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Albert A Espiritu
Bicol University College of Nursing, Legazpi City, Philippines

Shaira Nicole L Lao
Bicol University College of Nursing, Legazpi City, Philippines

Jonathan Jaime G Guerrero
Bicol University College of Science, Legazpi City, Philippines

Correspondence
Jonathan Jaime G Guerrero
Bicol University College of Science, Legazpi City, Philippines

Burn wound healing potential of *Bixa orellana* Linn [Bixaceae] leaf extracts on albino mice

Albert A Espiritu, Shaira Nicole L Lao, Jonathan Jaime G Guerrero

Abstract
The use of natural remedy for burn wounds is common in tropical countries such as the Philippines. *Bixa orellana* of the family Bixaceae is traditionally applied to burn wounds for immediate and continued relief. This research investigated the healing potential of the plant on burn wounds using five replicates of mice per treatment as the animal model. Different preparation procedures were employed: crude, ethanoic and ointment. These were compared to the commercially available silver sulfadiazine (SSD). Results suggest that *Bixa orellana*, regardless of preparation procedure, were effective in treating burn wounds and is comparable to SSD. Percent wound contraction, re-epithelialization time, and histological features of the wound treated with *Bixa orellana* extracts were comparable to the control (SSD).

Keywords: *Bixa orellana*, Atsuete, Burn wound, Ointment, Ethanolic

1. Introduction
Wounds can be caused by a lot of factors including pressure, mechanical abrasions and trauma, metabolic ailments, animal or insect bites, scalds or burns. Healing properties of plants have been investigated on a variety of wounds. Common examples include *Aloe vera* [1, 2], *Psidium guajava* Linn. [3], *Moringa oleifera* Linn [4]. In the Philippines, these plants are alternative forms of cure because they are widely distributed, relatively cheap and readily available. Preparations include poultice, ointments, maceration, decoctions, infusions or combinations. Many households use herbal plants as part of traditional family practice. This research focused on the wound healing property of *Bixa orellana* on burn wounds.

Burn wound is a serious problem around the world, especially in low and middle – income countries [5], complicated by a variety of socio-economic factors [6, 7], with children, female and elderly at higher risk [8-10]. Thermal injuries when not given proper attention can lead to wound infections which harbor a variety of microbial load as cited by research articles from Bangladesh [11] and Philippines [12], to name a few. Atiye and colleagues [13] said that burn wounds remain to be endemic in low and middle income countries and associated with a high mortality rate.

Bixa Orellana, commonly known as *atsuete* in the Philippines, is a small tree of about five meters tall belonging to the family Bixaceae. Pigments are extracted from the seeds to produce paints and lipstick before the rise of synthetic dyes. Plant parts were evaluated to have various biochemical properties such as the antimalarial property of hairy root culture [14], antimicrobial activity of extracts from the leaves and seeds [15], antilipemic activity of seeds [16], analgesic and hypoglycemic activity of the leaves [17], antioxidant property of the bark [18] and antihistamine activities of the leaves [19].

This research aimed to add up to the existing literature on *Bixa orellana*, validate folkloric uses of the plant and contribute to possible applications on wound management using natural and traditional medicine. Specifically, it tried to compare the healing potentials of crude, ethanolic and ointment-based extracts of *Bixa orellana* on burn wounds of male albino mice.

2. Materials and Methods
2.1 Plant Material
Fresh leaves of *Bixa orellana* were collected from a tree present in the Area. Samples were thoroughly washed and air dried to remove excess water. Three extraction procedures were done. Preparation of aqueous extracts and ointment were done to mimic household preparations, while ethanolic extraction was done to get phytochemicals active in wound healing according to literature search. All extracts were stored in a refrigerator at 4C prior to use.

2.2 Ointment Preparation
Preparation of *Bixa orellana* ointment was done using a common household procedure in the Philippines. Fresh leaves weighing 100g were cut into small pieces and slowly fried until crispy in 200 ml of vegetable oil. Leaves were removed while the oil was mixed with two wax candles (Esperma 5) sliced into small cubes. All debris were strained. The wax was thoroughly mixed and allowed to melt and settle.

2.3 Crude Extract
Leaves of *Bixa orellana* were cut into small pieces then placed in a mechanical blender. Leaves were crushed until juice were present. Juice were squeezed out using a sterilized cheese cloth and collected in a sterile container.

2.4 Ethanolic Extract
Leaves of *Bixa orellana* were soaked for 24-48 h in ethanol in 1:2 ratio (w/v). Ethanol with plant extracts was filtered and concentrated in vacuum under reduced pressure using rotary evaporator. Residual solvent was allowed to evaporate at room temperature.

