EFFECTIVENESS TEST OF SOURSOP LEAF ETHANOL EXTRACT (*Annona Muricata L*) AS A HEALER OF INCISION WOUNDS ON MICE (*Mus Musculus*) SKIN

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ARTICLE INFORMATION

Received: 4 April 2025
Revised : 9 Mei 2025
Accepted: 25 Juni 2025
DOI:

KEYWORDS

Keywords: Soursop leaves (Annona muricata L), Incision wounds, Mice (Mus musculus).

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A B S T R A C T

Soursop leaves (Annona muricata L.) are one of the traditional plants that are useful for healing wounds. Wounds are a condition that is often experienced by everyone, whether with mild, moderate or severe severity. Wounds are damage to part of the body's tissue. This study aims to determine the effectiveness of soursop leaf ethanol extract (Annona muricata L.) as a healer of incision wounds on mice (Mus musculus) skin. The method used in making the extract is the maceration process of simplicia using 96% ethanol. The test animals consisted of 15 white mice (Mus musculus), which were divided into 5 treatments. The first treatment was negative control (aquadest), the second treatment was positive control (povidone iodine 10%), the third treatment was extract 0.1 grams, the fourth treatment was 0.2 grams and the fifth treatment was 0.3 grams. In each treatment, an incision was made on the back of the test animal. The treatment was given once a day for 14 days. The results of this study indicate that ethanol extract of soursop leaves (Annona muricata L.) can accelerate the healing of incision wounds in test animals, mice (Mus musculus), namely with the treatment of 0.3 grams of extract. The results of the anova statistical test on incision wounds in mice using soursop leaf extract obtained a significance value of P> 0.05 (at a level of 0.05, namely 0.000 <0.005), meaning that each treatment gave a meaningful difference (significant), for this it is necessary to continue with testing the average difference of each treatment with the Post hoc tests LSD test.

INTRODUCTION

Indonesia is one of the world's biodiversity communities. From Sabang to Merauke, there are around 40,000 types of plants that contain various synthetic substances that can be used as food, beauty care products, and medicines. Traditional medicine is again widely chosen as a medicine to cure various diseases because in addition to having no side effects, its efficacy is also promising. This is very beneficial for the people of Indonesia (Ariani et al., 2020). Indonesia has known and used plants as medicine. Many plants found in nature are always used as traditional medicine because these plants are efficacious in curing diseases. One type of plant that is easily found in the environment is the soursop plant. The soursop plant has properties as an astringent, antibacterial and anticonvulsant (Mardiana, 2004).

The use of natural ingredients is increasing every year (Yolandari & Mustiqawati, 2022). Traditional medicine is again in demand by the public as an alternative medicine. This is because traditional medicine does not require a large cost, while modern medicine using chemical drugs usually requires more expensive costs. In addition, traditional medicine can be obtained without a doctor's prescription, the raw materials do not need to be imported and medicinal plants can be grown by the user themselves, and the risk of side effects is less compared to chemical drugs. One type of medicinal plant that is often used as a traditional medicine by the community is Annona muricata L. or better known as soursop (Djauhariya 2004). Soursop plants contain antioxidants. Antioxidants are substances that can neutralize free radicals that cause atoms and electrons to pair up (Mustiqawati et al, 2022).

Soursop (*Annona muricata L*) is one of the fruit plants that tastes sweet and sour. Fruit plants that grow a lot in yards and fields up to an altitude of about 1000 m above sea level. Soursop has great benefits for humans, namely as a food ingredient, medicine and herbal insecticide (Sari et al., 2014).

Soursop plants (*Annona muricata L*) contain toxic substances for insects. Raw fruit, seeds, leaves and soursop roots contain anonian 2 and resin can work as stomach poison and insect contact poison. Soursop leaf extract can be used to overcome grasshopper pests. In addition, the leaves and seeds can act as insect repellents and antifeedants for insects (Moniharapon et al., 2015).

