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Evaluation of Wound Healing Potential of Ethanol-Seed Extract of *Chrysophyllum albidum* in Wistar Rat

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ABSTRACT

Wound healing activities of the ethanol-seed extract of Chrysophyllum albidum have been established. However, scientific evaluation of its wound healing potential at different concentrations is lacking. Thus, the present study aimed to assess the wound healing activity of ethanol extract of Chrysophyllum albidum seeds at different concentrations. The seed cotyledon was extracted with ethanol by continuous cold extraction. Phytochemicals, antioxidant and antimicrobial activities of the extract were assayed by standard methods. Five groups of male wistar rats consisting of five animals each was used for the animal studies. The group 1 served as the negative control (untreated). Groups 2, 3 and 4 was treated with ethanol extract at varying dose of 2g/ml, 4g/ml and 6g/ml respectively, serving as the test groups. Group 5 animals served as the positive control (reference). All animals were experimentally wounded in the sterilized shaved dorsal fur region. Comparative wound healing efficiency of the control, test and reference groups were assessed.

Quantitative phytochemical analysis of the extracts showed that alkaloid content is 11.14mg/100g dried sample. The scavenging effect of seed extracts and standard (L-ascorbic acid) on the DPPH radical is L-ascorbic acid 51.44 % > Ethanol 49.05% at concentration of 0.6 mg/mL respectively. The ethanol extract of the seed showed high MIC against Serratia fonticola and Aeromonas veronii microorganism with 25 mg/mL concentration. In all groups, the feed intake increased gradually through the weeks. The decreasing order of wound healing capacity of the groups was obtained to be group 5 > group 4 > group 3 > group 2 > group 1 respectively. This implies that the higher the concentration of the ethanol extract of C. albidum seed, the higher the healing potential, the less the feeding and the greater the weight of animals in the post wounding days as applied to the reference groups (group 1). The results of this study offers pharmacological evidence of the folk use of Chrysophyllum albidum seed for wound healing. The study recommended that the usefulness of the seed extract as a cream should be examined in further research.

Keywords: Wound, Ethanol-seed, Chrysophyllum albidum, Wistars rat

INTRODUCTION

Evaluation of the wound healing potential of natural extracts holds significant importance in both traditional and modern medicine. Among the various botanical sources, Chrysophyllum albidum, commonly known as African star apple or white star apple, has garnered attention due to its purported medicinal properties. The ethanol-seed extract of Chrysophyllum albidum has been particularly intriguing for its potential wound healing effects. This research aims to delve into the evaluation of such potential, particularly in Wistar rats, which serve as a common model for wound healing studies (Ogbu, et al., 2024). Morakinyo, et al. (2023) demonstrated the antioxidant potential of Chrysophyllum albidum seed extract, attributing it to its high phenolic content. This antioxidant activity is crucial in wound healing, as oxidative stress can impede the natural healing process by causing cellular damage.

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The antimicrobial properties of Chrysophyllum albidum extracts have been documented in several studies. Odewade & Oluwatosin (2023) investigated the antimicrobial activity of the ethanol extract of Chrysophyllum albidum against common wound pathogens, revealing its potential as an adjunct therapy in wound management. This antimicrobial action is pivotal in preventing wound infections, which can significantly delay the healing process and lead to complications. In addition to its antioxidant and antimicrobial properties, Chrysophyllum albidum extract has shown promising anti-inflammatory effects. Inflammation is a critical component of the wound healing cascade, playing a dual role in both the initiation and resolution of the healing process (Adetoun et al., 2023). Morakinyo et al. (2023) demonstrated the anti-inflammatory activity of Chrysophyllum albidum leaf extract in an experimental model, suggesting its potential in modulating the inflammatory response during wound healing.

Building upon these findings, the present study seeks to evaluate the wound healing potential of the ethanol-seed extract of Chrysophyllum albidum in Wistar rats. Utilizing animal models allows for the investigation of the extract's effects in a controlled environment, providing valuable insights into its efficacy and safety profile. Through comprehensive evaluation encompassing histological, biochemical, and molecular parameters, this research aims to contribute to the growing body of evidence supporting the therapeutic potential of Chrysophyllum albidum in wound management (Adebayo, et al, 2010).

