Kontagora International Journal of Educational Research (KIJER) ISSN: 3027-0618
Volume 2, Issue 1, January 2025
https://fuekjournals.org/index.php/kijer

The Effect of *Tamarindus Indica Linn*. Seeds Extracts on Epidermal Wound Healing in Rabbit (*Oryctolagus Cuniculus*)

[1]Salami, J. U., [1]Oti, E. C., [2]Adediran, M. B. & [1]Solomon, B.

Corresponding author: **salamijenny@yahoo.com**

[1]Department of Biology, Federal University of Education, Kontagora, Niger State

[2]Department of Integrated Science, Adeyemi Federal University of Education, Ondo, Ondo State.

DOI: https://doi.org/10.5281/zenodo.14799574

To cite:
Salami, J. U., Oti, E. C., Adediran, M. B., & Solomon, B. (2025). The effect of *Tamarindus indica* Linn. seeds extracts on epidermal wound healing in rabbit (*Oryctolagus cuniculus*). *Kontagora International Journal of Educational Research, 2*(1), 421–432.

Abstract

Wound healing efficiency of Tamarind seed was evaluated. Different solvents: water, methanol and ethanol were used to prepare the extract. Vertical wound of 3cm was inflicted on the dorsal part of the body of sampled Oryctolagus cuniculus. Extracts were applied topically once daily. Wound area was measured using ruler and Tecno BC2C shot of 5.0mp digital camera was used to take the wound pictures. The different effect of the tamarind extracts on wound healing was observed. All extracts exhibited significant increase in the rate of healing with Water extract as the most effective. At day 9, all treatments were significant and demonstrated high percentage of wound reduction with Water and Ethanol surpassing 50% and Methanol at 37%. Treatment with all extracts resulted in complete wound healing at day 18 as compared to 21 days for the natural healing process. Phytochemical screening for Tamarind seeds reveals the presence of Phytate, Cyanide, Tannin, Saponin and Oxalate in all extracts.

Keywords: Tamarindus indica L. seeds, Extracts, Wound, Healing, Rabbits

Introduction

Tamarindus indica is an evergreen tropical tree as every part of it including roots, stems, fruits, and leaves not only have rich commercial and nutritional values but also in Trado-medicine for the treatment of wide array of diseases (Martinello *et al*., 2006). This tree remains one of the most interesting plants in Africa, particularly for its huge potential disease-healing components. It has immunomodulatory effect and lacks carcinogenic and cytotoxic activities (Sano *et al*., 1996). Water extract of tamarind seed was found to reduce blood sugar level in streptocin- induced diabetic male rats (Mori *et al*., 2004). In addition, Tamarind seeds are known to have high inhibitory activity against human neurotrophic elastase (Fook *et al*., 2005). Tamarind seed polysaccaharide (TSP) has been shown to improve dry eye syndrome, to assist release of drug in human body and intraocular penetration of rufloxacin (Ahelardi *et al*., 2004)

Wound healing is a process that occurs after injury, of which the bleeding is accompanied by blood clotting, inflammation, proliferation and remodeling set in (Nagle *et al*., 2023).

Materials and Methods

Collection of *Tamarindus indica* Seeds

Raw tamarind pulps were purchased from Kontagora New Market, Kontagora in Niger state, Nigeria. The average size of the pulps were approximately 3x2x2 cm in length and 1x2x2 cm in breadth, most of the pulps were not of the same size. The tamarind pulps were soaked in water for 24 hours, washed and the seeds removed. The seeds were further sundried for 12 hours to remove moisture for further usage.

Preparation of the Extracts

Three types of extracts were prepared namely; water, methanol and ethanol extracts. The air-dried seeds were ground until it became homogenous powder. The cold maceration method of extraction was used for the extraction of the plant sample (Tamarind seeds) with three different solvents: distilled water, methanol and ethanol respectively. Approximately 100g of the sample each was separately weighed in to three clean 2000ml conical flasks. To the three conical flasks, 1000ml of

distilled water, methanol and ethanol were added, respectively and allowed to stand at room temperature for 72 hours with constant shaking using water bath shaker. After 72 hours, the extracts were separately filtered into three clean beakers using Whatman filter paper. The aqueous extract was dried using LGJ-Freeze-dryer, while the methanolic and ethanolic extracts were air-dried at room temperature, and were then packed in to sample bottles for further use.

