effects on wounds and kill bacteria (Karliana & Wikanta, 2019). Determination of the total phenolic content of MeOH, Ethac, and $CHCl_3$ fractions in bidara leaves (*Zizyphus mauritiana* L.) was determined using the *Folin-Ciocalteu* principle based on redox reactions. Gallic acid is a stable and natural phenolic compound used as a standard solution. According to Sari & Ayuchecaria (2017), gallic acid is one of the phenolic compounds derived from hydroxybenzoic acid and is classified as a simple phenolic acid. *Gallic acid* reacts with *Folin-Ciocalteu* reagent to produce a yellow color indicating the presence of phenol, which then adds Na_2CO3 solution to it as a base atmosphere giver. During the reaction, the hydroxyl group of the phenolic compound reacts with *Folin-Ciocalteu* reagent to form a blue molybdenum-tungsten complex whose structure is unknown and can be detected using a spectrophotometer. The blue color becomes more intense depending on the concentration of phenol ions. That is, the higher the concentration of phenolic compounds, the more phenolic ions that reduce heteropoly acids (phosphomolybdate-phosphotungstat) into tungsten molybdenum complexes, so that the color obtained is more intense (Wirasti, 2019).

The total flavonoid content of the plant is expressed in QE (quercetin equivalent), and the total phenolic content is expressed in GAE (gallic acid equivalent), which is the equivalent of milligrams of quercetin or gallic acid in one gram of sample **(Figure 1)**.

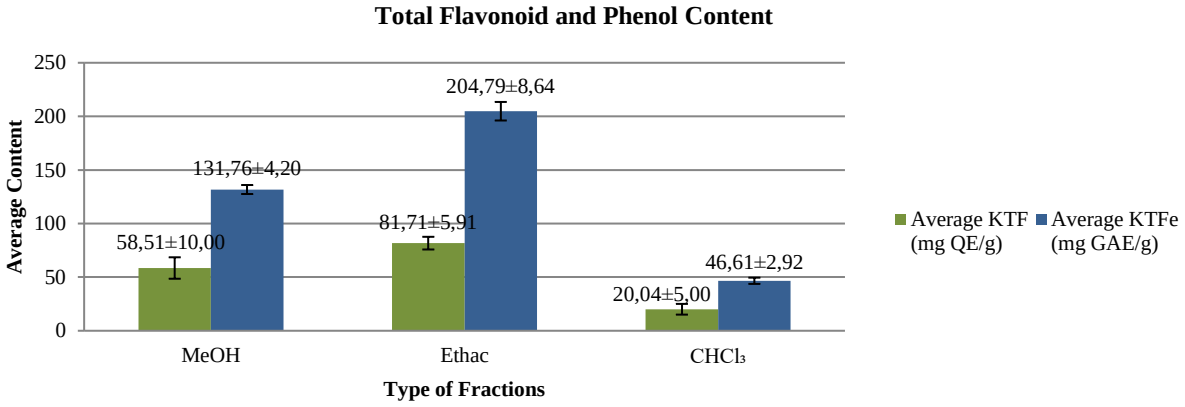

Figure 1. Average total flavonoid and phenolic content of bidara leaf fraction (*Zizyphus mauritiana* L.)

The results of determining the content of flavonoid compounds and total phenols, it is known that the sample of the ethyl acetate fraction of bidara leaves (*Zizyphus mauritiana* L.) has a higher content of flavonoid and phenol compounds, namely as much as 81.71 ± 5.91 mg QE/g extract and 204.79 ± 8.64 mg GAE/g extract, this is in line with the screening results where the ethyl acetate fraction has the results of phenol and flavonoid tests with the most concentrated color compared to other fractions. Followed by the methanol fraction as much as 58.51 ± 10.00 mg QE/g extract and 131.76 ± 4.20 mg GAE/g extract, the screening results in the flavonoid test having color results with almost the same concentration as the ethyl acetate fraction. Then, for the chloroform fraction with a content of 20.04 ± 5.00 mg QE/g extract and 46.61 ± 2.92 mg GAE/g extract, with the screening results in the phenol and flavonoid tests had a less concentrated color.

