Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Wound Healing Potential of Jatropha Multifida Leaf Ethanolic Extract and Ointment to Burn Wound

Wresler C. Pascua[1], Ariel M. Alcones[2]

[1]*Research and Development Unit, Apayao State College, Conner, Apayao, Philippines*
[2]*BS Agriculture Department, Apayao State College, Conner, Apayao, Philippines*

This research investigated the healing potential of the coral bush plant leaf ethanolic extract and ointment to burn wounds. The physical characteristics of the leaf ethanolic extract, physico-pharmaceutical evaluation of the formulated ointment, acute dermal toxicity, pus formation, percent wound contraction, percent epithelialization and white blood cell count were determined through the conduct of experiment using 24 male wistar albino rats as the animal model using 4 treatments (T1- Calmoseptine, T2- Jatropha Ethanolic Ointment, T3- Pure Jatropha Extract, and T4- Water) with 5 replicates and 4 albino rats were used for the dermal toxicity test. The data were analyzed using One-way ANOVA. Evaluation on the parameters like percent wound contraction that resulted into high significant level of the calmoseptine (T1) and ointment (T2), epithelization period that resulted in high significant levels in the ointment (T2), white blood cell count showing high significant levels in ointment(T2), pure extract(T3) and water control (T4) and the pus formation showed negative results of abcess formation. The ointment (T2) has confirmed that it has no toxic effects for the test animals since they did not show severe and enduring signs of distress and pain. For the congruency of the study, the conduct of physico- pharmaceutical evaluation of the ointment like viscosity and spreadibilty, conduct histopathological study, and conduct anti-microbial property of the leaf ethanolic extract on gram-negative bacteria were recommended.

Keywords: Burn wound, ethanolic extract, experimental design, ointment.

1. Introduction

The Philippines was endowed with indigenous herbal plants. Many of these plants have not yet been explored and some are neglected and remain underutilized.

Jatropha multifida, commonly known as coral bush, is a single-trunked evergreen shrub or small tree. This plant is now grown in tropical to sub-tropical areas throughout the world as an ornamental shrub featuring large, lobed and divided, orbicular leaves plus a long showy bloom of coral-red flowers including the Philippines. It has been known for its ornamental and potential medicinal uses. The coral-red flowers are used for ornamental purposes. In a larger scale it also used for landscaping. The plant is a rich source of phytochemicals such as

alkaloids, terpenoids, lignoids and cyclic peptides having a broad range of biopotency. The phytochemicals can also be utilized in agricultural, nutritional and pharmaceutical industries. Jatropha possess various metabolites of which terpenoids are the major part.The plants of this family are an important source of medicines and toxins. One of the major metabolites, diterpenoids, possess therapeutic potentiality as antibacterial, antihypertensive, anti-inflammatory, antileukemic, antioxidants, antiretroviral, antitumor, analgesic, cytotoxic, hallucinogens, sweeteners and may stimulate contraction of the uterus.

Although rarely defined, a wound is generally acknowledged as all manner of tissue damage resulting in the disruption of the original tissue architecture and homeostasis [1,2]. Wounding is followed by the activation of various extracellular and intracellular events that ultimately act to restore tissue integrity and physiological equilibrium [2]. Details of the wound repair process are complex and varied, and influenced by numerous features such as the size, severity and nature of the injury, and the site of the wound. Despite this diversity, the overall process and sequence of repair events is often broadly similar [2]. The primary goal of wound healing is to re-establish homeostasis and restore tissue architecture.

In the Philippines, fire-related death ranks among the 15 leading causes of death in children and young adults of 5-29 years old. Burn injury cases peaked during months of April to June 2012 and it was stated that Region III- Central Luzon has the highest burn injury cases followed by the National Capital Region and Cordillera Administrative Region. Most burn injuries happen at home and higher incidence of burn injuries occur in males than females [3].

2. Methodology

2.1. Experimental animals

Male Wistar Albino rats were used and assigned in groups. Rat cages with water dispenser, rodent feeds, purified water, and animal house with proper temperature was provided.

2.2. Preparation of Jatropha multifida leaves ointment

In the preparation of Jatropha multifida leaf ethanolic ointment following the fusion method, the following materials was used: Jatropha multifida leaves ethanolic extract, hard beeswax, lanolin, white soft paraffin, ceteryl achohol, hard paraffin wax, digital balance, beaker, electric stove, hot plate, saucepan, spatula, strainer, stirrer, cheesecloth, pH meter and ointment container.

