Research Article

Study on wound healing effect of Milk Bush (*Euphorbia tirucalli* Linn) in albino mice

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Abstract

Objective: This study aimed to determine the wound healing effect of *Euphorbia tirucalli* Linn. latex on albino mice. **Material and methods:** Phytochemical screening and wound-healing effect of milk bush was done. Test for alkaloids, saponin, anthraquinone, tannins, and steroids were done to profile the phytochemical screening of milk bush. In wound-healing, betadine was used as the positive control. Albino mice were evaluated if the efficacy of milk bush as wound-healing is greater than the commercial product. **Results and conclusion:** Examination of the physical properties of *E. tirucalli* Linn. showed it has a white color, with an unpleasant odor, a density of 2.6233 g/mL, a pH of 5.92, which is acidic, and was miscible in water, while immiscible in hexane and acetone. As for its chemical properties, it was positive in alkaloids, flavonoids, and steroids, but was negative in tannin, saponin, and antraquinone. The pure latex of the plant showed positive wound healing effect as manifested by the formation of scar tissue which effectively led to wound closure and eventual healing after six days of observation. Based on the analysis of variance, there was a significant difference that exists between and among the different concentration levels of *E. tirucalli* L. latex and the commercial antiseptic "Betadine". Impliedly, the plant latex is not comparable with the commercial preparation. However, the researchers believe, as borne out by the results, that the latex was effective in healing wounds and skin lacerations. The researchers recommend further studies using other part of *E. tirucalli* L. to determine its potential medicinal applications, in-depth characterization of the phytochemicals present in the latex of *E. tirucalli* L., and the use of the plant latex as a first aid application for wounds and lacerations should be studied further, considering that the substance is reputed to be poisonous.

***Keywords**:* Wound healing effect, antiseptic, milk bush, albino mice

Introduction

Biological based therapies consist primarily of herbal therapies or remedies, botanicals, and dietary supplements. Herbal remedies are a major component of all indigenous forms of medicine; prior to the development of pharmaceuticals at the end of the nineteenth century, people everywhere in the world relied on materials from nature for pain relief, wound healing, and treatments of a variety of ailments.

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Euphorbia tirucalli L. (Euphorbiaceae) is a succulent plant commonly distributed to tropical areas and rainforests in the Amazon, Madagascar, and South Africa. The plant is commonly called as "Vajradruma" (Sanskrit), Indian tree spurge or Milk bush (English) and "Bontakalli" (Kannada). The latex is used as an application for warts, rheumatism, neuralgia, and tooth ache (Abate et al., 2000).

Euphorbia tirucalli L., one of the 8,000 species within family Euphorbiaceae, is a shrub or a small tree endemic to tropical areas with pencil-like branches from which it derives its vernacular name, the pencil-tree. *E. tirucalli* is generally evergreen since its stems and branches remain green all year round and are rarely fed on by herbivores. It bears a white poisonous latex, which may possibly account for the low herbivore pressure and medicinal features.

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Figure 1. *Euphorbia tirucalli* Linn.

According to Schmelzer and Gurib-Fakim (2008) and Van Damme (1989), in East Africa, the latex is used against sexual impotence, warts, epilepsy, toothache, hemorrhoids, snake bites, extraction of ecto-parasites, and cough among others. In Peninsular Malaysia, a poultice of the roots or stems is applied to nose ulceration, hemorrhoids, and swellings. Root scrapings mixed with coconut oil are taken for stomach-ache. In India, Kumar (1999) notes that it is an unavoidable plant in most traditional homesteads and used as a remedy for ailments, such as spleen enlargement, asthma, dropsy, leprosy, biliousness, leucorrhoea, dyspepsia, jaundice, colic, tumors, and bladder stones. Although the latex is emetic in large doses, it is purgative in small doses and is applied against toothaches, earaches, rheumatism, warts, cough, neuralgia, and scorpion bites. The same author points out that its branch and root decoctions are administered for colic and gastralgia, while ashes are applied as caustic to open abscesses. Duke (1983) and Van Damme (1989) mention that in Brazil, *E. tirucalli* is used against cancer, cancroids, epitheliomas, sarcomas, tumors, and warts, although they argue that this has no scientific basis since the same tree is known to be co- carcinogenic.