Statement of the problem

The evaluation of the wound healing potential of ethanol-seed extract of Chrysophyllum albidum in Wistar rats addresses several critical questions in wound care research. Despite the long-standing traditional use of Chrysophyllum albidum in folk medicine for wound healing, there remains a dearth of scientific evidence supporting its efficacy and safety. Oluwole, Alagbe, Ibidapo, Samuel, Li & Shen, (2017) have characterized the phytochemical composition of Chrysophyllum albidum extracts, highlighting their antioxidant properties due to their phenolic content. However, the specific effects of these extracts on wound healing outcomes, particularly in vivo, have not been comprehensively elucidated. Additionally, while antimicrobial resistance poses a significant challenge in wound management. George, Adenipekun, Fasogbon & Oparanozie, (2018), have shown promising antimicrobial activity of Chrysophyllum albidum extracts. Nevertheless, the translation of these in vitro findings to in vivo wound healing efficacy remains to be explored. Therefore, the present study aims to address these gaps by evaluating the wound healing potential of ethanol-seed extract of Chrysophyllum albidum in Wistar rats, shedding light on its mechanisms of action and therapeutic implications for wound care.

Human beings and animal gets wounded inescapably or accidentally that needs treatment to heal fast because of infection. Most wound care products are expensive or ineffective due to the presence of other ailment in the body system. Also, there is the need to improve on the local use of *C. albidum* seed by investigating the active components in it.

Objective of the study

This proposed research is aimed at:

(i) Extraction of the uncoated seeds of *Chrysophyllum albidum* with ethanol and applying it on the induced wound of wistar rats to examine its wound healing potential.
(ii) Determining the phytochemical properties of the extract and to discover the pathways that these phytochemicals regulate to enhance wound repair and skin regeneration.
(iii) Determining the antioxidant activity of the extract that could enhance wound healing.
(iv) Determining the antimicrobial activity of the extract.

Significance of the study

This proposed research will be significant in:

* Making *C. albidum* seed useful rather than considering it as a waste i.e converting waste to wealth.

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- Solving wound healing problems.
- Creating job opportunity for the chemist to produce the wound healing ointment commercially.
- It will serve as source of income for the chemist.
- Reducing waste in our environment.
- Generally increasing the rate of wound healing in living organism.

MATERIALS AND METHODS

Materials: *Chrysophyllum albidum* seed, filter papers, experimental animals, polypropylene cages, standard wound healing ointment, distilled water.

Reagents : Ethanol, Ethyl Acetate, Conc. HCL, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), NaOH, H_2O_2, $CuSO_4$, Hydrozylproline, DimethylSulphoxide (DMSO) etc.

Apparatus and Equipments : Test tubes, measuring cylinders, Conical flasks, Beakers, Soxhlet extractor, Rotary evaporator, water bath, microwave, Oven, UV-Visible Spectrophotometer, heating mantle, dessicator, petri dishes etc.

Seed collection and preparation

The seeds of *Chrysophyllum albidum* were collected from local areas and deshelled. Seeds were shade-dried and blended. It was then subjected to extraction with 95% ethanol in soxhlet apparatus by continuous hot extraction and the extract was concentrated using rotary evaporator under reduced pressure.

Phytochemical investigation and screening

Qualitative and quantitative phytochemical investigation and screening of wound healing activities were carried out on the extract. Qualitative determination was carried out followed the method below.

Table 1: Qualitative phytochemical analysis methods.

Phytochemicals	Test	Observation
Alkaloids	Dragendorffs reagent	Yellow precipitate
Terpenoid and Steroid	Libermann-burchard test	Blue and purple respectively
Tannin	Iron III chloride	Blue black precipitate
Flavonoids	Ethyl acetate and ammonia solution	Yellow precipitate
Triterpenes	Chloroform, warm and conc. H_2SO_4	Reddish-brown colour
Saponins	Boil with H_2O	Frothing

Quantitatively, the phytochemicals was determined by preparing standards, taking absorbance, drawing the calibration curve and extrapolating for their concentrations using UV- Visible spectrophotometer which was carried out in a standard public laboratory where the facilities are available.

Investigation of the antioxidant activity

(1) DPPH Free Radical-Scavenging Assay: This assay has been used to investigate the scavenging activity of antioxidant compounds. In fact, DPPH is a stable free radical that can be reduced by a proton-donating substrate like an antioxidant, causing the discoloration of DPPH and reduction of the absorbance at 517 nm.

