

TEST OF THE HEALING EFFECT OF ETHANOL EXTRACT OF AFRICAN LEAVES (*Vernonia amygdalina Del.*) ON CUTS ON MICE (*Mus musculus*)

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A B S T R A C T

Natural medicine preparations as part of Indonesia's national cultural heritage are now increasingly important in everyday life. People have used various plants as traditional medicines. This study identified secondary metabolic compounds and tested the effectiveness of 96% ethanol extract of African leaves (*Vernonia amygdalina Del.*) in healing cuts in mice (*Mus musculus*). African leaves (*Vernonia amygdalina Del.*) are medicinal plants found in East Kalimantan. This experimental study used phytochemical screening and effectiveness test methods, as well as one-way ANOVA analysis. The test animals were mice (*Mus musculus*), which were divided into five groups. They consisted of a positive control group using 10% povidone iodine, a negative control group using distilled water, and a treatment group with extract concentrations of 0.1 grams, 0.2 grams, and 0.3 grams. The results showed that 96% ethanol extract of African leaves (*Vernonia amygdalina Del.*) contained alkaloids, flavonoids, saponins, and tannins. In addition, in the effectiveness test of 96% ethanol extract of African leaves (*Vernonia amygdalina Del.*) can reduce cuts in test animals mice (*Mus musculus*), with the results of the wound length test showing significant results from the one way ANOVA analysis ($F > F_{crit} = 151.549 > 1.961$) which shows that the effect of 96% ethanol extract of African leaves is effective for healing cuts with the best extract concentration being 0.3 g extract.

INTRODUCTION

Indonesia is famous for its wealth of natural resources, including various plants that have medicinal properties. Therefore, various studies and tests have been conducted to ensure that the benefits of medicinal plants are sufficiently accepted and trusted by the community (Wahyulianingsih et al., 2016).

Natural medicine preparations as part of Indonesia's national cultural heritage are now increasingly important in everyday life. People increasingly believe in the health benefits of natural ingredients and often choose them because they are cheaper, easy to find, and their use has been inherited from their ancestors. In addition, the negative impacts of synthetic materials also encourage people to return to using natural ingredients (Imansyah & Alam, 2021).

The use of medicinal plants in Indonesia is increasing, both directly by small-scale industrial communities and by large industries (Teheni et al., 2024). The use of plants as traditional medicine is based on the experiences of the community. Until now, traditional medicine is considered quite effective in treating various diseases (Yolandari & Mustiqawati, 2022).

Various plants have been used by the community in traditional medicine. One of the medicinal plants found in East Kalimantan is the African leaf (*vernonia amygdalina del.*) (Mashunah et al., 2020). The African plant (*Gymnanthemum amygdalinum Del.*) is one of the many plants in Indonesia that has medicinal properties. This plant, which originates from Africa, is one of the plants from the Compositae tribe which is used for traditional medicine from the genus *Vernonia*. The African leaf plant has properties such as antibacterial, antifungal, antimalarial, antioxidant and anticancer (Lubis & Dalimunthe, 2022).

African leaf (*Veronia amygdalina Del.*) varies in height from 2 meters to 10 meters, has light brown bark, is slightly rough and peels lengthwise, and branches are brittle. The leaves of this plant are lanceolate-oval in shape, the leaf area can reach 28x10 cm, green to dark green with or without fine hairs on the surface of the leaves and has a distinctive odor and bitter taste (Mardhiyah, 2015).

According to research (Ruslim et al., 2017) ethanol extract of *V. Amygdalina* has the effect of accelerating wound healing since the wound is closed after tooth extraction of *Rattus norvegicus* and its effective concentration is 5%.

According to research (Ginting et al., 2020) the use of a mixture of African leaf ethanol extract (EEDA) and oil palm leaf ethanol extract (EEDKS) obtained better effectiveness in healing rabbit burns compared to without the combination.