In Malabar (India) and the Moluccas, the latex is used as an emetic and anti-syphilitic, while in Indonesia, the root infusion is used for aching bones while a poultice of the roots or leaves is used to treat nose ulcers, hemorrhoids, and extraction of thorns. Wood decoctions are applied against leprosy, and hands and feet paralysis following childbirth (Duke, 1983). The same author states that in Java, the plant latex is used to cure skin ailments and bone fractures.

The plant is known in Brazil as “aveloz”, and its latex has been used by the native people in traditional medicine as anti-helminthic, antisyphilitic, and anti-tumor (Hecker, 1968; Silva, 2007). Some biological properties of *E. tirucalli* have been confirmed, such as its larvicidal, mollusicidal, bactericidal, and antiherpetic activities (Tiwari, 2003; Fürstenberger, 1986). These activities are likely related to the presence of phytosterols and triterpenes (Khan and Ahmed, 1987).

Materials and methods

Collection of Milk Bush pure latex

A total of one hundred twenty-four (124) mL of milk bush pure latex sample was collected and used in the entire study. The pure latex of the milk bush plant was collected by having plant parts cut off from the shoots using scissors and was collected into a clean container.

Determination of the physical properties color determination

Color determination of the milk bush pure latex using the sense of sight was done by a total of three respondents. About three (3) mL of milk bush pure latex was measured and put in a clear test tube. Then, using the sense of sight, the respondents were asked to describe the color of the pure latex contained in the test tube. The obtained perceptions of the color from the respondents were gathered for proper color determination. **Odor determination** of the milk bush pure latex was determined using olfactory sense of a total of three (3) respondents. About three (3) mL of milk bush pure latex was measured in a clear test tube. Then, using the sense of smell, the respondents were asked to describe the odor of the pure latex contained in the test tube. The obtained perception for the odor of the pure latex from the respondents was gathered for proper odor determination. **pH** of the milk bush pure latex was tested using a pH meter. About three (3) mL of the pure latex was placed in a beaker. Then, the pH meter was dipped into the pure latex. After a period of one minute, the value was recorded. The procedure was repeated thrice. Average pH was then computed. **Density determination,** about three (3) mL of the pure latex was weighed in a digital balance. Its weight was recorded and compared to the weight of an equal volume of water. This was repeated thrice for higher accuracy. **Miscibility determination**, for testing the miscibility of the milk bush pure latex, three (3) solvents were used, namely; hexane, water, and acetone. About three (3) mL of the pure latex was put into nine (9) clear test tubes. Then, two (2) mL each of hexane, water, and acetone was poured into the test tubes, taking into consideration the three replicates necessary to test each solvent. Hence, three of the nine test tubes were added with hexane, another three with water, and the last three test tubes were added with acetone as solvents. For one (1) full hour, the nine test tubes were observed to determine the miscibility of the pure latex in three (3) different solvents. After the given hour, the researcher recorded the results, whether it is (1) miscible,

(2) slightly miscible, or (3) immiscible with respect to the solvents tested.

Determination of chemical properties

The following procedures were taken from the Standard Methods by Guevara et al., (2005): **Test for Alkaloids**: Dragendorff's and Mayer's reagents were used in determining the presence of alkaloids. A positive result is indicated by the formation of an orange precipitate for Dragendorff's reagent, and while a white precipitate for Mayer's reagent is a positive indication. An equivalent of three (3) mL pure latex of milk bush was placed in an evaporating dish. Then, it was evaporated to a syrupy consistency over a steam bath. Five 5 mL of 2M HCl was added. Next, the solution was heated, with stirring, for about five minutes, after which the solution was allowed to cool. Then, about 0.5g NaCl was added. Then, it was stirred and filtered. The residue was washed with enough 2M HCl to bring the filtrate to a volume of 5 mL, and then the filtrate was separated into two parts. To the first part, 2-3 drops of Dragendorff's reagent, and to another part, 2-3 drops of Mayer's reagent, were also added. In this test, the reagents were used in determining the presence of alkaloids, a positive result of which is indicated by the formation of an orange precipitate in Dragendorff's reagent, and a white precipitate in Mayer's reagent. The result was recorded.