The DPPH free radical-scavenging potential of the extract was determined according to the reports of Baliyan, Mukherjee, Priyadarshini, Vibhuti, Gupta, Pandey & Chang, 2022). Radical-scavenging activity was expressed as the inhibition percentage and was calculated using the equation of DPPH radical scavenging activity.

$$\text{DPPH radical-scavenging activity (\%)} = \frac{A\text{control} - A\text{sample}}{A\text{control}} \times 100$$

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*A*control is the absorbance of the control reaction and *A*sample is the absorbance of oils/standard BHT samples. The IC50 value (mg sample/mL) is the effective concentration at which the DPPH radicals are scavenged by 50%. The test was carried out in duplicate.

(2) Ferric Reducing Antioxidant Power FRAP. The ability of the oils (0.06mg/mL to 1mg/mL) to reduce iron (III) was determined according to the method of Moalla Rekik, Ben Khedir, Ksouda Moalla, Kammoun, Rebai & Sahnoun, 2016).

The IC50 value (mg sample/mL) is the effective concentration at which the absorbance is 0.5 for the reducing power. BHT was used for comparison and all data values are the mean of duplicate analysis.

(3) β-Carotene Bleaching Assay. This spectrophotometric technique in the ultraviolet ray was developed by Marco (1968) and then slightly modified by Miller (1971). It consists in a measurement at 470 nm. The discoloration of β-carotene results from an oxidation by the linoleic acid.

Evaluation of the antimicrobial activity

The antimicrobial activity of the extract will be evaluated using a range of laboratory control stains: Bacillus subtilis (JN 934392) and Staphylococcus aureus (ATCC 6538), Escherichia coli (ATCC 25922) and Salmonella enteritidis.

Determinations of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) by microdilution method according to Diao, Hua, Zhang & Xu, (2014).

Wound healing experiment

Wistar male rats was selected and divided into five groups having five animals each comprising of the control group, standard group and the test group. They were housed in polypropylene cages and well-ventilated rooms in the animal house of Ajayi Crowther University under controlled conditions of 22–25°C, 60–70% relative humidity and 12 hours of dark-light cycle throughout the study. Animals were allowed access to standard food and water.

Group number (I) was untreated and served as a control (the wounds were cleaned with propylene glycol). Group (II), group (III) and group (IV) was treated with the *Chrysophyllum albidum* ethanol seed extract and would serve as the test groups, while group (V) was treated with standard cream and served as standard reference (positive control). The extract at a varying dose was administered to groups II, III, IV animals as a suspension in propylene glycol. All control animals received propylene glycol only.

A circular excision wound model of about 2.5 cm diameter was induced on the shaved rats' dorsal interscapular region.

Figure 3: Excision wound model (*source:* Ephrem *et. al.*, 2023)

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The day on which wound were created was considered as day 0, all the wounds was covered with a gauze dressing and treated until they were completely healed while adequately observing the healing pattern of the wounds.

Observation of wound healing rate

Photographs of the wounds from the first day of the wound induction until the day of Complete Wound Closure (CWC) was taken every four days to measure the rate of wound healing and comparative wound healing efficiency of the control, test and standard groups. A Canon Powershot 5.0MP digital camera was used for taken the wound photographs. A 15 cm ruler was used as a scale to measure the size of the wound.

Figure 4: Measurement of the size of wound using a scale

Percent relative healing efficiency (RHE) of the extract was calculated to measure how fast the extracts can completely heal the wound when compared with the time required for complete healing without any medicine of the control group and the standard group. Percent RHE was calculated using the following formula:

$$\% \text{ Relative wound healing efficiency} = \frac{T_N - T_E}{T_N} \times 100$$

T_N = Time required for natural wound healing i.e., CWC without any drug/extract. T_E=Time taken for wound healing i.e., CWC with drug/extract (Hanbisa, Tadesse & Abula, 2023).