2.3. Wound infliction

For wound infliction, the following materials was used: Albino rats, laboratory gown, gloves, masks and surgical cap, holding tray, clean cloth, Zoletil (General Anesthesia), lidocaine (local anesthesia), shaver, surgical scissors, betadine, forceps, syringe, alcohol and cotton balls.

2.4. Experimental Lay-out

The experiment was laid out using a Complete Randomized Design (CRD) using a total of twenty-four (24) rats with five (5) replicates each treatments equivalent of twenty (20) rats and the remaining four (4) Albino rats was used for acute dermal toxicity test.

T1- calmoseptine

T2- ethanolic ointment

T3- ethanolic extract

T4- distilled water

2.5. Collection of Plants

The researchers approximately collected a total of 30 leaves of coral bush with varied sizes at Conner, Apayao. The duration of collection was only one (1) day. The samples collected were authenticated by the Department of Agriculture, Plant Quarantine Station 2.

2.6. Acclimatization of experimental animals

This study was employed with experimental research method, using Male Wistar Albino rats, with an average weight of 100 grams. For the validity of using experimental animals, the researchers sought an animal permit from the Bureau of Animal Industry-Department of Agriculture Region 2. The rats were acclimatized for one weeks in an animal house. They were housed individually, in standard cages for an acclimatization period of seven days or one week before the commencement of experiment. During this period the animals were given free access to standard pellet diet and water in ad libitum in an ambient temperature of (24±2 C^{o}) and standard laboratory condition [4]. They were housed according to the experimental lay-out.

2.7. Leaf Ethanolic Extraction

The leaves were sorted out to obtain only fresh leaves and washed with distilled water without squeezing to remove debris and dust particle and then dry at room temperature under shade for 3 days. Then 193g of the grinded leaves was soaked in 245 ml of 70% ethanol for 24 hours without constant occasional stirring. Then the macerate will be filtered under vacuum. The residue was re-macerated three times to obtain maximum and pure ethanolic extract. To reduce concentration of the filtrate, the ethanol was evaporated using Rotary evaporator. The combined aqueous filtrate was then evaporated at 70 °C as the boiling point [5].

2.8. Formulation of Ointment using Fusion Method

In the preparation of 10% ointment, the researchers fused two grams (2g) Wool fat (Lanolin), two grams (2g) Hard Paraffin, two grams (2g) Cetearyl alcohol, thirty-four grams (34g) White Soft Paraffin. Each ingredient was melted at their melting point, mix and heated gently with constant stirring then cooled. Four (4g) of ethanolic extract from Jatropha leaves was added slowly to the above melted ingredients and stirred thoroughly until the mass cooled down and a homogeneous product was formed [6]. The ointment was packed in a wide mouth container.

2.9. Physico-Pharmaceutical Evaluation

The following evaluation on the physico-pharmaceutical properties was adopted from Panigrahi et. al (1997), the pH of various formulations was determined by using digital pH meter. 2.5 grams of sample was placed in 100 ml dry beaker and added 50 ml. The beaker was heated on water bath maintained at about 60c to 70c for 10 minutes, cooled to room temperature, then centrifuged at 3000rpm for 10 minutes [7]. The pH measurement of water

extract was measured by using digital pH meter by dipping the glass electrode into the ointment formulation [8].

2.10. Homogeneity

All the developed ointments were tested for homogeneity by visual inspection [9]. The appearance should be no lumps.

2.11. Color, odor, and smoothness

This evaluation of the formulated ointments was carried out by visual examination and through touch [9].

2.12. pH

The pH of various formulations was determine using Digital pH meter. The pH measurements were done using a digital type pH meter by dipping the glass electrode into the ointment formulation [8].

2.13. Administration of Drugs

Each Albino were anesthetized with Zoletil (60mg/kg) as general anesthesia before wound infliction. This was done with closed supervision of a licensed veterinarian. To assure of equal size of the wound per animal and to restrain the animal during wound infliction Lidocaine (.05 ml/rat) was administered subcutaneously prior to wound infliction at the dorsal thoracic region. Wound size was measured before infliction using plastic ruler and erasable marker [10]. After the wounding process, each Albino rats were caged individually and wrapped with sterilized cloth to avoid hypothermia due to anesthesia.

