INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 19, No. 4, p. 166-172, 2021

RESEARCH PAPER OPEN ACCESS

Wound Healing Activity of Ointment infused with Aqueous Extracts of Plants Used by the Agtas of Peñablanca and Malauegs of Rizal in the Province of Cagayan, Northern Philippines

Bernadette Y. Cauilan[1], Jamaica M. Danga[1], Marvin Henesse T. Lungan[1], Maria Louisa L. Pallingayan[1], Jinky Marie T. Chua[1*], Julius T. Capili[1], Ramelo B. Ramirez Jr[2]

[1]*College of Allied Health Sciences, Cagayan State University, Andrews Campus, Tuguegarao City, Cagayan North, Northern Philippines*

[2]*Natural Product Research and Innovation Center, Cagayan State University, Andrews Campus, Tuguegarao City, Cagayan North, Northern Philippines*

Key words: *Donax cannaeformis*, *Chlorophytum comosum*, Wound healing, Extraction, Ointment, Aqueous extracts.

http://dx.doi.org/10.12692/ijb/19.4.166-172 Article published on October 27, 2021

Abstract

Poor wound healing hinders the resolution of skin problems that become enlarged and infected that causes a significant negative impact on the quality of life. The study aimed to investigate the wound healing activity of the plants used by indigenous groups (Agtas and Malauegs) in the Philippines, *Donax cannaeformis* and *Chlorophytum comosus var. variegatum*. In the present study, ointments with aqueous extracts of these plants were administered topically to the Sprague Dawley rats. Evaluation of wound healing activity was done using the excision, incision, and burn wound models. In each model, Treatment A has 100% *D. cannaeformis*; B=100% *C. comosus*; C=50% *D. cannaeformis* and 50% *C. comosus*; D=75% *D. cannaeformis* and 25% *C. comosus*; E= 25% *D. cannaeformis* and 75% *C. comosus*; F=Positive control. All wound model rats were treated for fifteen days, and the wound healing activity was assessed by percentage wound contraction. The results showed that the ointments containing the plant extracts are not significantly different from the positive control. That means these can be used as an alternative herbal remedy to commercial products. However, tensile strength and histopathological studies of the granulation tissue and free radical scavenging activity are recommended to fully determine the activity of the plant extracts.

* **Corresponding Author:** Jinky Marie T. Chua ✉ jchua@csu.edu.ph

Introduction

The Philippines has been widely known for its plant diversity which displays an array of both potentially medicinal and therapeutic plants. Without prior researches and explorations, traditionally, these plants have been used as alternatives for medicine and therapy against diseases and ailments by indigenous groups. Medicinal plants have been used since time immemorial for the treatment of various ailments of skin and dermatological disorders, especially cuts, wounds and burns. Plants and their extracts have immense potential for the management and treatment of wounds (Kumar *et al.,* 2007; Modak *et al.,* 2017; Sharma *et al.,* 2012).

The phytomedicine for wound healing is not only cheap and affordable but is also purportedly safe as hypersensitive reactions are rarely encountered with the use of these agents. These natural agents induce healing and regeneration of the tissue by multiple mechanisms. However, there is a need for scientific validation, standardization and safety evaluation of plants of traditional medicine before they could be recommended for healing of wounds (Narayan, Sasmal, & Mazumder, 2011). Considering the fact that the pharmaceutical industries allot an enormous investment of research and money, the discovery of useful medicinal plants which have been used for millennia is very promising. Studies on these plants can lead to the development of products that are safer, more efficient, cost-effective and display a lower risk of adverse reactions.

Donnax cannaeformis and *Chlorophytum comosum var. variegatum* are employed by Agtas of Peñablanca and Malauegs of Rizal in the Province of Cagayan in folk medicine for wound healing, but both are not proven and infused to a skin formulation. *D. cannaeformis* is evaluated for antioxidant activity and is found to contain phenolic compounds, alkaloids, tannins, phytosterols, cardiac glycosides, terpenoids, steroids, steroids, saponins, and flavonoids (Daud, 2011; Azliza and Ong, 2012). *C. comosum var. variegatum* in Chinese traditional medicine is used for bronchitis, fractures, and burns (Braria, Ahmad, & Harikumar, 2014). With these, the study aims to evaluate the wound healing activity of the leaf extracts infused in ointment in animal models and compare them to commercially available drugs.