RESULTS AND DISCUSSION

The formulations yield was 125g and was found to be sticky thick brown colour. This indicates that in every 2500g of the chrysophylum albidum seed cotyledon extract with ethanol, 5% yield is expected after concentrations or evaporations of the solvents. The chemical components extracted from the seed gives its sticky form and the colour originates from the cotyledon innermost colour while its outer colour is yellowish. Qualitative and quantitative phytochemical analysis, antioxidant properties (DPPH, TAC and OH^-) and antimicrobial activity of the extract served as the preliminary in-vitro analysis carried out. The qualitative phytochemical screening revealed that alkaloid, steroid, tannin, flavonoid and triterpene were found to be present in the extract. Quantitatively however, the extract contains 11.14, 190.85, 94.44, 218.51, 204.35 mg/100g of alkaloid, steroid, tannin, flavonoid and triterpene respectively.

Formulations yield

Table 2: %Yield of ethanol extract of *C. albidum* seed

Extract	Total weight of sample (g)	Total volume of solvent (ml)	Total yield (g)	Yield (%)
Ethanol	2500	6410	125	5

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Table 3: Results for quantitative phytochemical analysis

Phytochemical mg/100g	Ethanol
Tannin	94.44
Steroids	190.85
Flavonoid	218.51
Alkaloid	11.14
Triterpene	204.35

Antioxidant analysis

The inhibition of DPPH scavenging radical of the extract was found to be 49.05% and standard (galic acid) was 51.44% at 0.6mg/ml concentration respectively. This shows that there is no significant difference in the DPPH scavenging radical of the extract and the standard making the extract to have excellent activities of this antioxidant. The hydroxyl (OH^-) scavenging radical of extract in 50mg/100g was found to be 9.23% lower than that which was found for n-hexane extract, 11.05% (Babatunde, Oladeji and Ajayi (2019).

The total antioxidant capacity of 10mg/ml of the ethanol extract is 95.91% higher than that which was found for n-hexane extract, 73.02%. The results of the antioxidant activities of the extract revealed excellent property of antioxidative enzymes which helps to maintain balance on the wound site.

Table 4: DPPH % scavenging activity

Concentration (mg/ml)	Ethanol	Standard (Garlic acid)
0.2	29.34	37.70
0.4	36.45	
0.6	49.05	51.44
0.8	50.95	
1	51.53	85.73

Antimicrobial activity

The antimicrobial susceptibility of the extract to the bacterial isolates; *Lactobacillus Delbruecki, Campylobacter and Serratia liquefaciens but it is not susceptible to Klebsielle pneumonia.*

The extract showed a minimum inhibitory concentration (MIC) activity of 25, 6.25, 12.5, 6.25, and 12.5 g/ml against *Serratia fonticola, Serratia Liquefaciens, Pseudomonas, Klebsielle Pneumoniae and Aeromonas Veronii* organisms respectively.

Table 5: Antimicrobial susceptibility (mm)

Bacterial Isolates	Ethanol
Lactobacillus delbruecki	4
Campylobacter	2
Klebsielle pneumonia	-
Serratia liquefaciens	6

Table 6: Activity level of extract using MIC technique

Test Organism	Ethanol
Serratia fonticola	25
Serratia liquefaciens	6.25
Pseudomonas	12.5
Klebsielle pneumoniae	6.25
Aeromonas veronii	12.5

*values are concentration in mg/ml of the extracts

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Wound healing experiment results

The group 1 served as the negative control (untreated). Groups 2, 3 and 4 was treated with ethanol extract at varying dose of 5g/ml, 10g/ml and 15g/ml respectively, serving as the test groups. Group 5 animals served as the positive control (reference or standard group). Average increase of animal weight and feed intake per week was observed in the post wounding. The average animal weight increases in the post wounding weeks on descending through the groups. The negative control (untreated) animals had the least average weight increase for the weeks studied after wound induction meanwhile they had the highest feeding increase and the longer healing days. In all groups, the feed intake increased gradually through the weeks. The decreasing order of wound healing capacity of the groups was obtained to be group 5 > group 4 > group 3 > group 2 > group 1 respectively. This implies that the higher the concentration of the ethanol extract *of C. albidum* seed, the higher the healing potential, the less the feeding and the greater the weight of animals in the post wounding days as applied to the reference groups (group 1). The phytochemical components of the *C. albidum* seed might have aided in the regeneration of lost tissue. The granulation tissue section of negative control animals showed decreased epithelialization, fibrosis and more aggregation of macrophages with less collagen fibers indicating incomplete healing. The tissue section from animals treated with povidone–iodine ointment (standard) and the extract showed complete healing with prominent epithelialization and increased collagenation and fibroblast.