From the results of the description above, researchers are interested in conducting research entitled "Test of the healing effect of African leaf extract (*Vernonia amygdalina Del.*) On mice (*mus musculus*)".

METHODOLOGY

This study is an experimental study that uses phytochemical screening methods and effectiveness tests and uses *one-way ANOVA* analysis involving mice as test animals which are divided into five groups.

Tool

The tools used are laboratory glassware, analytical scales, razor blades, scissors, stirring rods, scalpels, test tubes, droppers, clamps, bunsen burners, porcelain cups, glass jars, separating funnels, spoons, spatulas, maceration containers, mortars and pestles, rotary evaporators, animal cages, blenders, filter paper, vernier calipers and sieves.

Material

The materials used are African leaf extract, water, distilled water, 10% povidone iodine, 70% ethanol, 96% ethanol, ether, concentrated HCl, 2N HCl, magnesium powder, FeCl₃, Mayer's reagent, alcohol swab, dragendrof reagent and mouse food.

Time and Place Of Research

This research was conducted at the Phytochemical Pharmacognosy Laboratory of Mandala Waluya University and the Pharmacy Laboratory of Baubau Politeknik which was carried out from July to August 2024.

Work Procedures

Sample Peparation

African leaves (*Vernonia amygdalina Del.*) were taken from Waruruma Village, Kokalukuna District, Baubau City. Samples were taken in the morning at around 08.00 to 10.00 WITA. A total of 2 kg of African leaves were taken for research. The African leaves were then processed into simplicia by drying them naturally until they were completely dry. After the drying process was complete, the African leaves were crushed with a blender until smooth, then sieved. The ready-to-use African leaf simplicia was stored in a tightly closed glass jar to maintain its quality.

Sample Extraction

A total of 250 grams of fine simplicia is stored in a maceration jar then added 2000 mL of 96% ethanol solvent. After that the container is tightly closed and left in a place protected from light for 3 days while stirring occasionally. After the soaking time, filter the sample through filter paper to obtain the initial filtrate and residue. The residue is then reprocessed through a maceration process for 2 days to obtain the second filtrate. After both filtrates are obtained, the filtrate is then evaporated to remove the solvent contained in the African leaf extract using a rotary evaporator. The concentrated African leaf extract is stored in a glass container protected from sunlight.

Phytochemical Screening

Alkaloid

A total of 1 mL of African leaf extract was added to 2 mL of 2N HCl and shaken. The extract was then divided into two test tubes for different treatments. One drop of Mayer's reagent was added to reaction vessel I and Dragendorff's reagent was added to reaction vessel II. The results obtained in the form of white deposits in tube I and reddish deposits in tube II indicate the presence of alkaloid compounds (Endarini, 2016).

Flavonoid

A total of 1 mL of African leaf extract was reacted with 0.2 g of magnesium powder and 2 mL of concentrated HCl. A positive flavonoid is indicated if the solution changes color to red, orange or green (Endarini, 2016).

Saponin

A total of 1 mL of African leaf extract was added with hot distilled water and the mixture was stirred for 30 seconds. It is indicated as positive for containing saponins if the foam produced shows stable foam for 30 seconds (Saxena et al., 2013).

Tannin

A total of 1 mL of African leaf extract was reacted with FeCl₃. It is indicated as positive for containing tannin if there is a change in the color of the solution to bluish black or green (Endarini, 2016).

Treatment Of test Animals

Incision of Test Animals

Mice were anesthetized with ether, then the fur on the back that would be injured was cleaned. The area was swabbed with alcohol, and a 15 mm incision was made using a scalpel on each mouse.

Grouping of Test Animals

A total of 15 mice were divided into 5 test groups, each containing 3 mice, with different treatments for each group. Treatment was given once a day. Group 1 as a positive control received 10% povidone iodine, group 2 as a negative control was given distilled water, group 3 was given an extract with a concentration of 0.1 grams, group 4 with a concentration of 0.2 grams, and group 5 with a concentration of 0.3 grams. After treatment, observations were made every day on the length of the incision in the five groups for 14 days.