Effectiveness of Wound Healing

Wound healing is a multi-step process that occurs when the normal anatomy and function of skin tissue are disrupted. Inflammation, granulation, wound reduction, collagen formation, epithelial closure and scar formation are all part of the process. The smooth running of these stages promotes wound healing and restores the previously disrupted condition and function of the skin anatomy (Zulkefli et al., 2023). In recent years, plants and their products have been used to stimulate natural repair mechanisms by shortening the prolonged

inflammatory phase, enhancing fibroblast migration and proliferation, stimulating angiogenic processes, and thus promoting the re-epithelialization process of wound healing. Numerous studies on the potential of natural products as wound healing agents have scientifically validated their antibacterial, antioxidant, and anti-inflammatory properties. In this regard, WHO recommends the use of scientifically validated plant-based medicines. Natural products from traditional medicine and ethnopharmacology are important sources of new leads in developing pharmaceutical research for therapeutic applications. Bioassay-based fractionation and isolation is one of the most effective methods to discover new therapeutic compounds from active fractions of plant extracts (Bhat et al., 2023).

Wound observations were made by photographing the wound using a mobile phone camera every day until the rabbit healed and measuring the area and diameter of the wound area using the *Macbiophotonic Image J* program. Observations of wound healing ability characterized by a decrease in wound diameter reinforced by a decrease in the area of the incision on the rabbit's back were carried out with 3 replications for each treatment group for 8 days of testing. From the data obtained, the average reduction in the diameter of the incision wound area was calculated **(Figure 2)**.

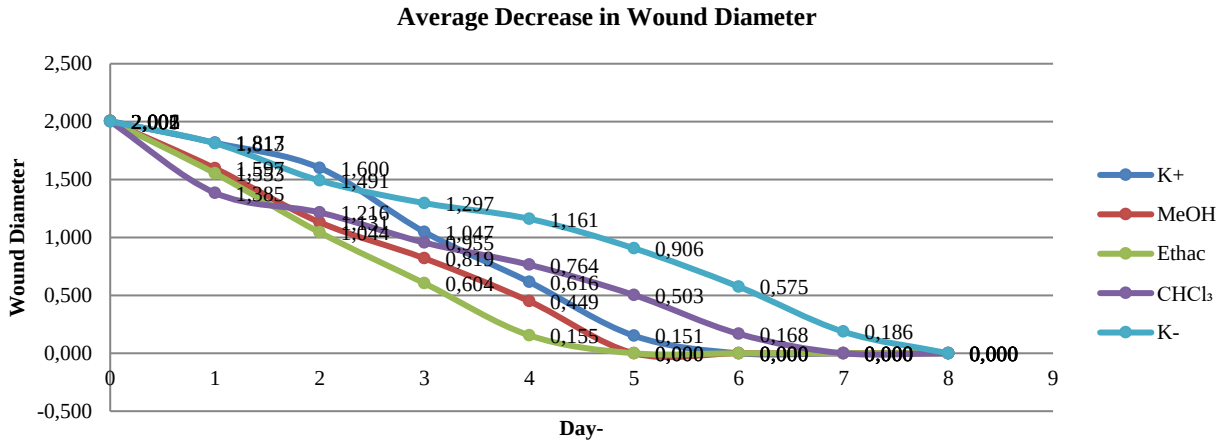

Figure 2. Decrease in incision wound diameter.

Five treatment groups consisting of negative control in the form of DMSO, positive control in the form of povidone iodine 10%, MeOH fraction, Ethac fraction, and $CHCl_3$ fraction of bidara leaves (*Zizyphus mauritiana* L.) are known to give different results in shortening the diameter of the incision every day.

