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DIABUCURE (Effectiveness Test of *Caesalpinniasappan* L wood and *Acalypha indica L* Leaf as Alternative Spray for Ulcus Diabetic Type 2 in *Musmuculus*)

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ABSTRACT

Caesalpinia sappan L wood and *Acalypha indica* L leaves contain flavonoids as the main compounds that significantly contribute to accelerating the healing of diabetic ulcers in *Mus muculus* and can impact the quality of life of the affected individuals. The aim of this research is to test the potential of *Caesalpinia sappan* L wood and *Acalypha indica* L leaves in the healing of diabetic wounds in mice and to determine the most effective formulation for Diabucure. The method used involves extracting the compounds from *Caesalpinia sappan* L wood and *Acalypha indica* L leaves to create Diabucure, inducing diabetic compounds (Alloxan monohydrate), creating wounds with a length of 1 cm and infecting them, and applying sprays with different compositions such as 100% pure extract, pure extract to alcohol ratios of 1:2 and 2:1 for 7 days. The research findings show that the wound lengths in groups T1, T2, and T3 are 0.7, 0.2, and 0.6 respectively, following the application ofDiabucure sprays containing flavonoids, terpenoids, tannins, and saponins. The spray formulation of 1:2 (T2) has a more significant effect compared to other treatments, showing a drastic reduction in wound length within 7 days. Thus, sprays containing *Caesalpinia sappan* L wood and *Acalypha indica* L leaves have the potential to be an alternative therapy for diabetic ulcers.

Keywords: *Diabetic Ulcers, Caesalpinniasappan L, Acalypha indica L, Spray, Musmuculus*

1. Introduction

1.1 Background

Diabetes mellitus is a chronic medical condition characterized by high blood sugar levels in the body due to impaired production or use of insulin(American Diabetes Association, 2020).

According to International Diabetes Federation (IDF) 2021, as many as 19,47 million people in Indonesia have diabetes. With a population of 179,72 million, this means that the prevalence of diabetes in Indonesia is 10.6%.

Diabetic wounds are a serious complication that can occur in people withtype 2 diabetes

mellitus. Due to high blood sugar levels and problems with bloodcirculation caused by diabetes, wounds on the feet or other areas tend to healslowly. This can lead to infection, gangrene and even amputation if not treatedproperly(Boulton, A. J.*etal*, 2020).

Indonesia is an agricultural country and has many herbal plants,which are *Caesalpiniasappan*Lthat containsflavonoids, brazilin,alkaloids saponins, tannins, phenyl propane, and terpenoids (Nomer, et. al., 2019). And also *Acalypha indica* L. which are known as weed plantsthecomponents contained in this plant are β-sitosterol and daucosterol, saponins,tannins,flavonoids andessentialoils(Anonymous, 2009).

In this study, diabetes-induced mice (*Musmuculus*) were used as subjects(testanimals).Theuseofmice astestanimalsisbecausetheyhavebee nrecognized as a good experimental animal model, easy to handle, can be obtainedinlargequantities, and provide reliable repeatvalues (Gelfand, 2002).

1.2 Research Objectives

1.2.1 To determine the process of making a combination of *Caesalpinnia sappan* L extractandearring leaves as an inhibitorof type 2 diabetes mellitus.

1.2.2 To determine the process of testing the effectiveness of a combinationspray of *Caesalpinnia sappan* L extract and *Acalypha indica* L leaves as an inhibitor of type 2diabetesmellitus.

1.2.3 To determine which comparison is most effective in healing type 2diabetesmellitus wounds.

1.3 Aim

1.3.1 Researcher

Obtain information on the effectiveness of a combination of nano-particle extracts of *Caesalpinnia sappan* L and *Acalypha indica* L leaves as an inhibitor of type 2diabetesmellitus.

And obtainedinformationaboutthepr ocessofmakingacombinationspr ayofnano-particleextractsof*Caesalpinnia sappan* Land*Acalypha indica* Lleavesasaninhibitorof type 2 diabetes mellitus.

Also for knowingtheshortcomingsthatex istinthecombinationofnano-particleextractof*Caesalpinnia sappan* Land*Acalypha indica* Lleavessothatitcanbeusedasane valuation material to beused as an improvement.