Materials and methods

Collection and preparation of plant materials

Freshly harvested leaves are collected at the Municipalities of Peñablanca and Rizal, Province of Cagayan, Northern Philippines. The identity of these plants was confirmed by a taxonomist from the Botany Department, National Museum, Manila, Philippines. The samples are washed with distilled water and dried at 60°C for three consecutive days until constant weight.

The dried plant materials were subjected to grinding through mortar and pestle and poured in a blender to make them finer. The plant powders were then stored and sealed in plastic bags in a cool dark place.

Aqueous Extraction of Plants

Every five grams of dried finely powdered plant material was mixed with 200ml of distilled water. The mixture was heated on a hot plate with continuous stirring at 30°-40 °C for 20 minutes.

The water extract was filtered through filter paper and the filtrate was used for the ointment formulation.

Ointment preparation

Beeswax and liquid paraffin were mixed and heated first and Span 60 and Tween 80 were added and heated gently with a stirring rod, then cooled. Aqueous extracts are added slowly to the above-melted ingredients and stirred thoroughly until the mass cools down and a homogeneous product is formed. The 50-gram ointment will then be packed in ointment jars. Five different ointments were formulated: A=100% *D. cannaeformis*; B=100% *C. comosum*; C= 50% *D. cannaeformis*, 50% *C. comosum*; D= 75% *D. cannaeformis*, 25% *C. comosum*; E= 25% *D. cannaeformis*, 75% *C. comosum*.

Animal preparation

Five-week-old male Sprague-Dawley rats weighing 150 – 180 grams were used in the experiment. The animals were purchased from the Food and Drug Administration Animal Facility. They were housed in a ventilated animal housing facility with ambient temperature at 250C with a 12-hour light-dark cycle. They were given equal amounts of standard mouse pellets everyday with water ad libitum. After purchasing the rats, they were allowed to acclimatize to laboratory conditions for 1-2 weeks before experimentation.

Creation of wound

All the surgical interventions were carried out under sterile conditions under general anesthesia. The predetermined area for wound infliction at the back of the animal was prepared for surgery by removing hairs using a razor. The animal was anesthetized intraperitoneally and placed on the operation table in its natural position. The animals were allowed to recover, housed individually in their cages, and monitored for respiration, color, and temperature. They are maintained under standard husbandry conditions and on a uniform diet and managed throughout the experimental period. Animals are closely observed for any infection; those who showed signs of infection are separated and excluded from the study. The laboratory animals are periodically weighed before and after the experiments.

a. Excision Wound Model is inflicted on the dorsal thoracic region 1–1.5 cm away from the vertebral column on either side and 5 cm away from the ear. After wound area preparation with 70% alcohol, using a sterile round seal of 2.5 cm diameter or a surgical blade or 5–8 mm biopsy punch, the circular skin from the predetermined area on the depilated back of the animal are excised to its full thickness to obtain a wound area of about 200–500 mm^2 diameter and 2 mm depth. Hemostasis is achieved by blotting the wound with a cotton swab soaked in normal saline. The ointments are administered topically to the animals of respective groups until complete epithelialization starting from the day of operation.

b. Incision Wound Models are made after preparation with 70% alcohol. Two longitudinal paravertebral incisions are made through the skin and cutaneous muscles at about 1.5 cm from the midline on either depilated side of the vertebral column with a sterile sharp surgical blade. Each incision made is 4–6 cm in length, and after complete hemostasis, the parted skin is stitched with interrupted sutures, 0.5–1.0 cm apart using black braided silk surgical thread (no. 000) and a curved needle (no. 11). The continuous threads on both wound edges are tightened for good closure of the wound. The wounds are left undressed and mopped with a cotton swab. The respective ointment is administered topically to the animals of respective groups until the 7th–9th day starting from the day of operation. The sutures are removed on the 7th day.

c. Burn Wound Model. A cylindrical metal rod (10 mm diameter) is heated over the open flame for 30 seconds and pressed to the shaved and disinfected surface for 20 seconds on the selected dorsal area of the animal under light anesthesia. Animals are placed in individual cages after recovery from anesthesia.

The respective ointment is administered topically to the animals of respective groups until the day of scab falling, starting from the day of operation.