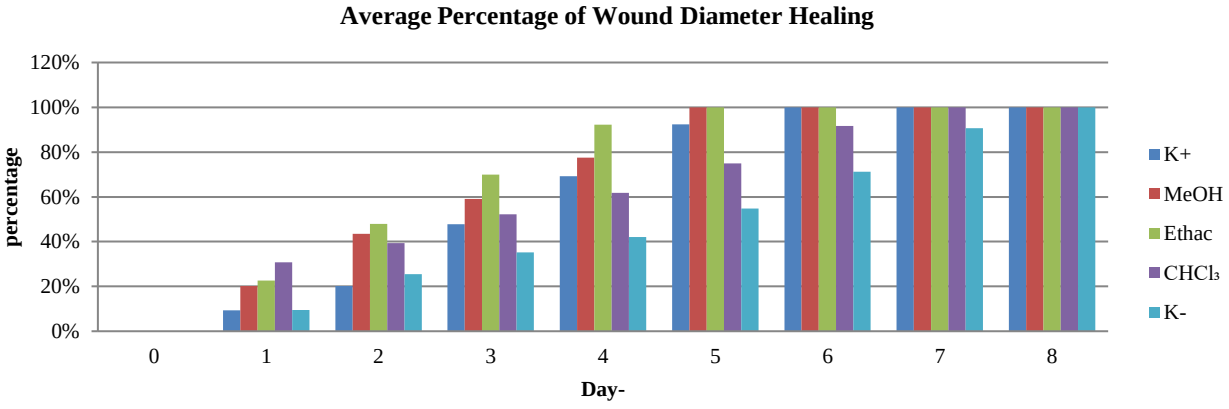

Figure 3. Percentage of incision wound diameter healing.

The percentage of wound diameter healing shows the closure of the wound on the rabbit's back due to the treatment in wound care. Based on Figure 3, the best healing on day 1 was shown by the $CHCl_3$ fraction, but

on day 2 until the maximum healing rate was reached, it was shown by the Ethac fraction. The Ethac fraction group which has the highest percentage of healing based on the area and diameter of the incision wound shows the best potential as a wound healing agent.

Based on the results of testing the effect of research groups on wound diameter reduction, the MeOH and Ethac groups could heal wounds the fastest, followed by the positive control and $CHCl_3$ fraction, and finally the negative group. The fractionated sample from the thistle leaf showed faster healing because povidone iodine 10%, which has less healing effect, was used as a positive control. Povidone iodine is a commonly used antimicrobial agent that contains polyvinylpyrrolidone iodine, a water-soluble complex with elemental iodine bound to a synthetic polymer. In vitro evidence shows that iodine not only has broad antimicrobial effects but also suppresses inflammation caused by pathogens and host responses. This includes anti-inflammatory effects, which are considered multifactorial and have been shown to be clinically relevant.

Based on the results of phytochemical screening, the MeOH fraction contains the highest amount of active components such as saponins, tannins, and steroids compared to other fractions, so that it has a good healing rate. On the other hand, due to the high content of flavonoids and phenols, the Ethac fraction has a good healing rate based on phytochemical screening and total content test compared to other fractions. The content of these active ingredients has advantages in supporting wound healing. Saponins have a mechanism to increase hemolytic activity and play a role in antibacterial, antiviral, and antioxidant properties. By increasing the ability of TGF-β receptors, fibroblasts can bind TGF-β strongly (Karliana & Wikanta, 2019). Tannins play an important role in the transcription and translation process of vascular endothelial growth factor (VEGF). Steroids by increasing the rate of epithelial formation in the body (Dewi & Wicaksono, 2020).

Phenolic compounds and flavonoids have antioxidant, anti-inflammatory, antibacterial, and antifungal properties that prevent tumor growth. Flavonoids have a wound healing mechanism with a protective effect against reperfusion of body tissues caused by ischemia. The antioxidant properties of flavonoids can reduce the degree of lipid peroxidation and promote the re-epithelialization process. Its astringent and antibacterial functions play an important role in wound contraction and increase the rate of body epithelialization (Dewi & Wicaksono, 2020; Kemalasari et al., 2018). Many studies have shown that flavonoids have wound healing properties due to their well-known anti-inflammatory, angiogenesis, re-epithelialization, and antioxidant effects. They are able to act on the wound healing process through the expression of biomarkers of each pathway which mainly include Wnt/β-*catenin*, *Hippo*, *Transforming Growth Factor-beta* (TGF-β), *Hedgehog*, *c-Jun N-Terminal Kinase* (JNK), NF-E2-*related factor 2/antioxidant responsive element* (Nrf2/ARE), *Nuclear Factor Kappa B* (NF-κB), MAPK/ERK, Ras/Raf/MEK/ERK, phosphatidylinositol 3-kinase (PI3K)/Akt, *Nitric oxide* (NO) (Zulkefli et al., 2023).