2.14. Creation of Burn Wound

Hair were shaved at the back area and disinfected with 70% isopropyl alcohol. Mice was anaesthetized using Lidocaine anesthesia. A cylindrical steel of 1.2 cm diameter was heated in a 100 C boiling water and then contacted with the skin of the rats for 3 seconds and will be remove thereafter [11].

2.15. Administration of Treatment

Each rat received their first topical treatment (day 1) after evaluation of the burn wound to be of the right depth and size. The rats were grouped into the following:

Positive Control Group (T1): received calmoseptine once a day.

Ointment Group (T2): received Jatropha ethanolic ointment once a day.

Pure Extract Group (T3): received Jatropha ethanolic extract once a day (.05 ml).

Negative Control Group(T4): received distilled water (dH2O) twice a day.

Each group has five replicates. Treatment was administered once a day for fourteen days.

2.16. Skin preparation for dermal toxicity studies

The skin at the dorsal thoracic area of the rats was clipped under general zoletil (50 mg/kg) and lidocaine (5 mg/kg) anaesthesia with an electric clipper followed by manual shaving using

a blade. Based on OECD guidelines 402 and 410 [12], not less than 10% of the body surface area were cleared for the application of the test substance. The extract was applied to dorsum area.

2.17. Acute Dermal Toxicity Test

For the Acute dermal toxicity, a total of 4 albino rats were used. Animals showing normal skin texture was housed individually in a cage and acclimatized to the laboratory condition for five days prior to the test. Then around 10 % of the body surface area fur was shaved from the dorsal part 24 h before the study. Then extract ointments (5% and 10%) were applied on the shaved area. At the end of the exposure period (24 h), the residual test substance was removed and the animals was observed for 24 h and for the next 14 days for development of any adverse skin reactions like inflammation, irritation or redness. In addition, skin toxicity of extract ointments was also observed when the ointment was applied for several days (> 10 days) [13,14].

2.18. Pus Formation

Pus is a thick, opaque, usually yellowish white fluid matter formed in association with inflammation caused by the invasion of the body by infective microorganisms (such as bacteria) [15]. It is composed of degenerating leukocytes (white blood cells), tissue debris, and living or dead microorganisms. An area of tissue becomes infected and initially looks like a hard boil. If ignored, white blood cells move into the infected area and collect within the damaged tissue and pus forms. If further ignored, the infected area could increase and break/ breach the skin leading to formation of an ulcer. If overlooked, the site could become infested with maggots after which gangrene could set in. This could result in amputation of the limb or infected part.

For the Pus Formation, it was monitored and observed through visual inspection by the number of days required as treated per treatment [16].

2.19. Parameter Evaluation

The parameters observed in the study were as follows:

2.19.1. Wound Contraction

It was noted by following the progressive changes in wound area planimetrically, excluding the day of the wounding the size of the wounds was measured using Vernier caliper, throughout the monitoring period [17]. The evaluated surface area was employed to calculate the percentage of wound contraction, taking the initial size (diameter) of the wound by using the following equation:

$$WC\ (\%) = \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

2.19.2. Epithelialization Period

It was monitored by noting the number of days required for the eschar to fall off from the burn wound surface without leaving a raw wound behind using the following equation [18]:

$$\text{Ephithelialization} = \frac{\text{Open wound area}}{\text{Total wound area}} \times 100\%$$

2.19.3. White Blood Cell Count (Under CBC)

White blood cell count is lying under the process of complete blood count (CBC) which is a blood test used to evaluate your overall health and detect a wide range of disorders, including anemia, infection and leukemia. A complete blood count test measures several components and features of your blood, including red blood cells, which carry oxygen, white blood cells, which fight infection, hemoglobin, the oxygen-carrying protein in red blood cells, hematocrit, the proportion of red blood cells to the fluid component, or plasma, in your blood platelets, which help with blood clotting [19].

Complete Blood Count was conducted before and after wound infliction to the experimental animals at DA-CVIAL, Regional Animal Diagnostics Disease Laboratory to test wether the number of WBC before treatment would have a significant difference after the treatment on the wounded skin of the rats.

2.19.4. Statistical Analysis

The data were analyzed using One-way ANOVA. The efficacies were obtained by comparing the time of wound contraction using the four treatments and the values were converted into percentage using mean index. The treatments with means that are significant were compared using Least Significant Differences (LSD).