Ointment administration

Application of the different treatments to the wound area was made once a day at the group-dependent time intervals after cleaning with sterile surgical cotton wool in the experimental group animals for a period of 15 days.

The ointments were applied at a dose of 100 mg/kg/day to 500 mg/kg/day with a sterile cotton swab. To prevent the possible thermic effect of the application, the temperature of the ointments is kept at approximately 37 °C. The commercial ointments, silver sulfadiazine for burns and Bactroban for incision and excision, in the same quantity, can be applied daily to wounds of reference group animals, respectively.

Evaluation of wound contraction

The progressive reduction in the wound area was monitored plan metrically by tracing the raw wound boundaries initially on a sterilized transparency paper sheet in mm^2 without causing any damage to the wound area, and then, the wound area recorded are measured using a graph paper on every 2–4-day interval.

The period of epithelialization is expressed as the number of days required for falling off the eschar (dead-tissue remnants) without any residual raw wound is considered as the endpoint of complete epithelialization. Percentage wound contraction is calculated as:

$$\%\ \text{wound contraction} = \frac{\text{initial wound size} - \text{specific day wound size}}{\text{initial wound size}} \times 100$$

Statistical analysis

One-way Analysis of Variance (ANOVA) was used to determine if there is a significant difference between the treatments. Statistical analysis was performed using SPSS version 20 software at a 0.05 level of significance.

Results and discussion

In evaluating the effect of extract treatment ointments, percentage wound contraction was determined from Day 3 to Day 15. Wound contraction is a hallmark of healing full-thickness cutaneous wounds. There are interplay between the complex cellular, biochemical, and biomechanical phenomena which result in wound contraction and it can be a beneficial or deleterious consequence of wound healing (Tranquillo and Murray, 1991).

Table 1 shows the percentage wound contraction of the excision wound model after 15 days. Among the different treatments, Treatments A and G showed the fastest rate of wound contraction on the 3rd day, while C displayed the fastest rate of wound contraction having a 94.25% rate on the 12th day apart from the controls. Phytochemical screening of these plants showed that both are positive for tannins which promote wound healing through several cellular mechanisms; chelating of the free radicals and reactive species of oxygen, promoting contractions of the wound and increasing the formation of capillary vessels and fibroblasts (Boakye, Agyare, Ayande, Titiloye, Asiamah, & Danquah, 2018).

Table 1. Percentage Wound Contraction for Excision.

Treatment	Days (Percentage Contraction)				
	3	6	9	12	15
A	38.33	45	60	68.33	100
B	24.07	53.7	62.96	72.22	100
C	28.81	49.15	76.27	94.92	100
D	9.8	21.57	74.51	84.31	100
E	19.05	45.24	54.76	83.33	100
F	7.55	64.15	96.23	100	100
G	57.69	65.38	92.31	100	100

A= 100% *D. cannaeformis*; B=100% *C. comosus*; C=50% *D. cannaeformis* and 50% *C. comosus*; D=75% *D. cannaeformis* and 25% *C. comosus*; E= 25% *D. cannaeformis* and 75% *C. comosus*; F=Ointment Base; G=Bactroban.

The result on the 12th day may be due to the synergistic effect exerted by combining both extracts at a concentration of 50% *D. cannaeformis*-50% *C. comosum* plant extract. Using the One-Way Analysis of Variance, a statistic F-value of 0.152794 is obtained which gave a p-value of 0.8713 (Table 2). Since the p-value is greater than alpha=0.05, the researchers do not reject the null hypothesis. At a 5% level of significance, there is no sufficient evidence to say that there is at least one significant pairwise difference among the treatments. This is consistent with several studies (Chua, 2019; Capili and Pastores, 2017); since the null hypothesis is not rejected, the ointments infused with plant extracts have no significant difference against the positive control on percentage wound contraction for excision.

Table 2. One Way Analysis of Variance for the Excision Percent Wound Contraction.

Type	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2369.087	6	394.8478	0.402002	0.871346
Within Groups	27501.7	28	982.2036		
Total	29870.79	34			

Table 3 shows the percentage wound contraction of the incision wound model after 15 days.