1.3.2 Community

Helping people with type 2 diabetes mellitus to get treatment at a more affordable cost, also helps reduce complications that occur in people with type 2 diabetes mellitus.

1.3.3 Environtment

Utilizing *Acalypha indica* L leaves, which are weeds, as herbal medicine.

2. Basic Theories

2.1 Diabetes Mellitus

More than 90% of diabetics have type 2 diabetes mellitus, characterized by gradual loss of pancreatic beta cell function due to insulin resistance. This resistance in muscle, adipose tissue, and liver is called insulin resistance. Insulin levels may be low or within normal limits, making it non-insulin dependent. Factors such as decreased muscle mass, physical inactivity, changes in body composition, dietary habits, and neurohormonal changes contribute to insulin resistance in the elderly.

2.2 *Caesalpinniasappan* L

Caesalpiniasappan L.) is a small tree from the Caesalpiniaceae family. It contains phenolic compounds such as triterpenoids, alkaloids, tannins, phenols, saponins, steroids, and flavonoids, including *Caesalpiniasappan*, which have antioxidant, anti-diabetic, anti-inflammatory, antibacterial, and anticancer effects.

2.3 *Acalypha indica* L

Acalypha indica L is a medicinal weed that grows wild on roadsides, lawns, and hills to mountains. It has bitter, cold, and astringent taste. It contains alkaloids, saponins, phenolic compounds, steroids/triterpenoids, tannins, volatile oils, methylellagic acid, β-sitosterol-β-D-glucoside, cyanogenetic glycoside, triacetocalypine, n-octasanol, β-sitosterol acetate, kaempferol, kebractyl, resin, cyanitic acid, and essential oil. Phytochemical tests have been conducted on *Acalypha indica* L extracts by Vijayarekha et al. (2015).

2.4 Extraction

Active substances in plants and animals are more soluble in organic solvents. During extraction, organic solvents penetrate the cell wall and dissolve the active ingredient within the cells, creating a concentration difference. Maseration is a method where the material is turned into coarse powder, mixed with solvent in a closed container, and left at room temperature for at least 3 days.

2.5 *Musmuculus*

Mice (*Mus musculus*) are small, adaptable mammals found worldwide. They have a compact body, pointed snout, round ears, and a long tail. Their sharp incisors continuously grow, allowing them to gnaw and chew on different materials. Mice are generally small in size, ranging from a few centimeters to a few inches long.

2.6 REEDA Scoring

The REEDA scoring system assesses postpartum healing. It includes Redness, Edema, Ecchymosis, Discharge, and Approximation. Redness (Inflammation indicates infection or healing issues), Edema (Swelling shows healing progress and complications), Ecchymosis (Bruising or discoloration shows tissue

trauma and healing progress), Discharge (Fluid or exudate reveals healing and infection presence), Approximation (Evaluates wound closure and alignment. Poor approximation suggests delayed healing or further intervention). The REEDA scoring system aids in monitoring healing, allowing timely intervention for complications and promoting recovery

2.7 Spray

Spray is the act of spreading liquid or particles in mist form using aerosol sprays or other equipment. It can involve water, chemicals, and more.

3. Method and Experimental Details

This research was conducted from April to June 2023. The sampling locations for CaesalpiniaSappan L wood were SMAN 3 Semarang area, while Acalypha indica L leaves were sampled in Halmahera, East Semarang. The study was carried out at the Chemistry Laboratory of SMAN 3 Semarang, Chemistry Laboratory of Semarang State University, and Biology Laboratory of Semarang State University. 8-10 week-old mice, weighing 35-40 grams obtained from the Biology Laboratory of Semarang State University were used as the test animals. The sample testing was performed at the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Semarang State University. Sampling was conducted in April, while sample testing and other data analysis were carried out from May to June 2023.