Data Analysis

The data from the research on the length of the incision wound were then analyzed using *the one-way ANOVA* analysis method to determine whether there were differences between the treatments in each group.

RESULTS AND DISCUSSION

African leaves (*Vernonia amygdalina*) are shrubs that grow in the African continent, especially in countries like Nigeria, Cameroon, and Zimbabwe. They also grow in more tropical places like Indonesia. These plants are often found in yards, on the edges of forests, around rivers and lakes, and in grasslands. African leaves help your health in many ways, such as reducing blood sugar levels, preventing cancer, acting as an antibacterial, treating malaria, controlling blood pressure, and preventing heart disease (Wibowo et al., 2023).

This study examines the bioactive compounds and how African leaf extract (*Vernonia amygdalina Del.*) heals cut wounds in mice (*Mus musculus*). The part of the plant used in this study is the African leaf (*Vernonia amygdalina Del.*). Knowing the secondary metabolic materials in African leaves can potentially be used as a cure for cut wounds.

Wounds are defined as tissue damage that disrupts normal cellular processes or disrupts the continuity and integrity of body tissue, often accompanied by the loss of some tissue. Wounds can be considered as a loss of continuity or tissue damage (Mansjoer and Arif, 2000). Cuts (*Vulva scissum*) are wounds characterized by regular straight lines with clear boundaries. This injury usually occurs due to trauma or direct contact with sharp objects. Cuts are common during daily activities, and lack of awareness of the presence of sharp objects in the environment is one of the causes of injury (Pazry, 2016). Cuts are wounds that occur due to being cut by a sharp instrument. Signs of cuts include open wounds, pain, and the length of the wound is greater than the depth of the wound (Berman et al., 2009).

African leaves contain secondary metabolic compounds such as flavonoids, tannins and saponins known to have a positive effect on wound healing. Phytochemical screening results indicate that African leaves contain positive alkaloids, flavonoids, saponins, and tannins. The results of phytochemical tests of African leaf extracts can be shown in table 1 below:

Table 1. Results of Phytochemical Screening of African Leaf Extracts (*Vernonia amygdalina Del.*)

Secondary Metabolites	Reagent	Extract Result	Information
Alkaloid	Mayer's reagent, Dragendorff's reagent	+	There is white sediment There is a reddish deposit
Flavonoid	Concentrated HCl, Mg Powder	+	Color changes to orange
Saponin	Distilled water	+	The presence of stable foam for 30 seconds
Tannin	FeCl ₃ 1%	+	The color of the environment becomes bluish black

Source: Primary data, 2024

Next, testing was conducted to treat wounds in mice. The treatment consisted of a positive control in the form of 10% povidone-iodine, a negative control in the form of distilled water, and treatment with three different extract concentrations, namely 0.1 grams, 0.2 grams, and 0.3 grams. Measurement of the length of the mouse incision wound can be seen in the following table:

Table 2. Observation of the Size of Incision Wounds in All Treatments

Treatment Group	Duration of administration Day 1 to day 14 (mm)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Positive Control (Povidone Iodine 10%)	15,0	13,0	11,1	10,0	9,2	8,5	7,3	15,6	3,8	1,0	0,0	0,0	0,0	0,0
Negative Control (Aquadest)	15,0	14,5	13,4	12,7	11,8	10,9	9,2	7,6	6,6	5,3	4,5	3,1	1,7	0,6
Extract Concentration (0,1 g)	15,0	14,4	13,2	12,4	11,5	10,8	9,0	7,3	6,0	4,0	3,7	2,0	0,9	0,0
Extract Concentration (0,2 g)	15,0	14,0	12,8	11,7	10,6	9,7	8,5	6,9	5,4	3,6	2,7	1,2	0,0	0,0
Extract Concentration (0,3 g)	15,0	13,8	12,6	11,5	10,4	9,3	7,9	6,1	4,8	2,0	0,8	0,0	0,0	0,0