All treatments showed complete wound contraction during the 12th day. However, Treatment D (25% *D. cannaeformis*-75% *C. comosum*) showed the fastest rate of wound contraction, having 85.33% wound contraction on the 9th day. This implies that the combination at this concentration could have exerted a synergistic effect on wound contraction, as shown in the result on percent wound contraction on the 9th day.

Table 3. Percentage Wound Contraction for Incision.

Treatment	Days (Percentage Contraction)				
	3	6	9	12	15
A	34.25	67.12	75.34	100	100
B	29.11	59.49	72.15	100	100
C	21.95	57.32	78.05	100	100
D	41.84	64.29	69.39	100	100
E	6.67	62.67	85.33	100	100
F	33.33	49.43	59.77	100	100
G	4.62	33.84	53.85	100	100

A= 100% *D. cannaeformis*; B=100% *C. comosus*; C=50% *D. cannaeformis* and 50% *C. comosus*; D=75% *D. cannaeformis* and 25% *C. comosus*; E= 25% *D. cannaeformis* and 75% *C. comosus*; F=Ointment Base; G=Bactroban.

Using the One-Way Analysis of Variance, a test statistic F-value of 0.152794 is obtained which gave a p-value of approximately 0.9869 (Table 4).

Since the p-value is greater than alpha=0.05, the null hypothesis is not rejected. At a 5% level of significance, there is no sufficient evidence to say that there is at least one significant pairwise difference among the treatments. Since the null hypothesis is not rejected, the treatments have no significant difference against the positive control on percentage wound contraction for incision.

Table 4. One Way Analysis of Variance for the Incision Percent Wound Contraction.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	985.1675	6	164.1946	0.152794	0.986908
Within Groups	30089.19	28	1074.614		
Total	31074.35	34			

Table 5 shows the percentage wound contraction of the burn wound model after 15 days. Among the different treatments, silver sulfadiazine showed the fastest rate of wound contraction, having 100% contraction on the 12th day, while treatment C containing 50%-50% extracts showed the slowest rate. Treatment A (100% *D. cannaeformis*), Treatment B (100% *C. comosus*), and Treatment E

(25% *D. cannaeformis*-75% *C. comosus*) showed 100% wound contraction on the 15th day. From Day 12 to 15, Treatment E shows the highest increase in the rate of wound contraction. It implies that this concentration may show a synergistic effect between the two extracts as compared to other concentrations, which slowed down the rate of wound contraction.

Table 5. Percentage Wound Contraction for Burn Wound Model.

Treatment	Days (Percentage Contraction)				
	3	6	9	12	15
A	30.77	35.38	50.77	56.92	100
B	14.89	4.26	19.15	42.55	100
C	6.88	26.52	20.74	30.89	77.78
D	1.32	16.69	21.00	38.75	85.29
E	1.96	5.88	15.67	23.53	100
F	7.69	55.77	61.52	100	100
G	2.0	60.00	90.00	100	100

A= 100% *D. cannaeformis*; B=100% *C. comosus*; C=50% *D. cannaeformis* and 50% *C. comosus*; D=75% *D. cannaeformis* and 25% *C. comosus*; E= 25% *D. cannaeformis* and 75% *C. comosus*; F=Ointment Base; G=Silver sulfadiazine.

Using the One-Way Analysis of Variance, a test statistic F-value of 1.5747 is obtained which gave a p-value of approximately 0.1914 (Table 6). Since the p-value is greater than alpha=0.05, the null hypothesis is not rejected. At a 5% level of significance, there is no sufficient evidence to say that there is at least one significant pairwise difference among the treatments. Since the null hypothesis is not rejected, the researchers can say that the treatments have no significant difference against the positive control, Sulfur sulfadiazine, on wound contraction using the burn wound model.

Table 6. One Way Analysis of Variance for Burn Percentage Wound Contraction.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10412.8	6	1735.466	1.5747	0.191434
Within Groups	30858.62	28	1102.094		
Total	41271.42	34			

Conclusion

D. cannaeformis and *C. comosus* have wound healing activities for all wound models (excision, incision, and burn). The ointments with the aqueous plant extracts are not significantly different against the positive control. Among the treatments with the plant extracts, the combination of extracts showed the fastest rate in wound contraction. For excision, the 50% *D. cannaeformis* and 50% *C. comosus* showed the fastest rate in wound contraction. For incision, the 75% *D. cannaeformis* and 25% *C. comosus,* and for burn wound model, the *25% D. cannaeformis* and 75% *C. comosus*. But these results showed no significant difference against 100% *D. cannaeformis* and 100% *C. comosus*. This suggests that the performances of these treatments concerning wound contraction do not vary significantly.