3. Results and Discussion

3.1. Physical Characteristics Evaluation of the Extract

As gleaned on the table 1, results showed that the color and odor of the ethanolic extract were similar to the standard observation as odorless and clear in color [9]. These indicators showed purity and the liquid has no impurities and no chances of having microbial growth.

Table 1. Physical characteristics evaluation of the extract

	Standard	Observation
odor	odorless	odorless
color	crystal clear	white and clear

3.2. Physico-Pharmaceutical Evaluation of the Formulated Ointment

Table 2 shows the physico-pharmaceutical evaluation of ointment. In terms of the pH, the ointment measured 5.62 which was classified as acidic and falls under the standard values [8]. Meanwhile, the odor of the ointment is like a candle wax due to its paraffin component and still conforms to the standard odor of ointment. Smoothness and grittiness of the ointment appeared to be the same as the standards which was assessed smooth with no lumps. The ointment is also homogeneous and appeared to be non-greasy and yellow in color. The overall evaluation of the ointment conforms with the standard basis.

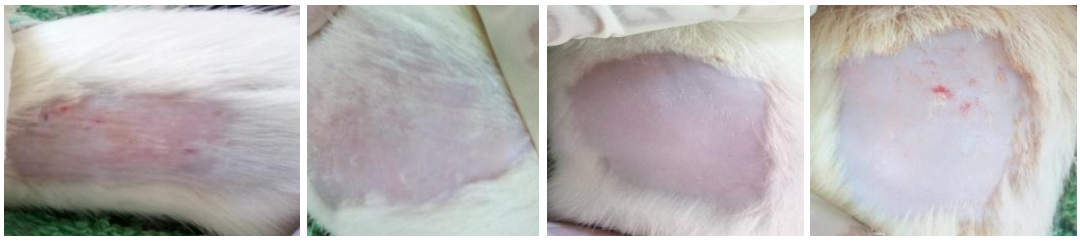
Table 2. Physico-Pharmaceutical Evaluation of the Ointment

	Standard	Observation
pH	5-7	5.62 (acidic)
Odor	No specific	Like a candle wax
Smoothness	smooth	smooth
Grittiness	No lumps	No lumps
Homogeneity and appearance	Homogeneous, opaque and non-greasy	Homogenous, non-greasy yellow in color

3.3. Acute Dermal Toxicity

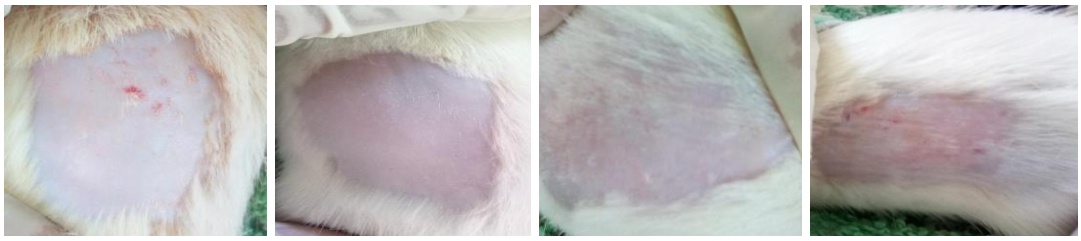
As the result of dermal toxicity on plate 1 and plate 2, it appears that there is virtually non-toxic since no signs of toxic effects on the rats' skin nor shown any feelings of pain and distress as what the principle is trying to figure out. Dermal toxicity test is vital in the conduct of the study as it ascertains the possible effects of the extracts and the ointment which can cause redness, rashes, and inflammation which can lead to pain and distress to the test animals [12]. As per OECD guidelines, the results of the acute dermal toxicity test were basis for the administration and was converted the mg/kg to g/g depending on the weight of the rat [13,14].