Table 7: Average animal weight (g) increase per week after wound induction

Week	Group 1	Group 2	Group 3	Group 4	Group 5
1	0	0	1	4	10
2	1	2	3	6	14
3	2	4	6	9	17
4	4	5	8	11	20
5	6	8	12	15	24

Table 8: Feed weight (g) per week after wound induction

Week	Group 1	Group 2	Group 3	Group 4	Group 5
1	77	79	82	84	66
2	93	91	94	174	98
3	111	109	105	181	124
4	152	145	166	218	145
5	206	188	190	225	187

Surface area of wound

Table 9: Percent (%) wound healing rate

Day	Group 1	Group 2	Group 3	Group 4	Group 5
4	18	25	39	43	47
8	31	40	52	74	87
12	48	58	64	89	98
16	66	77	81	93	100
20	75	89	96	100	-
24	89	98	100	-	-
28	96	100	-	-	-
32	100	-	-	-	-

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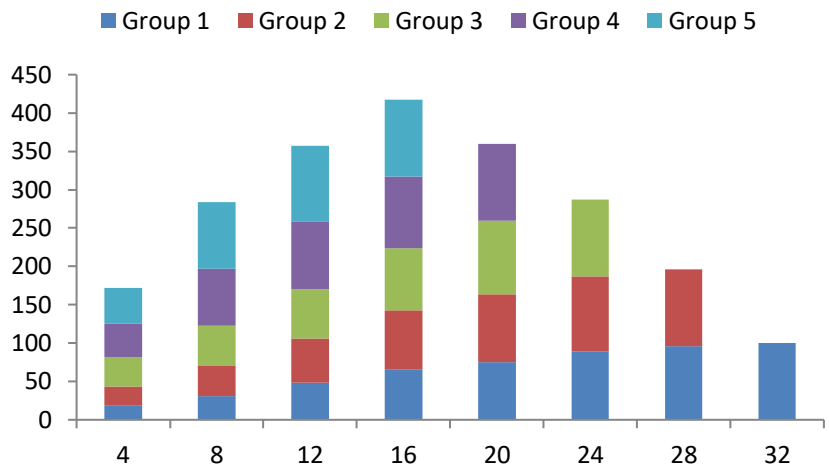

Fig. 5: percent wound healing rate for the groups

Group 1: Untreated (negative control)

Fig. 6: Pictures of wound every 4th day after induction for negative control

Group 2: Test group (5 g/ml ethanol extract)

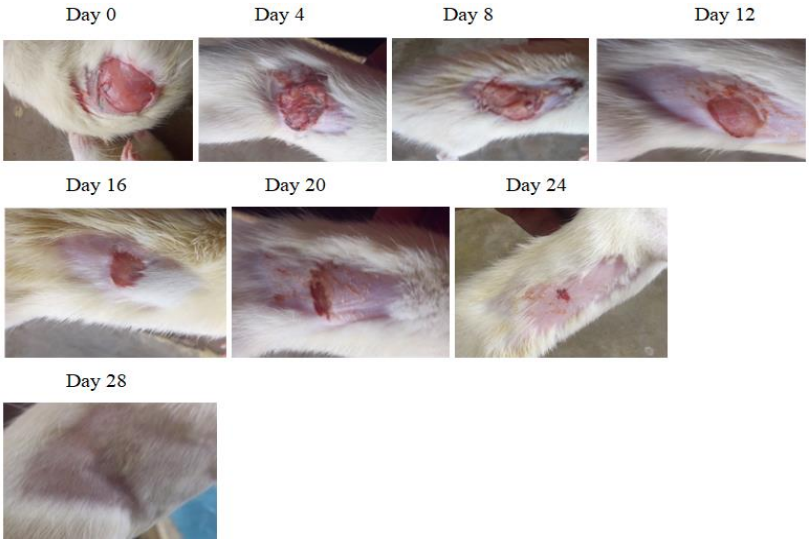

Fig. 7: Pictures of wound every 4th day after induction for test group

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Group 3: Test group (10 g/ml ethanol extract)