However, tensile strength and histopathological studies of the granulation tissue are recommended for the incision wound model, while the type of wound closure and free radical scavenging activity for the excision model is recommended. In all models, hydroxyproline content in the scab is recommended.

References

Azliza MA, Ong HC. 2012. Ethno-medicinal Resources Used by the Temuan in Ulu Kuang Village. Studies on Ethno-Medicine **6(1),** 17-22. https://doi.org/10.1080/09735070.2012.11886415

Boakye YD, Agyare C, Ayande, GP, Titiloye N, Asiamah EA, & Danquah KO. 2018. Assessment of Wound-Healing Properties of Medicinal Plants: The Case of *Phyllanthus muellerianus*. Frontiers in pharmacology **9,** 945. https://doi.org/10.3389/fphar.2018.00945

Capili JT, Pastores NM. 2017. Effectiveness of Spider Plant (Chlorophytum comosum var. variegatum) in Skin Burns of Albino Rats (Rattus norvegicus strain Sprague Dawley) Compared to Silver Sulfadiazine. *IAMURE* International Journal of Ecology and Conservation, **22(1).** Retrieved from http://ejournals.ph/form/cite.php?id=12495

Chua JT. 2019. Wound Healing Activity of Aqueous Extracts of Common Legumes. *IAMURE* International Journal of Ecology and Conservation, **29(1).** Retrieved from http://ejournals.ph/form/cite.php?id=14896

Daud JM. 2011. Phytochemicals Screening and Antioxidant Activities of Malaysian Donax Grandis Extracts. European Journal of Scientific Research, **61(4),** 572-577.

Garg VK, Khosa RL, Paliwal SK. 2009. Wound healing activity of aqueous extract of Cynodon dactylon. Pharmacologyonline **1,** 1246-55.

Jha RK, Garud N, Nema RK. 2009. Excision and incision wound healing activity of flower head alcoholic extract of Sphaeranthus indicus Linn. in albino rats. Global Journal of Pharmacology **3(1),** 32-7.

Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P. 2007. Ethnopharmacological approaches to wound healing--exploring medicinal plants of India. Journal of Ethnopharmacology, **114(2),** 103-13. https://doi.org/10.1016/j.jep.2007.08.010

Martin P. 1997. Wound healing--aiming for perfect skin regeneration. Science **276(5309),** 75-81. https://doi.org/10.1126/science.276.5309.75

Modak M, Barde M, Modak MD, Barde MP. 2017. Efficacy of an Ayurvedic Formulation for Mild-to-moderate Osteoarthritis: A Phase 3, Randomized Controlled Study. Alternative Therapies in Health & Medicine **23(1).**

Narayan S, Sasmal D, Mazumder PM. 2011. Evaluation of the Wound Healing Effect of Herbal Ointment Formulated With Salvia Splendens (Scarlet Sage). International Journal of Pharmacy and Pharmaceutical Sciences **3(3),** 195-199.

Odimegwu DC, Ibezim EC, Esimone CO, Nworu CS, Okoye FB. 2008. Wound healing and antibacterial activities of the extract of Dissotis theifolia (Melastomataceae) stem formulated in a simple ointment base. Journal of medicinal plants research **2(1),** 1011-6.

Priya KS, Arumugam G, Rathinam B, Wells A, Babu M. 2004. Celosia argentea Linn. Leaf extract improves wound healing in a rat burn wound model. Wound repair and regeneration **12(6),** 618-25. https://doi.org/10.1111/j.1067-1927.2004.12603.x

Rupesh T, Nitika J, Raghvendra P, Sardul SS. 2011. Practices in Wound Healing Studies of Plants. Evidence-Based Complementary and Alternative Medicine, 2011. https://doi.org/10.1155/2011/438056

Sharma S, Mehta BK, Mehta D, Nagar H, Mishra A. 2012. A review on pharmacological activity of Syzygium cumini extracts using different solvent and their effective doses. International Research Journal of Pharmacy **3(12),** 54-58.