Source: Primary data, 2024

In this study, observation and measurement of the length of the cut wound showed a decrease in the length of the wound from day 1 to day 14 in all treatment groups. In group 1, the positive control containing 10% povidone-iodine, the wound healed on day 11. In group 2, the negative control with distilled water, the wound length was still 0.6 mm on day 14 and had not healed. In group 3, where 96% African ethanol extract (*Vernonia amygdalina Del.*) was used at a concentration of 0.1 g, the wound healed on day 14. Group 4 which used ethanol extract of African leaves at a concentration of 0.2 g experienced wound healing on day 13. While the last group, namely group 5 which used ethanol extract of African leaves at a concentration of 0.3 g showed the disappearance of the cut wound on day 12. This proves that the higher the concentration of 96% African ethanol extract (*Vernonia amygdalina Del.*), the faster the wound healing process occurs. Based on previous research, several secondary metabolite compounds have been found that have the potential to be used as wound healers. For example, flavonoid compounds have a mechanism in wound healing by increasing the number of platelets, which function to prevent bleeding. When platelets rupture, the thrombokinase enzyme is activated, which then converts fibrinogen into fibrin monomers with the help of vitamin K and Ca ions in blood plasma (Risa et al., 2018). Tannins can act as astringents, narrowing skin pores, hardening skin tissue, preventing exudate, closing wounds, and preventing bleeding that usually occurs in wounds (Robinson, 1995). Saponins have been studied to help the formation of important collagen by increasing the number of macrophages that migrate to the wound area and increasing the production of cytokines that activate fibroblasts in wound tissue (Risa et al., 2018).

Wound healing can be inhibited by internal (intrinsic) or external (extrinsic) factors. The wound healing process consists of four stages. The first stage is coagulation, where the blood clots. The second phase is inflammation and focuses on improving hemostasis, removing dead tissue, and preventing infection by pathogenic bacteria. The third stage, proliferation, occurs 4 to 21 days after trauma, and keratinocytes around the wound undergo phenotypic changes. The final stage is remodeling and is the longest stage of healing (Sjamsuhidat dan Jong, 2003).

The reduction in the length of the incision wound in mice is inseparable from the role of bioactive compounds found in African leaves, such as flavonoids, tannins, and saponins, which play a synergistic role and contribute to the wound healing process. Meanwhile, in the positive control, 10% povidone-iodine has a working mechanism by slowly releasing iodine elements after direct contact

with tissue, inhibiting bacterial enzymatic metabolism, disrupting bacterial growth, thereby weakening bacteria (Risa et al., 2018).

The wound length measured until day 14 was analyzed using *one way ANOVA* analysis to determine whether there was a significant difference in the effect of the extract on wound healing in mice. The results of the *one way ANOVA* analysis are shown in the following table:

Table 3. One way ANOVA analysis results

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1391,764	13	107,059	151,549	0,000	1,961
Within Groups	29,67	42	0,706			
Total	1421,434	55				

Source: Primary data, 2024

Based on the results of *the Anova One Way* data analysis, it was obtained that the F value was greater than F Crit ($151.549 > 1.961$), this indicates a significant difference and positive effect of African leaves. Thus, it can be concluded that 96% ethanol extract of African leaves (*Vernonia amygdalina Del.*) is effective in reducing the length of the cut wound.

CONCLUSION

From the research results, the following conclusions were obtained:

96% ethanol extract of African leaves (*Vernonia amygdalina Del.*) is proven to contain alkaloids, flavonoids, saponins, and tannins. 96% ethanol extract of African leaves (*Vernonia amygdalina Del.*) shows activity in healing cuts. With an F value greater than F Crit ($151.549 > 1.961$), H_0 is rejected and H_1 is accepted. The healing time at a concentration of 0.1 g was achieved on the 14th day, a concentration of 0.2 g on the 13th day, and a concentration of 0.3 g on the 12th day.

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