Soursop is the easiest type of plant to grow among other Anonna species and requires a warm and humid tropical climate (Arief, 2012). This plant can grow at an altitude of up to 1200 m. Soursop plants will grow well in climate conditions with temperatures of 22-28 ° C, with humidity and rainfall ranging from 1500-2500 mm per year (Bilqisti, 2013). Soursop trees have spread to various countries. This plant was brought by the Spanish to the Philippines and has been proven to grow in most tropical countries, including Benin, Cambodia, China, Cote d'Ivoire and including Indonesia (Zuhud, 2011). Soursop (Annona muricata L) is a plant originating from the Caribbean, South America and Central America. Soursop plants in Indonesia originate from Mexico and are thought to have been brought to Indonesia by the Dutch during the colonial period, now soursop plants are spread throughout Indonesia, but are more commonly found as house yard plants. In Java, the distribution of soursop plants can be found in West Java, especially Raja Mandala and South Bandung and Karanganyar in the Central Java region (Sunarjono 2005).

Wounds are a damage to the integrity of the skin that occurs when the skin is exposed to temperature, chemicals, friction, and radiation. Wound healing is the body's response to various injuries with a complex regeneration process that results in continuous restoration of anatomy and function (Suryadi et al., 2013). The shape of the wound varies according to the cause, there are open and closed wounds. Closed wounds are characterized by internal bleeding and open wounds are characterized by lacerations on the skin with external bleeding such as incision wounds. An incision wound is a wound made using a scalpel to open deeper tissue or organs by considering the size, location, and purpose of making the wound (Alphama & Suhaymi, 2021). Treatment of incision wounds generally uses conventional drugs such as topical antibiotics. The use of medical drugs that are used for a long period will cause dangerous side effects for the body so that other alternatives are needed by using herbal plant medicine. Herbal plants contain active compounds that are efficacious as treatment.

In previous research, soursop leaf extract ointment on the back skin of mice infected with *S. aureus* showed that the ointment preparation was able to inhibit the growth of the test bacteria, which healing time of infection was marked by the disappearance of pus until the back wound on the skin of the mice dried (Hasmila et al., 2015). Based on the background above, the researcher wants to research "Test of Ethanol Extract Activity of Soursop Leaves (*Annona muricata L*) as a Healer of Incision Wounds on Mice (*Mus musculus*) Skin" because the compounds contained have the same activity as previous research.

METHOD

The research conducted in this study was an experimental research type by testing the effectiveness of ethanol extract of soursop skin (*Annona muricata L*) as a healer of incision wounds on the skin of mice (*Mus musculus*).

Time and Location of Research

This research was conducted in July-August 2024. The extract was made at the Mandala Waluya University Laboratory, Kendari and the concentration weighing was carried out at the Baubau Polytechnic Laboratory.

Sampling Location

The samples used in this study were from soursop leaves (*Annona muricata L*) taken in Baubau City.

Tools

The tools used in this study were stirring rods, vessels for maceration, blenders, Erlenmeyer flasks, scissors, handscoons, gauze, mouse cages, cotton, filter paper, masks, ovens, hair clippers, rulers, droppers, rotary evaporator vacuums, scalpels, syringes, test tubes, digital scales, tissues and glass jars.

Materials

The materials used in this study were distilled water as a negative control, 96% ethanol, ether, standard feed, 10% povidone iodine ointment as a positive control and dried soursop leaf simplicia.

Working Procedure

Making Simple Drugs

Take 2 kg of soursop leaves and do wet sorting, then wash the soursop leaves until clean and cut into small pieces then dried by airing. Then the dried soursop leaves are weighed and made into powder using a blender and finally stored in a glass jar.

Making Soursop Leaf Extract (*Annona muricata L*)

Prepare the tools and materials to be used, grind 500 grams of soursop leaf simplicia, put the simplicia powder into a maceration vessel, then macerate the powder with 2.5 liters of 96% ethanol with several stirrings, then cover and let stand for 3 days while stirring occasionally and this maceration process is carried out for 1x24 hours, then the maceration results are filtered and the filtrate is taken and evaporated using a rotary evaporator at a temperature of ±40ºC so that a thick soursop leaf ethanol extract is obtained.

Weighing the Concentration of Soursop Leaf Extract

Concentration 0.1 grams, weigh 0.1 grams of soursop leaf ethanol extract then put it in a small container, then stir until homogeneous, concentration 0.2 grams, weigh 0.2 grams of soursop leaf ethanol extract then put it in a small container, then stir until homogeneous, concentration 0.3 grams, weigh 0.3 grams of soursop leaf ethanol extract then put it in a small container, then stir until homogeneous.