Experimental Animals

Twenty (20) male rabbits (*Oryctolagus cuniculus*) weighing (525±12.5) at wound infliction, (526±12.6) on the 3rd day and (528±12.6) at 15th day post-infliction, were used as test samples to observe the wound – healing rate. The rabbits were purchased at Kontagora old market, Kontagora, Niger state, and housed under standard experimental condition of light/dark cycle. They were allowed free access to normal diet (Spinach, Maize brawn and groundnut feed) and water throughout the period of experiment. The rabbits were 3-4 months old at the time when wound was inflicted and were randomly placed in four (4) groups of five (5). The first group was treated with iodine and Vaseline and others with water, ethanol and methanol extracts respectively.

Wound Infliction and Extracts application on test Animals.

The fur was shaved off using razor blades, scissors and shaven stick. Before incision, a thread and ruler was used to measure the length required for the wound to be made. They were partially anaesthetized as a vertical epidermal wound of 3cm length was made at the dorsal part of the rabbits using tooth-forcep and razor blades. Cotton wool and ethanol pads were used to clean the blood and skin, and let to dry. The extract was applied 30 minutes after infliction and not immediately to avoid flush out by the wound. 1ml/100g of each extract was measured daily using weighing balance. The extract was mixed with Vaseline using spatula to provide moisture and soothing feeling as the spatula was used in applying the extract on the wound surface once in a day. A Techno BC2C Shot 5.0mp digital camera was used to picture distinct features of the wound. The wound area and reduction were noted each day and measured using Millimeter scale ruler and percentage was calculated as;

$$\text{Wound reduction (\%)} = \frac{\text{Initial size of wound- size of wound at specific day}}{\text{Initial size of wound}} X100$$

Phytochemical Composition of Tamarind Seeds

A collection of tests was used for quantitative analysis of the phytochemicals present in the extract. The test used in this experiment was to determine the presence or absence of phylate, cyanide, tannins, alkaloids, saponins and oxalate.

Determination of Phytate

4.0g of the sample was soaked in 100ml of 2% HCL for 5hours then filtered 25ml of the filtrate was taken into the conical flask and 5.0ml of 0.3% HN_4SON solution was titrated with a standard solution of $FeCl_2$ containing 0.00195 Fe/ml until a brownish yellow colour persisted for five minutes. The Phytate content was then calculated using the multiplying value of phytin-phosphorus by 3.55,

$$Concentration\ of\ phytate\ (mg/100g) = \frac{TV \times Equ, Wt \times D.3.55 \times 1000}{W}$$

$TV = titrate\ value$ $Equ.wt = equivalent\ weight$

$D. = dilution\ factor\ 1000$ $W = weight\ of\ sample$

Determination of Oxalate Content

2.5g of the sample was extracted with 100ml, 20% HCL 5ml of concentration NH_3 and precipitated, was then washed with 20ml of 25% determine the concentration of oxalate until a pink end point was observed (1ml of 0.045g oxalic acid).

Concentration of oxalate (mg/100g) = (TV×Equ,Wt×D.3.55×1000)/W

Where TV=titrate value Equ. Wt= equivalent weight

D = dilution factor W = weight of sample.

Test for Tannin

0.5g of the sample was weighed into a plastic bottle, 100ml of distil water was added and shaken for 1 hour in a mechanical shaker. This was filtered into 10ml volumetric flask and made up to the mark then 1ml of filtrate was pipetted into test tube and mixed with 0.4ml of 0.1ml $FeCl_2$ in 0.1 NHCL and 0.008m potassium Ferro cyanide.