Test for the presence of Saponin: Capillary test was used to determine the presence of saponin. If the level of the pure plant latex in the capillary tube is half or less than the other tube containing water, the presence of saponin may be inferred. Load the capillary tube with the plant latex by immersing the tube to a height of 10 mm in the pure plant latex. Likewise, load another capillary tube with distilled water and lift the capillary tube to keep both in vertical position to allow the liquid inside to flow freely. And then after sometime, compare the heights of the liquids in the two tubes.

Confirmatory test for Saponin: A positive result is inferred when more than 2 cm 'honey comb' froth persists after 10 minutes on the surface of the pure latex. About 1 g of the pure latex of the milk bush plant was taken and added with 10 mL of 80% ethyl alcohol. This was allowed to stand for 30 minutes. It was then filtered. An equivalent of three (3) mL pure latex was then transferred to a test tube. In a separate test tube, 1 mL of the pure latex served as the standard. In the other test tube, 10 mL of distilled water was added. The test tube was then stoppered and shaken vigorously for 30 seconds. The result was compared with that of the standard.

Test for Anthraquinone: The modified Borntrager's Test was used to determine the presence of Anthraquinone. In this test, a pink color indicates a positive result for antraquinone.

The test was done by separately taking 3 mL of the stock plant latex prepared and evaporates to incipient dryness over a steam bath. Ten milliliters (10 mL) of distilled water was added to the residue, and it was filtered separately. The residue was discarded. The aqueous filtrate of the pure latex was extracted separately with 5 mL portions of benzene. The benzene extracts of pure latex were divided into 2 portions. One portion was reserved as the control. The other portion was treated with 5 mL ammonia solution and shaken. Then it was compared with the control tube for color determination.

Test for Tannins: Into three test tubes, each with three (3) mL of milk bush pure latex was added with a few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins.

Test for Flavonoids: About three (3) mL pure latex was added with 2 mL ethanol. Three pieces of magnesium chips were added, followed by 2-3 drops of concentrated hydrochloric acid. A pink, orange, or red to purple coloration is the evidence of the presence of flavonoids.

Test for Steroids: About three (3) mL pure latex was added with 2 mL chloroform; then, 2-3 drops of concentrated sulfuric acid was carefully added. A reddish brown color indicates the presence of steroids.

Wound healing Study

A total of forty-five (45) albino mice were used to evaluate the wound healing effect of *Euphorbia tirucalli* Linn. pure latex. A commercial antiseptic iodine preparation, was used as thepositive control, while distilled water was used as the negative control.

Acclimatization of animals

Experimental animals used were forty-five (45) healthy, young albino mice, each weighing between ten to twenty-five grams (10-25g). They were housed in groups of five (5) animals each, in standard cages for an acclimatization period of seven (7) days or one (1) week to let them adjust to the environment before the commencement of the experiment. Albino mice were periodically weighed before and after the experiment.

Preparation of E. tirucalli Linn. pure latex

A fresh stem for latex extraction was collected in Barangay Baybay, Catarman, Northern Samar. After cutting the stem of *E. tirucalli* L., the pure latex was directly collected into a clean container. There were three (3) levels of concentration

of *Euphorbia tirucalli* Linn. pure latex. 1. 100% concentration- pure latex. 2. 75% concentration- a mixture of 1.5 mL of pure latex, added with 0.5 of distilled water to achieve a 75% concentration of the solution. 3. 50% concentration- a mixture of 1 mL of pure latex added with 1 mL of distilled water was prepared to arrive at a 50% concentration of the solution. The different concentrations of *Euphorbia tirucalli* Linn. pure latex was placed in sterile bottles and was labeled for easy identification.

Wound Infliction/Creation

The dorsal skin was shaved and cleaned with a commercial antiseptic prior to the experiment. The wound was made on the back- dorsal thoracic region - using surgical scalpel and scissors. An open, full-thickness wound that was approximately 5 mm long, with a depth of about 0.84 mm, was cut up to the level of the subcutaneous adipose tissue. After the wounding process, each albino mice was caged individually.

Results and discussion

The latex contained the secondary metabolites; alkaloids, flavonoids and steroids, but was negative in tannin, saponin and antraquinone. The presence of such secondary metabolites could account for its wound healing properties.

The following figure (Figure 2), consisting of a rectangular graph, was used as the basis for determining the healing effect of the treatments. It shows the wound length (red box) and the formation of scar tissue (yellow) around it until finally the wound healed completely (yellow box) on the sixth day of observation. Results show that different concentration of the latex initiated scar tissue formation that was comparable to the commercial antiseptic.