Wound Making Stages

Before the incision is made, the location for the incision is first determined, namely at one third of the body length from the mouse's head, after the incision is made, the fur around the mouse's back is shaved, then the mouse's back is disinfected using an alcohol swab, then the mouse is anesthetized using ether, then continued with making an incision on the mouse's back. The wound is made in a square shape with a wound area of 1 cm, by first lining the skin. Then pinched with tweezers using the left hand and the right hand incises with a scalpel to the subcutaneous part.

Wound Care Stages

The incision wound was treated openly, then wound care was carried out on all research samples, in the wound treatment, ethanol extract of soursop leaves was smeared with 0.1 grams, 0.2 grams and 0.3 grams, then in negative control mice, wound care was carried out by giving distilled water, while in mice with positive control, wound care was carried out using 10% povidone iodine ointment, this treatment was carried out once a day for 14 days.

Wound Healing Observation

Observation is done by observing the presence/absence of redness in the wound, swelling in the wound and the area of the wound. This observation is done by observing visually every 1 day until the 14th day by measuring with a caliper, the wound is considered healed if the diameter of the wound reaches the nearest 0 cm or closes the wound.

Data analysis

The data analysis technique used in this study is the analysis technique using the Anova-one-way test with one treatment one sample group. Anova one way (one-way analysis of variance) is usually used to test the average/effect of treatment from an experiment using one factor, where one factor has three or more groups.

RESULTS & DISCUSSION

In this study, soursop leaf samples (Annona muricata L) were used, obtained from Bukit Wolio Indah Village, Wolio District, Baubau City. Ethanol extract of soursop leaves (Annona muricata L) was given to test animals, mice (Mus musculus) to see the healing period of incision wounds. The results of the study are stated in the following table:

Table 1. Incision Wound Size in All Treatments

Treatment	Wound healing day (cm)															Average
	To-	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Negative Control (Aquadest)	1	1	1	0,96	0,91	0,88	0,86	0,83	0,78	0,76	0,71	0,65	0,59	0,53	0,42	0,79
	2	1	0,99	0,95	0,92	0,88	0,86	0,84	0,78	0,76	0,70	0,67	0,58	0,52	0,40	0,86
	3	1	0,98	0,95	0,92	0,87	0,85	0,84	0,79	0,75	0,71	0,65	0,59	0,53	0,40	0,77
Positive Control (Povidone 10%)	1	1	0,90	0,85	0,79	0,67	0,57	0,45	0,32	0,28	0,10	0	0	0	0	0,42
	2	1	0,91	0,85	0,77	0,68	0,58	0,43	0,31	0,27	0,11	0	0	0	0	0,42
	3	1	0,90	0,86	0,77	0,67	0,59	0,43	0,32	0,29	0,10	0	0	0	0	0,42
Extract 0,1 gram	1	1	1	0,90	0,86	0,81	0,75	0,70	0,66	0,60	0,59	0,53	0,45	0,30	0,20	0,67
	2	1	1	0,91	0,85	0,81	0,75	0,69	0,67	0,61	0,57	0,51	0,45	0,30	0,20	0,67
	3	1	1	0,90	0,85	0,82	0,76	0,70	0,66	0,61	0,57	0,51	0,45	0,29	0,20	0,67
Extract 0,2 gram	1	1	1	0,88	0,83	0,75	0,66	0,56	0,50	0,42	0,32	0,25	0,19	0,09	0	0,53
	2	1	0,98	0,89	0,83	0,76	0,67	0,57	0,49	0,44	0,33	0,25	0,18	0,10	0	0,54
	3	1	1	0,88	0,84	0,76	0,66	0,56	0,49	0,44	0,33	0,24	0,19	0,09	0	0,53
Extract 0,3 gram	1	1	0,95	0,90	0,83	0,75	0,68	0,61	0,51	0,41	0,33	0,23	0,10	0	0	0,52
	2	1	1	0,89	0,83	0,74	0,67	0,59	0,50	0,41	0,34	0,23	0,10	0	0	0,52
	3	1	0,95	0,89	0,84	0,74	0,68	0,59	0,50	0,42	0,33	0,23	0,10	0	0	0,48

Source: Primary Data, 2024

In this study, wound care was carried out using topical soursop leaf ethanol extract which was given to the backs of mice that had been given incision wounds. The acceleration of the healing time of incision wounds in test animals was due to the presence of active compounds derived from soursop leaf extract. The anti-inflammatory activity of soursop leaf extract is due to the presence of flavonoids, tannins, saponins and alkaloids. Flavonoids as antibacterials have a working mechanism that inhibits the function of bacterial cell membranes (Saputri et al., 2021).