Plate 1. Acute dermal toxicity results when treated with coral bush extracts



200 mg/kg 500 mg/kg 1000 mg/kg 2000 mg/kg

Plate 2. Acute dermal toxicity results when treated with coral bush ointment



200 mg/kg 500 mg/kg 1000 mg/kg 2000 mg/kg

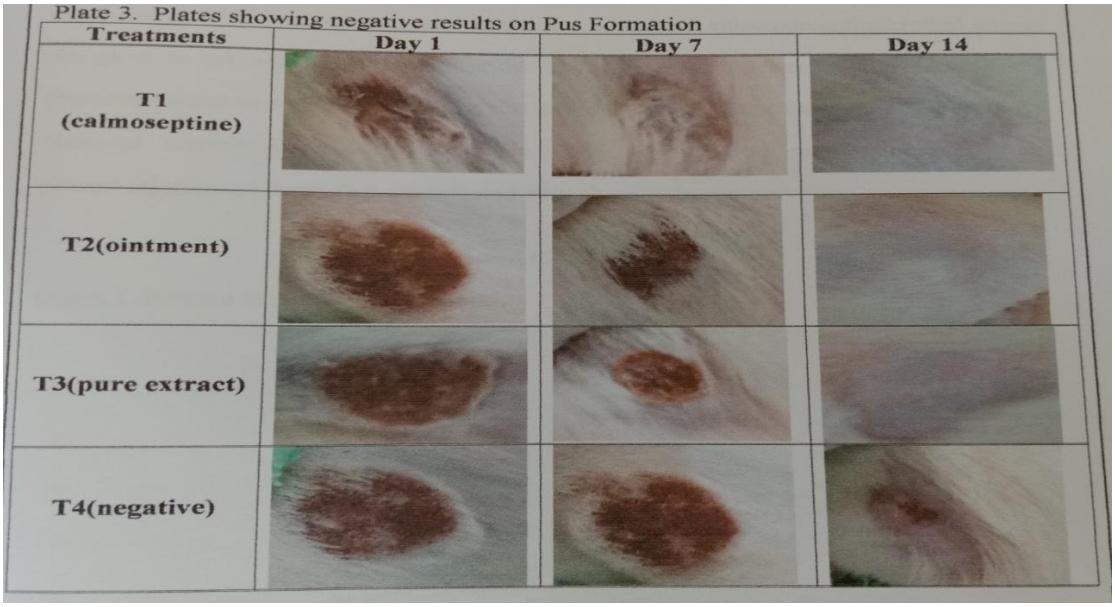
3.4. Pus Formation

Based on the observations within 14 days, the researchers have found that no pus formation insisted on the burn wounds of the experimental animals. T1 GROUP, T2 GROUP, T3 GROUP, and T4 GROUP are healed on their wounds in accordance to the treatment applied and no to prior formation of pus.

Pus is a major components of abscess that contains a mixture of dead tissue, white blood cells and bacteria [20]. The symptoms and signs of having pus are pain, heat, swelling, tenderness,

and redness. It is usually cause by germs, such as bacteria (Staphylococcus Aureus) and foreign substances entering the body through needles piercing the skin and contaminating the injection site.

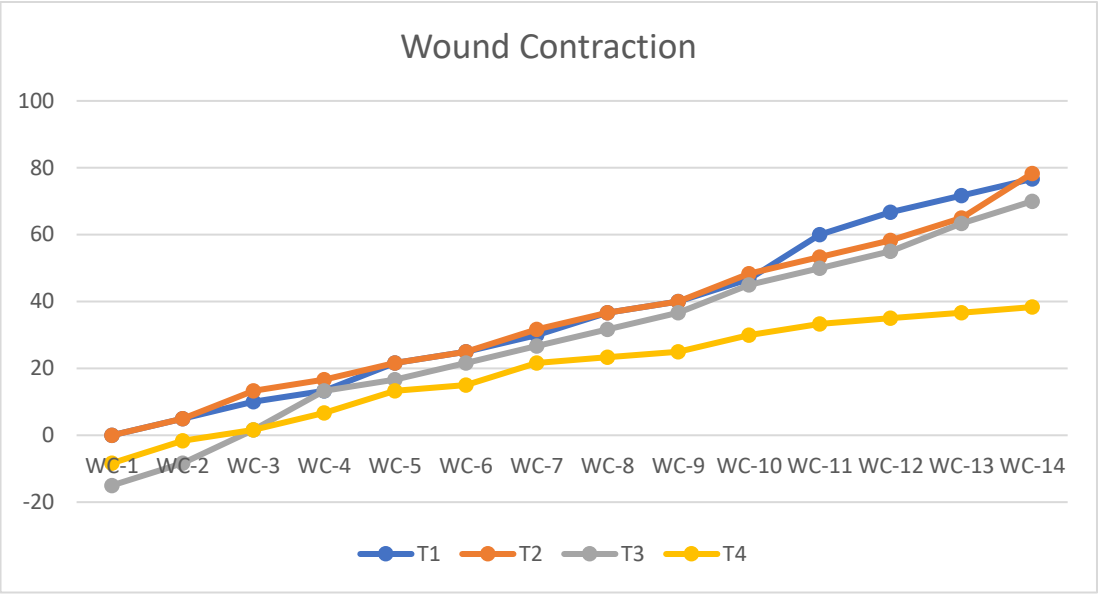
Pus formation can be cured depending on its severity. It has been produced a best practice statement on the use of topical antiseptic/antimicrobial agents designed to provide guidance for health practitioners on when to start and equally important when to stop using topical antimicrobial agents [21]. According to the data from the National Center for Health Statistics in 2000, 8.6 million unintentional of burn related injuries are listed that are infected with abscess formation.