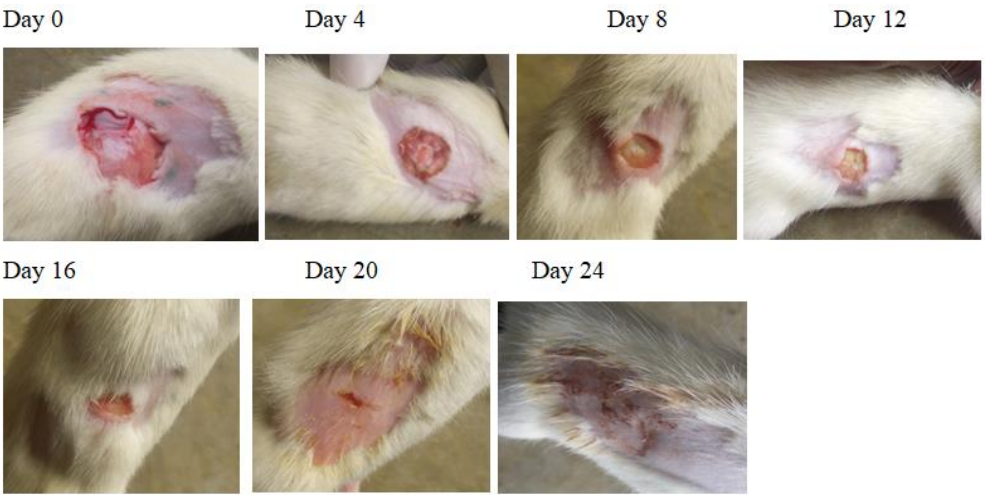

Fig. 8: Pictures of wound every 4th day after induction for test group

Group 4: Test group (15g/ml ethanol extract)

Fig. 9: Pictures of wound every 4th day after induction for test group

Group 5: standard (positive control)

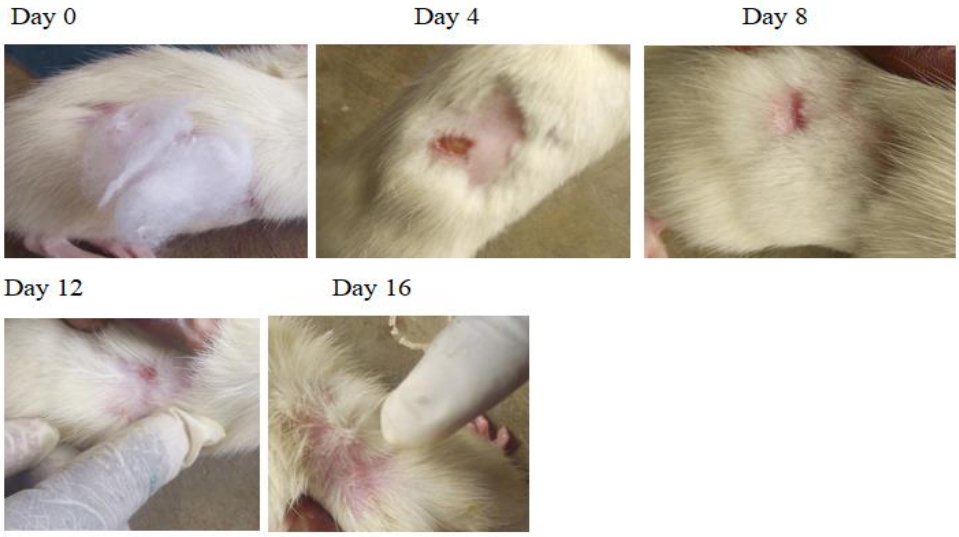

Fig. 10: Pictures of wound every 4th day after wound induction for positive control

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CONCLUSION

This study demonstrated the topical application of the ethanol extracts of *Chrysophyllum albidum* seed for the acceleration of wound healing in wistar rat. According to this finding, extract showed accelerated wound healing activity better than the negative control (untreated) which is comparably close to the standard/positive control group. This may be due to high percentage of flavonoids and triterpene present in the extract. The results of this study strongly document the beneficial effect of the ethanol-seed extract for the acceleration of wound healing.

RECOMMENDATIONS

The usefulness of the seed extract as a cream should also be examined in further research.

The purification and characterization of the *Chrysophyllum albidum* seed extract should be done for further study in order to further ascertain its importance in solving wound problems.

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