Test for Alkaloids

Quantitative determination of Alkaloid was according to the methodology by Harborne (1998). 200cm of 10% acetic acid in ethanol was added to each tamarind powder sample of 2.50g in 250cm beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by additional of 15drops of concentrated ammonium hydroxide to the extract until the precipitation was complete, immediately after filtration. After 3hours of mixture sedimentation, the supernatant was discarded and precipitated was washed with 20cm of 0.1m of ammonium hydroxide and then filtered using gem filter paper (12.5cm). Using electronic weighing balance model B-218, the residue was dried in an oven and the percentage of Alkaloid is expressed mathematically as

$$\text{Alkaloid (mg)} = \frac{Weight\ of\ Alkaloid \times 100}{Weight\ of\ the\ sample}$$

Test for Saponins

Saponin quantitative determination was carried out using the method by Ejikeme *et al*., (2014) as reported Ademoye *et al*., (2018). Exactly 100cm of 20% aqueous ethanol was added to 5g of each tamarind powder sample in 250cm conical flask. The mixture was heated over hot water bath for 4hours with continuous stirring at a temperature of 55%. The residue of the mixture was re-extracted.

$$\text{Saponin (mg)} = \frac{Weight\ of\ saponin \times 100}{Weight\ of\ the\ sample}$$

Determination of cyanide

The determination of cyanide concentration in tamarind powder sample was conducted by addition of exactly 80% of aqueous methanol added to 2.50g of sample in a 250cm beaker covered and allowed to stand for 24hours at room temperature.

Data Analysis

Mean and Standard Errors of the means of the extracts for the treatments were determined. All data were subjected to one-way ANOVA using SPSS Software, 17 at significant level of $p \leq 0.05$

Result

Methanol extract of tamarind seed gave a yield of 14.00g freeze–dried powder, water extraction with 4.53g freeze-dried powder and ethanol extract of tamarind seed produced 14.45g freeze-dried powder in 100g of seed respectively.

Effect of Tamarind seed extracts on Wound Healing

Table 1: Percentage (%) of average healing rate (of 5 rabbits) at interval of 3 days.

Extract	3rd day	6th day	9th day	12th day	15th day	18th day	21st day
Untreated	3	16	30	50	67	80	97
Water	20	33	53	70	93	100	-
Methanol	10	23	37	63	73	97	-
Ethanol	16	30	50	68	80	100	-

The reduction of wound areas per day according to the extracts varied. The healing rate at 50% reduction of the initial wound area at 8, 9 and 11 days were observed for water, ethanol and methanol extracts respectively, compared to approximately 17 days for the untreated group and for 75% wound reduction, nearly 12, 14 and16 days were required (Table 1).

Table 2: Healing rate for 21 days

Extract	Wound size (cm)
Untreated	1.53±0.36
Water	0.99±0.28
Methanol	1.27±0.32
Ethanol	1.09±0.26

(Mean± SEM)

At day 3, all treatments showed noticeable reduction, water been the highest at 20%. At day 9, all treatments were significant and demonstrated high percentage of wound reduction with Water and Ethanol surpassing 50% and Methanol at 37%. Treatment with all extracts resulted in complete wound healing at day 18 as compared to 21 days for the natural healing process. Healing was fastest with water extract at 0.99±0.28 as compared to the standard control group (Table 2). The rate of healing of the different groups was not significantly different.