3.5. Percent Wound Contraction

Based on the graph, it shows that the trend of wound contraction of T2 (ointment) is approximately 78%, almost equal as the trend of wound contraction of T1(calmoseptine) with 79% which means that the wound healing potential of the formulated ointment is almost the same as the commercial ointment which is the calmoseptine. T3 (pure extract) also shows a high trend when it comes to wound contraction which is approximately 65% and is so it is positive and high in wound healing potential. T4(negative) is has the lowest trend of wound healing at approximately 39% since it has no emulsifying agents that helps in healing the wounds.

The high levels of wound contraction on both T1 and T2 was because of the emulsifying agents that helps in the retention of the burn wounds. T3 also has high wound contraction because the ethanolic extract of the leaves has three di-C-glycosidic biflavones were identified and the extract presented significant analgesic and anti-inflammatory properties [22]. T4 appears to be the lowest in healing properties because it has no emulsifying agents that helps in healing the wounds. Results would statistically conclude that the healing process of wounds was continuous in 14 days.



Graph 1. Percent Wound Contraction

Legend:

T1-Positive (Calmoseptine)

T2-Ointment

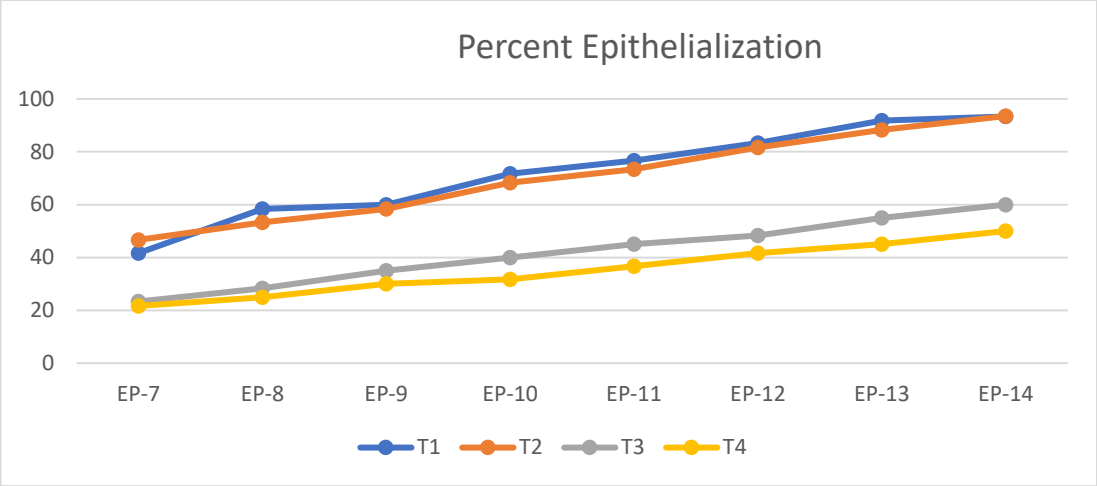
T3-Pure Extract

T4-negative (distilled water

3.6. Percent Epithelialization

The graph 2 shows that T1(calmoseptine) and T2(ointment) has high trend of epithelialization period at approximately 92% which means that the area of repaired tissues is high in using the commercial and formulated ointment, followed by T3 (pure extract) with 60% and lastly, by T4 (negative) with 50%.

The treatment with higher percentage of epithelialization has high potential in repairing the damage tissues if any. Re-epithelialization process is facilitated by the migration and proliferation of original uninjured epidermal cells from the wound margin [23]. The leaked fluid is rich in cytokines that stimulates new tissue growth [24].



Graph 2. Percent Epithelialization

Legend:

T1-Positive (Calmoseptine)

T2-Ointment

T3-Pure Extract

T4-negative (distilled water

3.7. Multiple Comparisons of the PRE-POST White Blood Cell Count

The table shows that T1 and T4 has highly significant difference on the initial and final WBC Count at <.050000 significant level of difference.