Diabucure was prepared by soaking 300 grams of *CaesalpiniaSappan*L wood powder in 3000 ml of 96% ethanol, and 100 grams of *Acalypha indica* L leaf powder in 1000 ml of the same solvent in separate maceration containers for 5 days, followed by filtration. The maceration result was made into pure extract using a Rotary Evaporator, and then used to create 4 spray samples. The first spray consisted of 25 ml of 70% alcohol, the second spray was a combination of 25 ml of pure *Caesalpiniasappan*extract and 1 ml of *Acalypha indica* extract, the third spray was a combination of pure extract with alcohol in a 1:2 ratio (7.7 ml of *Caesalpiniasappan* extract and 0.3 ml of *Acalypha indica* extract, dissolved in 16 ml of 70% alcohol), and the fourth spray was a combination of pure extract with alcohol in a 2:1 ratio (17.3 ml of *Caesalpiniasappan* extract and 0.7 ml of *Acalypha indica* extract, dissolved in 9 ml of 70% alcohol). The sprays were placed in spray bottles.

Type 2 Diabetes Mellitus induction in experimental animals was performed by acclimating the mice to cages. Alloxan induction was administered once by intraperitoneal injection of 175 mg/kg body weight alloxan monohydrate in 0.1 M sodium citrate buffer, followed by a waiting period of 3 days for the

mice's blood glucose levels to increase. The mice were then divided into 5 treatment groups: K (diabetic ulcer + infection + no treatment), K+ (diabetic ulcer + infection + 70% alcohol spray), T1 (diabetic ulcer + infection + pure extract spray), T2 (diabetic ulcer + infection + 1:2 spray), and T3 (diabetic ulcer + infection + 2:1 spray).

To create wounds, the hair on the dorsal area of the mice was removed, and a 1 cm long incision was made using a surgical blade. Infection was induced by allowing the wound to be infected with bacteria in the cage for one night. The spray application on diabetic ulcers in the mice was conducted for 7 days, once a day with 3 sprays in each application. Histological observations of the dermal ulcers that received the spray treatment were conducted, and conclusions were drawn regarding the most effective treatment for diabetic ulcers in mice.

4. Result and Discussion

No	Group	Blood Glucose Levels in Mice	
		Day 1	Day 3
1	K	117	159
2	K+	71	118
3	T1	116	195
4	T2	81	117
5	T3	77	101

Group	Number	Weight (gram)	SCORING DAY 1					
			R	E	E	D	A	TOTAL
K	1	31	1	1	1	3	2	8
	2	22	1	1	1	3	2	8
	3	34	1	1	1	3	2	8
K+	1	31	1	1	1	3	2	8
	2	40	1	1	2	3	2	9
	3	32	1	1	1	3	2	8
T1	1	30	1	1	1	3	2	8
	2	31	1	1	1	3	2	8
	3	33	1	1	1	3	1	7
T2	1	33	1	1	1	2	2	7
	2	27	1	1	1	3	2	8
	3	29	1	1	1	3	2	8
T3	1	35	1	1	1	3	1	7
	2	27	1	1	1	3	2	8
	3	27	2	1	1	3	2	9

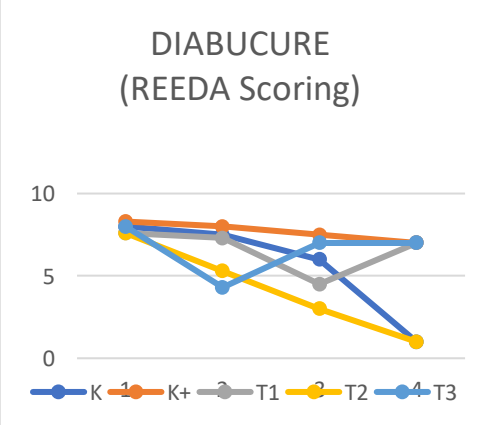
Group	Number	Weight (gram)	SCORING DAY 3					
			R	E	E	D	A	TOTAL
K	1	31	1	1	0	3	2	7
	2	22	1	1	1	3	2	8
	3	34	1	1	1	3	2	8
K+	1	31	1	1	1	3	2	8
	2	40	1	1	1	3	2	9
	3	32	1	1	1	3	2	8
T1	1	30	1	1	1	3	2	8
	2	31	1	1	0	3	2	7
	3	33	1	1	0	3	2	7
T2	1	33	1	1	0	3	1	6
	2	27	1	1	0	3	2	7
	3	29	1	1	0	0	1	3
T3	1	35	1	1	0	0	1	3
	2	27	1	0	0	0	1	2
	3	27	1	1	1	3	2	8