Table 2 presents the amount of scar tissue formed six days after wound infliction and application of the different treatments. Results show that pure latex had an almost similar effect when compared with the commercial

Table 1. Physical properties and phytochemical constituents of *Euphorbia tirucalli* Linn. pure latex

Physical properties		**Phytochemical constituents**	
Color	White	**Flavonoids**	Positive
Odor	Unpleasant	**Alkaloids**	Positive
pH	Acidic (5.92)	**Tannin**	Negative
Density	2.6223 mg/mL	**Saponin**	Negative
Miscibility with;		**Anthraquinone**	Negative
Water	Miscible	**Steroids**	Positive
Hexane	Immiscible		
Acetone	Immiscible		

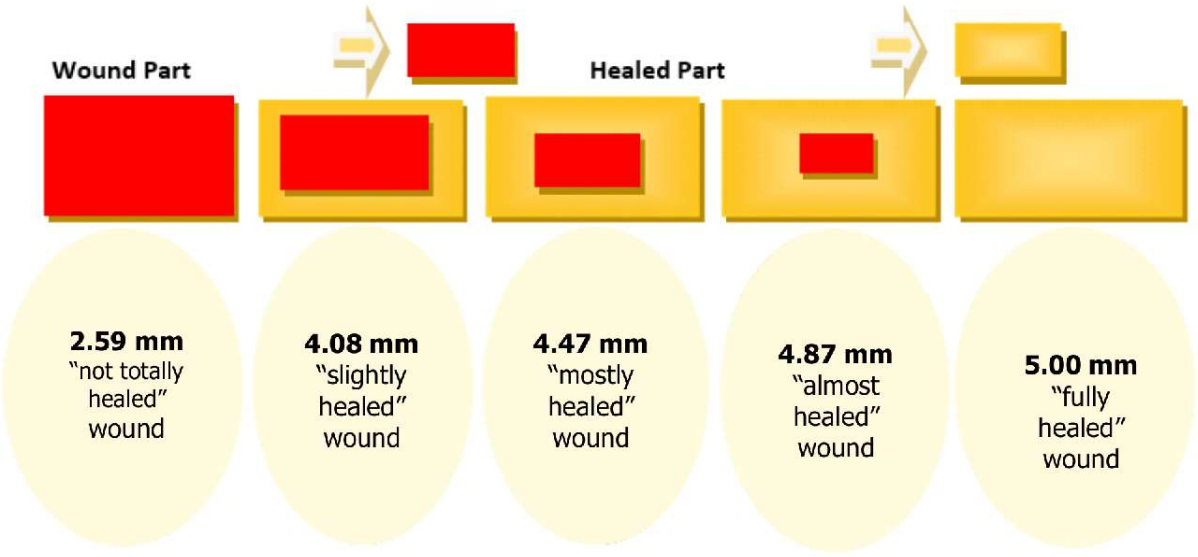

Figure 2. Healing effects of the treatments

Table 2. Scar tissue formation in albino mice using different treatments

Treatment	Average of Length, Scar Tissue *(After 6 days)*	Interpretation
Positive control	5.0 mm	"Fully healed" Wound
100% concentration	4.87 mm	"Almost healed" Wound
50% concentration	4.47 mm	"Mostly healed" Wound
75% concentration	4.08 mm	"Slightly healed" Wound
Negative control	2.59 mm	"Not totally healed" wound

antiseptic agent used as the positive control, while it was apparently significantly different with the effect of distilled water (negative control). Impliedly, therefore, the latex had positive wound healing effect on albino mice.

Conclusion

The pure latex of *E. tirucalli* Linn. has physical characteristics and contains phytochemical constituents or secondary metabolites that could account for its wound healing properties. This positive effect was manifested by the formation of scar tissue, effectively leading to wound closure and eventual complete healing. Statistical analysis indicates the plant latex was not significantly different in its effect when compared with a commercial antiseptic. This implies that the latex has positive wound healing effect and therefore, is a potential cure for wounds and/or skin lacerations.

Recommendation

It is recommended to utilize other plant parts of *E. tirucalli* Linn. to discover its potential medicinal applications is deemed necessary. Despite the poisonous reputation of the plant latex, its efficacy as a first aid treatment for wounds and lacerations is recommended.

Conflicts of interest: Not declared.

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