Also, the result on the initial test shows normal WBC number and has increased after the wound infliction. White Blood Cells only rise in number if there are inflammations and infections occurred in the body [19].

Table 3. Comparison of initial and final WBC Count

Variable	Mean Pre	Mean Post	p Variances
T1- Positive (Calmoseptine)	13.46000**	6.52500**	0.697119**
T2- Ethanolic Ointment	10.40000*	8.22500*	0.094805*
T3- Pure Extract	11.38000*	9.26667*	0.960540*
T4- Untreated (distilled water)	9.84000**	14.50000**	0.825578**

The mean difference is significant at <.050000 level

Red **: Highly significant

Black*: Significant

3.8. Test of Differences on Treatments

The data were analyzed using One-way ANOVA. The efficacies were obtained by comparing

the time of wound contraction using the four treatments and the values were converted into percentage using mean index. The treatments with means that are significant were compared using Least Significant Differences (LSD).

Based on the data analyzed in table 4, T1 and T3 posed highly significant differences on wound contraction. More so, there was significant difference on T2 and T1 on epithelialization. Finally, T1, T2, and T3 posed highly significant differences in WBC count.

Table 4. Significant difference between each parameter (ANOVA)

Treatment	Wound Contraction	Percent Epithelialization	WBC
T1- Positive (Calmoseptine)	76.67^{ac}	42.667^{ac}	6.525^{a}
T2- Ethanolic Ointment	78.33^{c}	46.667^{a}	8.225^{a}
T3- Pure Extract	70^{a}	23.333^{bc}	9.266^{a}
T4- Untreated (distilled water)	38.33^{b}	21.667^{bc}	14.500^{b}

The mean difference is significant at <.050000 level

a: Highly significant

b: Not Significant

c. Not Significant

3.9. Mean of Treatments

As gleaned on the table, T1 having the highest mean value has the greater wound healing potential. Further, T2 is comparable to the result of T1. T3 has also effect to wound healing close as to T1 because the retention of the applied extract is lesser compared to ointment. T4 has the lowest wound healing potential because it has no presence of any wound healing component but just distilled water.

However, there is no pus formation in all of the treatments applied and were able to heal eventually; yet the negative treatment (T4) took it slower than the 3 treatments because the experimental animals were healthy and the water applied was distilled, therefore aseptic.

Table 5. Treatments with their respective means

Treatment	Mean (All groups)
T1- Positive (Calmoseptine)	78.33333^{ac}
T2- Ethanolic Ointment	76.66667^{c}
T3- Pure Extract	70.00000^{a}
T4- Untreated (distilled water)	38.33333^{b}
Total	65.83333

The mean difference is significant at <.050000 level

a: Highly significant

b: Not Significant

c. Not Significant

4. Conclusion

Based on the results, the researchers concluded that the ethanolic ointment and the ethanolic extract has burn wound healing potential. The ethanolic ointment has the same percentage of wound contraction as the calmoseptine and the pure extract has also high percentage of wound contraction and closely the same as the trend of ethanolic ointment which means that they have wound healing capacity. Ethanolic ointment has also high rate epithelialization and almost close to calmoseptine. Also, all treatments showed negative results of pus formation and showed low levels of WBC's before the wound infliction and increases after inflicting the wounds which means that the cells are aggressive and aggregated. The ethanolic ointment has confirmed that has no toxic effects for the test animals since they did not show severe and enduring signs of distress and pain. Further, the overall evaluation of the ointment and the extracts conforms with the standard basis.

Funding Details

ASC Research and Development Unit

Disclosure Statement

The authors declare that there is no conflict of interest.