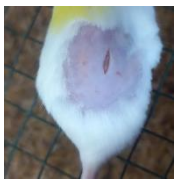
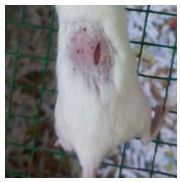
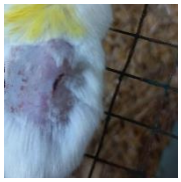
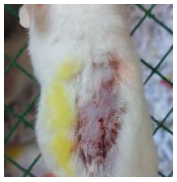
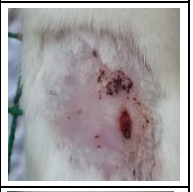
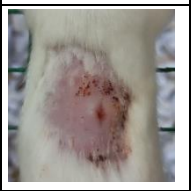
Group	Number	Weight (gram)	SCORING DAY 5					
			R	E	E	D	A	TOTAL
K	1	31	1	1	0	3	2	7
	2	22	1	0	0	3	2	6
	3	34	1	0	0	3	1	5
K+	1	31	1	1	0	3	2	7
	2	40	1	1	1	3	2	8
	3	32	0	0	0	0	0	0
T1	1	30	1	1	0	3	2	7
	2	31	1	0	0	0	1	2
	3	33	0	0	0	0	0	0
T2	1	33	1	1	0	3	0	5
	2	27	0	0	0	0	1	1
	3	29	0	0	0	0	0	0
T3	1	35	0	0	0	0	0	0
	2	27	0	0	0	0	0	0
	3	27	1	1	0	3	2	7

Group	Number	Weight (gram)	SCORING DAY 7					
			R	E	E	D	A	TOTAL
K	1	31	0	0	0	0	0	0
	2	22	1	0	0	0	1	2
	3	34	0	0	0	0	1	1
K+	1	31	1	1	0	3	2	7
	2	40	1	1	0	3	2	7
	3	32	0	0	0	0	0	0
T1	1	30	1	1	0	3	2	7
	2	31	0	0	0	0	0	0
	3	33	0	0	0	0	0	0
T2	1	33	0	1	0	0	0	1
	2	27	0	0	0	0	0	0
	3	29	0	0	0	0	0	0
T3	1	35	0	0	0	0	0	0
	2	27	0	0	0	0	0	0
	3	27	1	1	0	3	2	7

Based on the table above, it can be seen that there is an increase in blood glucose levels in mice after being induced with alloxan. Therefore, the mice have developed type 2 diabetes mellitus.

And a decrease of ulcusdiabeticum is observed in the following graph.



Day 1					
Day 3					
Day 5					
Day 7					
GROUP	K	K+	T1	T2	T3

Ulcus diabetic day-1, day-3, day-5, and day-7

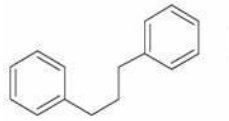
In this study, a comparison of diabetic ulcer healing time was conducted. The wound length decreased on the third day of spray application. The initial wound length was 1 cm. The pure extract spray (T1) showed a decrease to 0.7 cm on the fifth day, the 1:2 ratio spray (T2) showed a decrease to 0.2 cm on the fifth day, and the 2:1 ratio spray (T3) showed a decrease to 0.6 cm on the fifth day.

The analysis results indicated that the wood of *Caesalpiniasappan* L and the leaves of *Acalypha indica* L demonstrated the ability to accelerate the healing time of clean wounds. In this case, the observed factors were the time required for the skin color to return to normal and the decreasing wound length. The research results showed that the pure extract spray formulation (T1) was ineffective in wound healing due to the absence of antiseptic compounds that can kill or inhibit the growth of pathogenic microorganisms on the surface of diabetic wounds. One of the requirements for wound healing is the absence of infection in the wound itself. The T3 spray formulation (Pure Extract: Alcohol, (2:1) was also less effective in healing diabetic wounds in mice. This was because the research results indicated a higher amount of extract than antiseptic, making the extract

unstable and causing argonoleptic effects. This could be attributed to hydrolysis, where the components in the extract might be susceptible to hydrolysis in the presence of water contained in alcohol. The hydrolysis process can cause changes in the chemical structure of extract components, which in turn can affect stability and argonoleptic properties. Changes in polarity may also occur because alcohol has high polarity, and the addition of alcohol to pure extract can alter the overall polarity of the mixture. Precipitation can occur as well, where the addition of alcohol to pure extract can cause precipitation or deposition of certain compounds. This particularly happens when the concentration of alcohol used is very high. Precipitation can change the physical properties of the extract and cause changes in its argonoleptic properties.