2.5 Test Animal
Methodology employed in the care, use, inflicting of wound, administration of treatment, excision and evaluation of wound on the mice were done in strict compliance to the Code of Practice for the care and use of laboratory animals in the Philippines prepared by the Philippine Association for Laboratory Animal Science (PALAS). Likewise, a thesis review and appraisal committee approved the conduct of the study as part of the university's research protocol.

2.6 Creation of Burn Wound
Full thickness second-degree burn wounds were made using methods of *Cai and colleagues* [20] and Yaman and colleagues [21], modified accordingly. Hair was shaved at the back area and disinfected with 70% isopropyl alcohol. Mice were anaesthetized using xylocaine % topical anesthesia. A cylindrical steel of 1 cm diameter was heated in a 100 C boiling water. This was held in contact with the skin of the mice for 10 s and removed thereafter.

2.7 Administration of Treatment
Each mice received their first topical treatment (day 1) after evaluation of the burn wound to be of the right depth and size. Mice of ICR strain were grouped into the following:
Positive Control Group: received silver sulfadiazine once a day
Group I: received *Bixa orellana* crude extract twice a day
Group II: received *Bixa orellana* ethanolic extract twice a day
Group III: received *Bixa orellana* ointment twice a day
Negative Control Group: received distilled water (dH_2O) twice a day
Each group had four replicates. Treatment were administered twice a day for fourteen days.

2.8 Evaluation of Wound
Gross examination of the wound was done daily to check on color, presence of exudates, swelling and consistency of tissues surrounding the wound [22]. Wound size was computed based on the following formula:

$$\%\ wound\ closure = \frac{initial\ area\ of\ wound - final\ area\ of\ wound}{initial\ area\ of\ wound}\ x\ 100$$

2.9 Histological Examination
Burned skin tissue samples were collected after sacrificing the mice for histological examination following methods by Yaman and colleagues [23]. Tissues were fixed in 10% neutral – buffered formalin solution and brought to the Bicol Regional Teaching and Training Hospital (BRTTH) Pathology section for preparation of slides. Prepared slides stained with Hematoxylin and Eosin were observed under light microscope.

2.10 Statistical Analysis
Numerical data were analyzed using Analysis of Variance (ANOVA) at 5% level of significance and Duncan Multiple Range Test (DMRT) as post-hoc test. Statistical analysis was carried out using SPSS version 22.

3. Results
3.1 Wound Contraction
Wound contraction is a gross assessment of how much of the injury was healed. A significant area of the burn wound among the test animal was reduced at day 8 of topical application of ethanolic and crude treatments as well as those given silver sulfadiazine compared to those given the ointment and distilled water (p<0.05). Completely healed wound was observed on day 14 on all mice given *Bixa orellana* extracts and silver sulfadiazine.

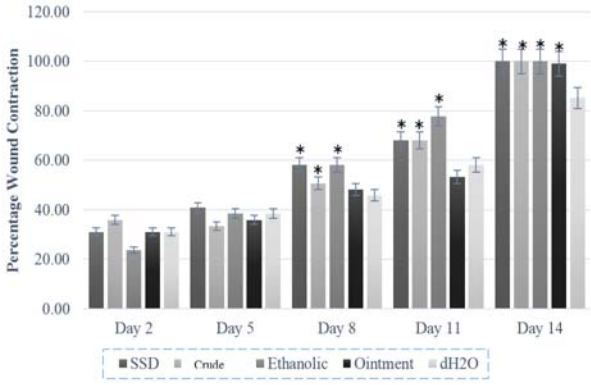

Fig 1: Effect of topical application of Bixa orellana extracts and control treatments on burn wound expressed as percentage of wound contraction. N=5. Values are mean ± SEM. * p<0.05 vs dH_2O

3.2 Epithelialization time

Table 1: Epithelialization time (days) of wound given topical treatments of Bixa Orellana

Groups	***Epithelialization time (days)****
SSD	13.5 ± 0.5[a]
Crude Extract	13 ± 0.5[a]
Ethanolic Extract	12.5 ± 0.5[a]
Ointment	13.5 ± 0.5[a]
Distilled H2O	14[b]

** – significantly different at p<0.05*
Values are means ± standard deviation
Values followed by similar letters are statistically comparable

Average epithelialization time was 13 days. Epithelialization of wound showed a significant difference against mice treated with distilled water. All extracts from *Bixa orellana* were statistically comparable to SSD.

3.3 Histological Examination
Burn wounds given silver sulfadiazine (Fig 2.A) showed a well-arranged and well-spaced re-epithelialization and moderate fibroblasts. *Bixa orellana* crude extract (Fig 2.B) elicited granulation, with concentration of cells in the lower dermis and extensive fibroblasts. Collagen and fibroblast are well-arranged in the granulation tissue of the wound applied

with ethanolic extract of *Bixa orellana* with minimal inflammatory cells (Fig 2.C). A huge number of inflammatory cells were observed from wounds given the *Bixa orellana* ointment (Fig 2.D) and with moderately defined granulation tissue. Wounds from mice given distilled water have no signs of extensive granulation, no to little collagen and fibroblast, with many inflammatory cells.