References

1. Stocum DL. Wound repair, regeneration and artificial tissues. New York: Springer-Verlag; 1995.
2. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature. 2008 May;453(7193):314-21.
3. Mekonnen A, Atlabachew M, Kassie B. Investigation of antioxidant and antimicrobial activities of Euclea schimperi leaf extracts. Chemical and biological technologies in agriculture. 2018 Dec;5:1-2.
4. Arts JW, Kramer K, Arndt SS, Ohl F. Sex differences in physiological acclimatization after transfer in Wistar rats. Animals. 2014 Oct 30;4(4):693-711.
5. Owoyele BV, Adebukola OM, Funmilayo AA, Soladoye AO. Anti-inflammatory activities of ethanolic extract of Carica papaya leaves. Inflammopharmacology. 2008 Aug;16:168-73.
6. Bhagurkar AM, Angamuthu M, Patil H, Tiwari RV, Maurya A, Hashemnejad SM, Kundu S, Murthy SN, Repka MA. Development of an ointment formulation using hot-melt extrusion technology. Aaps Pharmscitech. 2016 Feb;17:158-66.
7. Schmidt PC, Herzog R. Calcium phosphates in pharmaceutical tableting: 1. Physico-pharmaceutical properties. Pharmacy World and Science. 1993 Mar;15:105-15.
8. Patel RP, Patel H, Baria A. Formulation and evaluation. Int J Drug Deliv Technol. 2009;1:42-5.
9. Khullar R, Kumar D, Seth N, Saini S. Formulation and evaluation of mefenamic acid emulgel for topical delivery. Saudi pharmaceutical journal. 2012 Jan 1;20(1):63-7.
10. Ferrari L, Turrini G, Rostello C, Guidi A, Casartelli A, Piaia A, Sartori M. Evaluation of two combinations of Domitor, Zoletil 100, and Euthatal to obtain long-term nonrecovery anesthesia in Sprague-Dawley rats. Comparative medicine. 2005 Jun 15;55(3):256-64.
11. Cai EZ, Ang CH, Raju A, Tan KB, Hing EC, Loo Y, Wong YC, Lee H, Lim J, Moochhala SM, Hauser CA. Creation of consistent burn wounds: a rat model. Archives of plastic

surgery. 2014 Jul;41(04):317-24.
12. Grandy NJ. Role of the OECD in chemicals control and international harmonization of testing methods. InFundamentals of aquatic toxicology 2020 Aug 6 (pp. 763-773). CRC Press.
13. Mulisa E, Asres K, Engidawork E. Evaluation of wound healing and anti-inflammatory activity of the rhizomes of Rumex abyssinicus J.(Polygonaceae) in mice. BMC complementary and alternative medicine. 2015 Dec;15:1-0.
14. Kokane DD, More RY, Kale MB, Nehete MN, Mehendale PC, Gadgoli CH. Evaluation of wound healing activity of root of Mimosa pudica. Journal of ethnopharmacology. 2009 Jul 15;124(2):311-5.
15. Rezaei Hosseinabadi S, Parsapour A, Nouri Khorasani S, Razavi SM, Hashemibeni B, Heidari F, Khalili S. Wound dressing application of castor oil-and CAPA-based polyurethane membranes. Polymer Bulletin. 2020 Jun;77:2945-64.
16. Nasiri E, Hosseinimehr SJ, Azadbakht M, Akbari J, Enayati-Fard R, Azizi S. Effect of Malva sylvestris cream on burn injury and wounds in rats. Avicenna journal of phytomedicine. 2015 Jul;5(4):341.
17. Cukjati D, Reberšek S, Miklavčič D. A reliable method of determining wound healing rate. Medical and Biological Engineering and Computing. 2001 Mar;39:263-71.
18. Coulombe PA. Wound epithelialization: accelerationg the pace of discovery. Journal of investigative dermatology. 2003 Aug 1;121(2):219-30.
19. Onah IA, Onuigbo EB, Odimegwu DC. Adjuvant effect of Vernonia amygdalina leaf extract on host immune response to hepatitis B virus subunit vaccine. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2019 Mar 1;74(3):179-85.
20. Sullivan Giovanello K, Schnyer DM, Verfaellie M. A critical role for the anterior hippocampus in relational memory: evidence from an fMRI study comparing associative and item recognition. Hippocampus. 2004;14(1):5-8.
21. Upton D, South F. The psychological consequences of wounds—a vicious circle that should not be overlooked. Wounds UK. 2011 Nov 1;7(4):136-8.
22. Kalra S, Baruah MP, Sahay RK, Unnikrishnan AG, Uppal S, Adetunji O. Glucagon-like peptide-1 receptor agonists in the treatment of type 2 diabetes: past, present, and future. Indian journal of endocrinology and metabolism. 2016 Mar 1;20(2):254-67.
23. Singer AJ, Clark RA. Cutaneous wound healing. New England journal of medicine. 1999 Sep 2;341(10):738-46.
24. Rodriguez R, Redman R. More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. Journal of experimental botany. 2008 Mar 1;59(5):1109-14.