Polyphenols are a major component of plant metabolites and are important in the human health system. The healing properties of these phenolic compounds are mainly due to their strong antibacterial, anti-inflammatory, and antioxidant activities, and these activities are positively correlated with each other. They play an important role in enhancing healing activity by forming a barrier against microorganisms, reducing the chance of wound infection, and protecting cells from damage caused by reactive oxygen species (ROS). Several natural phenols such as flavonoids rutin and quercetin, phenolic acids, chlorogenic acid, carotenoids, and curcumin are known to have various therapeutic activities. These compounds also aid wound healing due to their free radical scavenging, antioxidant, lipid peroxidation inhibitor, immunomodulatory, angiogenic, antibacterial, and neurogenic properties (Micale et al., 2020). Phenol also acts as a wound antiseptic, which kills bacteria by denaturing bacterial cell proteins and plays a role in the epithelialization process by stimulating the regeneration process of skin tissue in the wound so as to accelerate wound closure with new skin (Karliana & Wikanta, 2019).

The negative control group, DMSO, which was used as a carrier solution in the fraction samples, showed the lowest wound healing effectiveness. The use of this negative control is intended to measure physiological wound healing in the body and make it possible to estimate the time it takes for a wound to heal without treatment. In addition, the negative control was also conducted to determine whether the carrier solution affects wound healing.

Data Analysis

Data processing was carried out based on the test data obtained to determine whether there were differences in the effect of incision wound healing in each treatment group. Tests carried out first test normality using the *Shapiro-Wilk* test and homogeneity test. The data obtained were normally and homogeneously distributed because it showed that ($p>0.05$).

Table 3. *One-way anova* test.

Treatment	Significance value (p)
Diameter	0.004

The ANOVA test results of wound healing from MeOH fraction, Ethac fraction, $CHCl_3$ fraction, positive control, and negative control showed a significance value of $p<0.05$, which means there is a significant difference between wound healing from each test sample **(Table 3)**.

Table 4. *Tukey HSD* test result.

Treatment group	Comparisons	Significance values (p)	Description
Positive control	MeOH fraction	0.630	No different
	Ethac fraction	0.135	No different
	$CHCl_3$ fraction	0.693	No different
	Negative control	0.056	Significant difference
MeOH fraction	Positive control	0.630	No different
	Ethac fraction	0.765	No different
	$CHCl_3$ fraction	0.029	Significant difference
	Negative control	0.006	Significant difference
Fraksi Ethac	Positive control	0.135	No different
	MeOH fraction	0.765	No different
	$CHCl_3$ fraction	0.018	Significant difference
	Negative control	0.001	Significant difference
Fraksi CHCl3	Positive control	0.693	No different
	MeOH fraction	0.029	Significant difference
	Ethac fraction	0.018	Significant difference
	Negative control	0.528	No different
Negative control	Positive control	0.056	Significant difference
	MeOH fraction	0.006	Significant difference
	Ethac fraction	0.001	Significant difference
	$CHCl_3$ fraction	0.528	No different

Based on the Tukey test results in Table 4, the wound healing by the tested samples in the MeOH and Ethac fraction showed similar results. However, qualitatively, the Ethac fraction has a good healing level based on phytochemical screening compared to other fractions shown and confirmed by testing the total flavonoid and phenol content. The similarity of healing results in MeOH and Ethac fractions can be influenced by the similarity of flavonoids contained in both so that they show the same effectiveness in healing cuts.

CONCLUSIONS

Methanol, ethyl acetate, and chloroform fractions of bidara (*Zizyphus mauritiana* L.) leaf extract have effectiveness in healing incision wounds in rabbits with wound healing ranging from 5-8 days, with compounds involved in the healing process in the form of flavonoids, phenols, saponins, tannins and steroids. The ethyl acetate fraction has a better effect in narrowing the wound based on the percentage of healing of the incision wound through a reduction in wound diameter compared to the methanol fraction and chloroform fraction.

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Competing Interests: The authors declare that there are no competing interests.

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