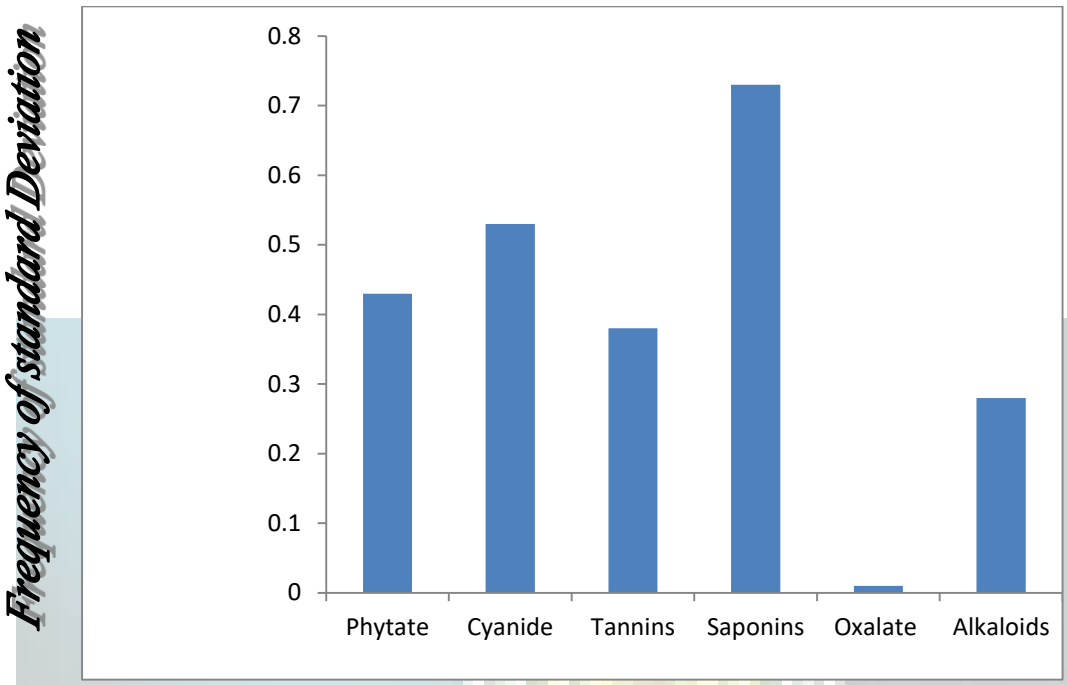

Figure 1: Shows the Photochemical Constituents and Concentration

Discussion

Wound is a disruption of the anatomical structure and function of tissues, of which inflammation is initiated at the early phase, followed by epithelialization, proliferation and remodeling (Nagle *et al*., 2023). The choice of plants for wound treatment depends on several factors including skin structure, good understanding of wound healing process and plant components.

Phytomedicine has become a primary and thriving means of healthcare especially by about 80% of world population in Asia and Africa (WIPO, 2015). Tamarind been considered an important plant have been recognized for its potency, as evident by various research. In this study, water, methanol and ethanol were used as the extraction solvent. Extracts were applied with Vaseline to provide sooth and moisture to the wound bed, which is essential for the enzymatic activity.

Extracts of Tamarind seeds in this study have shown faster wound healing process. This result was expected due to findings on its immunomodulatory, anti-toxicity and antioxidative activities (Sreelakha *et al*., 1991, Sano *et al*., 1996). Phytate in Tamarind seeds may be involved in stabilizing

highly reactive, potential harmful free radicals and protect cells from oxidative damage. The ability of antioxidant to destroy highly reactive free radicals serves to protect structural integrity of immune cell and prevent the loss of essential functions. This study proposes that the phytate might contain bioactive components which act as antioxidants responsible for neutralizing the effect of free radicals which are normally generated by oxygen dependant mechanism for intra-cellular killing by leukocytes as reported earlier by Sudjaroen *et al*., (2005). Water extract exhibits the fastest rate of healing, which contains highest bioactive contents.

Phytochemical result showed that alkaloids were present and additional tannin was present in water extract. Alkaloid is known to assist epithelialization of wound and chemotaxis in fibrosis as it could work with Tannin for the antioxidant, anti-inflammatory and antimicrobial properties for faster wound healing (Azeez *et al*., 2007, Adeola *et al*., 2010). Saponin on the other hand stimulates angiogenesis by modifying the balance of protease /protease inhibitor secretion in human endothelial vascular cells (Morisaki, 2000).

Water extract had the highest healing rate, which agrees with result from Rahman *et al*., 2012 which also conforms to the outcome of the application of Ethanol extract. According to Robab *et al*., (2015), Tannin promotes excisional wound healing by angiogenesis and coagulation. The presence of alkaloid, saponin and Tannin in water extract points to the efficacy of Tamarind to wound healing.

Conclusion

Extracts of *Tamarindus indica L.* seeds are sources of medical properties, with potential ability of healing wounds amongst treatment of other human ailments that has been investigated. This study revealed the natural antioxidants present in *T. indica* seeds and its possible impacts on wound healing as observed in *O. cuniculus*.

Application of the different extracts of *T. indica* seeds on the wound surfaces significantly enhanced healing. The phytochemical screening of the seeds elucidates constituents like Alkaloids, Saponins, Phytate, and Cyanids, which could result in faster wound healing process.

Recommendations

The use of different Tamarind seed extracts on the wound surface significantly enhanced healing, thus, recommended for use. This study validates the need to further explore Trado-medicine, hence, the call for investigation and developments of effective and safe drugs from various plants parts for epithelialization and healing of wound.