Tannins are useful as astringents or stop bleeding and accelerate wound healing. Saponins can reduce surface tension so that they can increase cell leakage and cause intracellular compounds to come out. Saponins have the ability as cleaners and antiseptics that function to kill and prevent the growth of microorganisms. Alkaloids have the ability as antibacterials. The suspected mechanism is by disrupting the peptidoglycan component of bacterial cells, so that the cell wall layer is not formed perfectly and causes lysis in bacterial cells (Lallo et al., 2020).

Giving 10% povidone iodine in wound care can reduce redness and its antibacterial content. Works directly by quickly killing germs, not inhibiting the growth of germs. Based on this theory, the therapeutic effect of povidone iodine as a broad-spectrum antiseptic can help the healing process of incision wounds in mice in the positive control group.

This study shows the results of changes in the length of incision wounds in mice (*Mus musculus*). This treatment was carried out by observing the length of the wound for 14 days, the wounds were observed once a day and given different extracts, namely positive control, negative control and ethanol extract of soursop leaves (*Annona muricata L.*) 0.1 grams, 0.2 grams and 0.3 grams.

From the results of this study, there has been a change in the length of the cut wound where the treatment with negative control (aquadest) and 0.1 gram extract had the slowest healing process. In the treatment of 0.2 and 0.3 gram extracts, the healing process of the cut wound increased slightly or was faster compared to the negative control and 0.1 gram extract. And in the positive control, it was the

treatment that provided the most repair process in the cut wound of mice with the final result of the length of the cut wound being 0 cm. The difference in the healing process of the cut wound was due to the first treatment, namely the negative control (aquadest) with its content that was not better than povidone iodine. In the second treatment, namely the positive control (10% povidone) with the content contained in it, it was effective for healing cuts. And in the third treatment, the healing was seen to be different because of the amount of ethanol extract in the 0.1, 0.2, 0.3 extract treatments, different amounts of extract. From the results of the study, it was found that 0.3 gram extract was the best concentration as a healer of cut wounds in mice, based on its speed in healing wounds.

Table 2. Results of One Way Anova Analysis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2770.933	4	692.733	123.702	.000
Within Groups	56.000	10	5.600		
Total	2826.933	14			

Analysis of variance (Analysis of variance) or anova is a statistical analysis method that is included in the branch of inferential statistics. Anova output is the end of the calculation used to determine the analysis of the hypothesis to be accepted or rejected. This anova test is used to determine whether the average of the four samples is comparable or different. Decision making in the one-way anova test used is if the significance value (Sig)> 0.05 means that the average is the same and if the significance value (Sig) <0.05 means that the average is different.

From the results of the one-way anova analysis of the length of the wound, the results of Sig <0.05 were obtained which means that there is a significant difference. This states that the administration of soursop plant extract to incision wounds has a positive effect that can heal wounds. Thus it can be concluded that soursop leaf extract has properties in healing cuts.

CONCLUSION

Based on the results of the research that has been done, it was found that soursop leaf extract (*Annona muricata L.*) is able to provide a healing effect on cuts. And the results showed that the most optimum extract was found in 0.3 grams of extract with the fastest healing of cuts compared to extract concentrations of 0.1 grams and 0.2 grams.

One of the limitations of this study is the limited variation in extract doses, so it has not been able to determine the truly optimal dose. Further research is needed to test more diverse doses and their long-term effects.

ACKNOWLEDGMENTS

My deepest gratitude to my supervisors Mrs. Apt. Sri Yolandari, S.Farm., M.Si and Mr. Suparman Supardi, S.Si., M.Si for the guidance and support given during the writing of this scientific journal. Without their valuable direction and advice, this research could not have been completed properly.

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