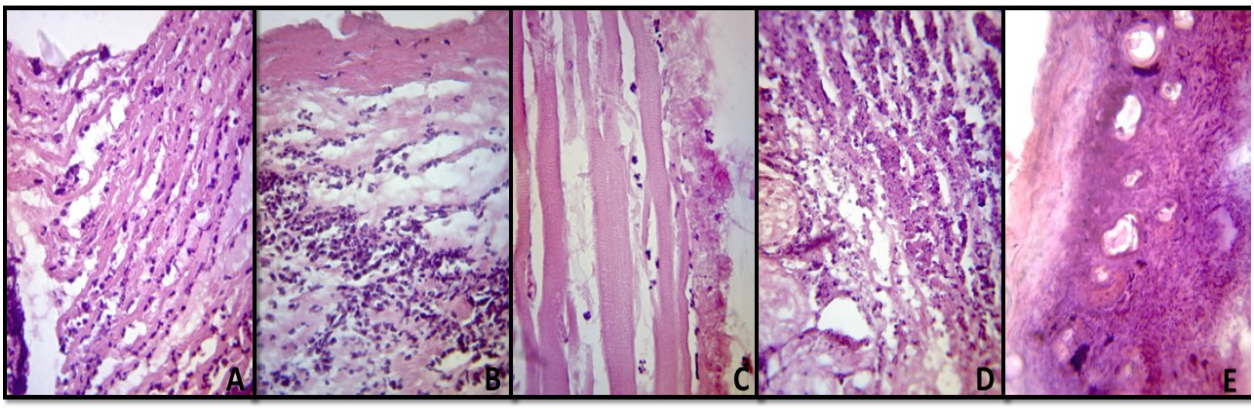

Figure 2. Excised wounds from mice treated with silver sulfadiazine (A), crude (B) and ethanolic (C) Bixa orellana extracts, Bixa orellana ointment (D), and distilled water (E). (H&E, 400x)

4. Discussion

The results of the research provide adequate evidence of *Bixa orellana* as a potential wound treatment with effects comparable to the commercially available silver sulfadiazine. The process of wound healing involves a dynamic interaction of physiological factors, spaced throughout four general phases: hemostasis, inflammation, proliferation and remodeling (or maturation) [24]. It would take approximately 21 days (or beyond depending on severity) for the affected area to move from phase 1 to maturation, encompassing the activities of platelets for clotting and the eventual actions of fibrocytes for the development of tensile strength [25]. Although burn wounds share many characteristics with other injuries, the addition of heat generally separates them and requires specific attention. Heat damages permeate beyond the skin, affecting other parts of the body, and healing depends on the depth of burns [26].

Wounds, whether they are caused by burns or mechanical abrasion, may almost always harbor microorganisms which can impede the process of healing. Topical antibiotics act on the wounds by promoting re-epithelialization on the damaged area. Silver sulfadiazine are commonly used because of its antimicrobial activity. Sulfonamides, known to be a broad-spectrum antibiotic, are released into the affected area which kills bacteria. A research by Fox and Modak [27] suggests that silver, not sulfadiazine, has antibacterial actions by a steady release of silver ions into the wound area. Other topical agents are being looked into as better than SSD such as zinc oxide [28] because it can shorten healing time, among other parameters.

Plant become a natural remedy for many diseases because it is a storage of active compounds. Many plant materials have been tested against SSD and have been found to be comparable or better in terms of percentage of wound contraction and collagen-fibroblast assemblage. These include *Arnebia euchroma* [29], *Aloe vera* [30, 31], *Punica granatum* [32] and *Plectranthus amboinicus* [33] among others. All these plants are locally available and relatively cheaper for a typical household.

Phytochemical investigation of *Bixa orellana* revealed the presence of various compounds in aqueous and ethanolic extracts including alkaloids, tannins, triterpenoids, steroids, saponins, and flavonoids [34]. Flavonoids have been known to reduce lipid peroxidation and thereby increasing collagen fiber viability due to increased vascularity [35]. Tannins, on the other hand, have been found to promote cutaneous wound healing by increasing vascularization (angiogenesis) and being antibacterial [36]. Saponin is found to be beneficial in cutaneous wound healing by increasing keratinocyte migration rate and reducing inflammatory cells in the wound area [37]. Extraction procedure and preparation, as shown in the results of this research, seem to have no significant effect on its wound healing activity.

5. Conclusion

Bixa orellana, regardless of extraction procedure and preparation, have been found to be significantly comparable to the commercially available silver sulfadiazine in treating burn wounds among albino mice.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

6. Acknowledgement

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