References

Ademoye, M.A., Lajide, L. & Owolabi, B.J. (2018). Phytochemical and Antioxidants screening of *Chrysophyllum albidum, Mezoneeuron benthamianum, Phyllanthus muellerianus* and *Acalypha fimbriata*. International Journal of Sciences, 7(11).

Adeola, A.A., Adeola, O.O., & Dosunmu, O.O., (2010). Comparative analysis of Phytochemicals and antimicrobial properties of extracts of wild *Tamarindus indica* pulps. African Journal of Microbiology Research, 4 (24), 2779-2797

Azeez, S., Amudhan, S. Adiga, S., Rao, N. & Udupa., L. A. (2007). Wound healing profile of Area Catechu extract on different wound models in wistar rats. Kuwait Medical Journal, 39 (1), 48-52

Ejikeme, C.M., Ezeonu, C.S., & Eboatu, A.N. (2014). Determination of physical and Phytochemical constituents of some tropical timbers indigenous to Niger Delta area of Nigeria. European Scientific Journal, 10 (18):247-270.

Fook, J. M. S. S. L., Macedo, L. L. P. & Moura, G. E. D. D. (2005). A serine proteinase inhibitor isolated from *Tamarindus indica* seeds and its effects on the release of human neutrophil elastase. Journal of Life Sciences, (76), 2881-2891.

Gherlaadi, E., Tavanti, A. & Davini, P. (2004). A muco-adhesive polymer extracted from Tamarind seed improves the intraocular penetration and efficacy of Rufloxacin in topical treatment of experimental Bacterial Keratitis, Journal of Antimicrobial Agents and Chemotherapy, 48, 3396-3401.

Harborne, J.B. (1998). Phytochemical Methods: A guide to modern Techniques of plant analysis. 5th Edition, Chapman & Hall Ltd., London, 21-72.

Martinello, F., Soares, S.M., Franco, J.J., Santas, A.C., Sugohara, A., Garcia, S.B., Curti, C & Uyemura, S.A. (2006). Hypolipemic and antioxidant activities from *T. indica L*. pulp fruit extract in hypercholesterolemic hamsters. Food Chemistry Toxicology, 44: 810-818

Morisaki, N., Watanabe, S. & Tezuka, M. (2000). Mechanism of angiogenic effects of saponin from ginseng Radix rubra in human umbilical vein and endothelial cells, British journal of pharmacology, 115:1188-1193

Mori, R., Kondo, T., Nishie, T., Ohshima, T. & Asano, M. (2004). Impairment of skin wound healing in -1,4-Galactosyltransferase-Deficient Mice with reduced leukocyte recruitment, American Journal of Pathology, 164: 4

Nagle, S.M., Stevens, K.A. & Wilbraham, S.C. (2023). Wound Assessment. National Library of Medicine. StatPearls Publishing, Treasure Island. https://www.ncbi.nlm.nih.gov/books

Rahman, M. T, Mohamas, M.Y., Akram, H.B. & Bero, D.N., (2012). Tamarind seed Extract Enhances Epidermal Healing. International Journal of Plant Science and Research, 11(1), 2349-2805

Robab, V., Ali. A.H., Gholamreza, H., Sara, B. & Bahadoram, M. (2015). Wound healing potential of *Althaea officinalis* flower mucilage in rabbit full lickness wounds. Asian Pacific Journal of Tropical Biomedicine, 15 (11), 937-943.

Sano, M., Miyata, E., Tamano, S., Hasiwara, A., Ito,N. & Shirai, T. (1996).Lack of carcinogenicity of Tamarind Seed Polysaccharide in B6C3F1 Mice, Food Chemistry Toxicology, 34 (5), 463-467.

Sreelekha, T. T., Vijayakumar, T., Ankanthil, R., Vijayan, K.K. & Nair, M.K. (1993). Immunomodulatory effect of a polysaccharide from *Tamarindus Indica,* Anticancer Drugs, 4(2), 209-212.

Sudjaroen, Y., Haubner, R., Wu, G. & Hull, W. E. (2005). Isolation and structure elucidation of phenolic antioxidants from Tamarind (*Tamarindus indica L.*) seeds and pericarp. Food and Chemical Toxicology, 43, 1673-1682.

WIPO. (2015). Intellectual Property and Traditional Medical Knowledge. World Intellectual Property Organization