Furthermore, the study obtained results indicating that the best ratio in the preparation of this spray was (Pure extract : Alcohol, 1:2), which showed significant healing results. In this case, the pure extract did not experience argonoleptic effects, and alcohol remained a good antiseptic, as evidenced by the absence of infection in diabetic wounds in mice. Active compounds, such as flavonoids, are believed to have the effect of reducing the number of days of inflammation or acting as anti-inflammatory agents by improving blood circulation throughout the body and preventing blockages in blood vessels. They also function as antioxidants and help reduce pain in case of bleeding or swelling.

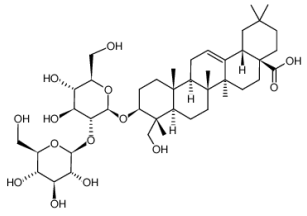
Flavonoids are known to have antioxidant activity as they can donate protons to unstable compounds, making them stable. Flavonoids stabilize reactive oxygen species (ROS), preventing them from attacking cells and inhibiting the process of



Flavonoid

angiogenesis, thus accelerating wound healing. The mechanism of flavonoids in wound healing involves increasing the proliferation of epithelial cells and collagen, thereby improving the wound healing process.

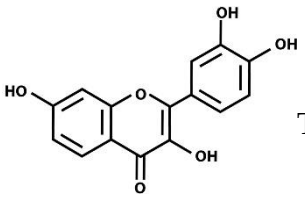
Saponins, which are triterpenoid compounds, have been identified to reduce inflammatory symptoms (such as erythema and edema), exhibit antimicrobial properties, influence collagen, and repair and strengthen skin cells. Another mechanism of saponins in wound healing is stimulating the formation of type 1 collagen, which plays a crucial role in the wound closure process and enhances tissue



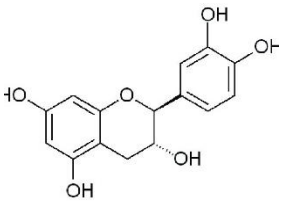
epithelialization. They also act as antimicrobials, antioxidants, and promote epith.

The presence of terpenoids also affects the wound healing process due to their anti-inflammatory properties. Excessive inflammation can hinder the wound healing process, and the presence of anti-inflammatory properties can help reduce inflammation in diabetic wounds. Terpenoids can also have antimicrobial effects, which means they have the ability to kill or inhibit the growth of pathogenic microorganisms.

Additionally, they have the ability to enhance cell proliferation and collagen synthesis, which are important in the formation of new tissue and wound closure. In addition, there are tannin compounds, which have astringent effects, meaning they have the ability to shrink and tighten tissues. This can help stop bleeding in wounds and aid in the formation of blood clots. Furthermore, tannins have antioxidant properties that can help protect cells from damage caused by free radicals and oxidative stress. Wound healing in diabetes is often hindered by oxidative stress, so the antioxidant properties of tannins can help accelerate healing.



Terpenoid



Tanin

5. Conclusion

Based on the research findings, it can be concluded that the administration of ethanol extracts from CaesalpiniaSappan L wood and Acalypha indica L leaves has an effect on reducing the length of diabetic wounds in mice. The Diabucure 1:2 spray formulation had the most significant impact compared to other ratio combination, achieving wound closure within 7 days. Therefore, the *Caesalpiniasappan* L wood and *Acalypha indica*L leaf spray have the potential as alternative therapies for diabetic ulcers.

For future research, it is expected that the spray formulation can be made less concentrated and colorless. Additionally, skin irritation tests should be conducted to determine the safety of Diabucure on the skin.

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hoping that this research is able to provide more benefits in the future.

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