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# EXCISION AND INCISION WOUND HEALING ACTIVITY OF DIFFERENT EXTRACT OF VITEX TRIFOLIA L IN ALBINO RATS.

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**Abstract:**
*Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin) is regenerated. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling. Traditionally, Vitex trifolia L is used for wound healing. Since no detailed scientific data are available regarding the wound-healing activity of Vitex trifolia L is the present study was designed to explore the same. The wound-healing efficacy of petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract of Vitex trifolia L is was evaluated in excision and incision wound models. The parameters studied include rate of wound contraction, period of complete epithelialization, and tensile strength of incision wound. Student's* t *test was used to analyze the results obtained from the present study and* $P<0.05$ *was considered significant. Ethyl Acetate extract of Vitex trifolia was found to possess significant wound-healing activity, which was evidenced by decrease in the period of epithelialization, increase in the rate of wound closer and skin-breaking strength. The present study has demonstrated that the Ethyl Acetate extract of Vitex trifolia has properties that render them capable of promoting accelerated wound-healing activity compared with placebo control.*

**Keywords:** *Vitex trifolia, wound healing, inflammation, proliferation, remodeling, Ethyl Acetate.*

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## INTRODUCTION:

A skin wound results from the breakdown of the epidermal layer integrity. Any tissue injury with anatomical integrity interruption with functional loss can be described as a wound. Wound healing mainly means healing of the skin. The wound healing begins immediately after an injury to the epidermal layer and might take years. This dynamic process includes the highly organized cellular, humoral, and molecular mechanisms. Wound healing has 3 overlapping phases which are inflammation, proliferation, and remodeling. Any disruption leads to abnormal wound healing (1). Wound healing is occasionally classified as primary healing and secondary healing. Uncomplicated healing of a non infected, well-approximated wound is defined as primary healing. Surgical wounds are the best example for primary healing. If the wound healing course in this wound is disrupted by infection, dehiscence, hypoxia or immune dysfunction, secondary healing stage begins. During secondary healing, granulation tissue formation and epithelization over this new tissue take place. These types of wounds are more susceptible to infections and poor healing (2). *Vitex trifolia L* Vitex trifolia L. is a shrub or shrubby tree that may grow up to 6 meters in height & belonging to Lamiaceae family. In literature it has been reported as an antibacterial activity (3), Anthelmintic activity (4) & antioxidant activity (5).

## MATERIAL & METHODS:

**Plant Material:**

Leaves of Vitex trifolia L. was collected from rural area of Bhopal (M.P), India in the months of March, 2021. The collected material is compared with published description of the drug, and identification is verified by an acknowledged expert. The plant material was identified and authenticated in the Department of Botany, Barkatullah University Bhopal (M.P). The leaves were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time. Then these plants materials were shade dried without any contamination for about 3 to 4 weeks. Dried plant materials were grinded using electronic grinder. Powdered plant materials were observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container.

**Extraction of Plant Material:**

The plant resources were primarily washed with distilled water and with the help of paper towel was dried in laboratory at room temperature for 24 h and pulverized into powder (coarse) by a mechanical grinder. Accurately weighed 1 Kg of the plant resources was firstly defatted with petroleum ether and was extracted with chloroform, ethyl acetate, ethanol and aqueous solvents in a soxhlet extractor. The standard extracts obtained from *Vitex trifolia* were packed in an air tight container and then kept in a refrigerator.

**Wound Healing Activity:**

**Ointment preparation:**

The dry plant extracts were used to prepare the ointment. Simple Ointment (British Pharmacopoeia) base was prepared as follows: white soft paraffin, cetostearyl alcohol, hard paraffin and lanolin were weighed in a crucible in 17:1:1:1 ratio. The crucible was placed in a water bath at 85°C to melt the contents, after which these were stirred constantly until the mixture solidified. The 10% and the 20% *Vitex trifolia* ointments were prepared by triturating 10 grams and 20 grams of various extracts respectively into 90 grams and 80 grams of the earlier formulated simple ointment. The trituration was done on a glass slab using a stainless steel spatula until a uniformly mixed ointment was achieved. The respective ointments were dispensed into separate labeled containers and stored at room temperature awaiting use. The above mentioned simple ointment (without plant extracts) was used on control groups.

**Wound Healing Activity:**

**Selection of model:**

Excision and incision wound model, using albino rats was selected for assessing the wound healing activity. This model was employed to study the rate of wound contraction, time of epithelization & tensile strength. These parameters were selected because of easy availability of albino rats and simplicity in handling them.

**Selection and procurement of animals:**

For the present study Albino rats (Wistar strain) of either sex with 150-250 g weight were used. The inbred animals were made used to the experimental situation in the animal house of the organization. The animal house was finely kept in average hygienic circumstances, at 22 ± 2ºC temperature, 60 % ± 10% room humidity through 12 h day and night cycle, with food and water *ad libitum*. They were offered by purified water and marketable food pellets **(6)(Odimegwu *et al*., 2008).** Animal preservation was as per the CPCSEA (Committee for the purpose of Control and Supervision of Experimentation of Animals) guidelines and every experiment on animals was performed as per the internationally established principles for laboratory animal usage and as per the experimental procedure.

**Excision wound model:**
The animals were at random separated into 7 groups, each containing of 6 animals. Group I was considered as untreated control and simple ointment was applied over it. Group II was considered as the standard group, framycetin sulfate cream (Soframycin, Aventis) 0.2%w/w was applied over it. Group III was applied with the pet ether extract (10% w/w) and Group IV was applied with the chloroform extract (10% w/w), Group V was applied with the ethyl acetate extract (10% w/w), Group VI was applied with the Ethanolic extract (10% w/w) and Group VII was applied with the Aqueous extract (10% w/w) of *Vitex trifolia* till absolute epithelization. The animals were previously anesthetized for creating the wounds, by means of 1mL of intravenous ketamine hydrochloride (10mg/kg). As explained by Morton and Malone the rats were inflicted with excision wounds **(7).** A mark was created on the dorsal thoracic area 5 cm away from ear and 1 cm away from vertebral column on the anaesthetized animal. An electric clipper was used to shave the dorsal far of the animals and the area to be wounded was marked on the posterior of the animals. A complete thickness of the excision wound of spherical area of 500mm and 2mm deepness was made by means of scalpel, toothed forceps and pointed scissors. Haemostasis was accomplished by blotting the wound by cotton swab flooded in normal saline. The complete wound was kept open **(8).** Every surgical event was performed in aseptic conditions. The medicaments were topically used one time daily, starting from the first day. The wound area was measured on 4th, 6th, 8th, 11th, 14th day and 16th day subsequent to wounding **(9).** This was accomplished by tracing the wound area on a graph paper. Decrease in the wound area was shown as percentage of the initial wound size.

**Measurement of epithelization time:**
The epithelization period was considered as the number of days needed for declining off of the dead tissue remaining with no any outstanding raw wound (10).

**Incision wound model:**
The rats were at random separated into 7 groups, every group having 6 animals. Group I act as control (untreated) and was treated with simple ointment. Group II was considered as standard group was applied with framycetin sulfate cream (Soframycin, Aventis). Group III was applied with Pet ether extract (10% w/v) and chloroform extract (10% w/v) was applied to Group IV, Group V was applied with ethyl acetate extract (10% w/v), Group VI was applied with Ethanolic extract (10% w/v) and Group VII was applied with aqueous extract (10% w/v) of *Vitex trifolia*. The animal's dorsal fur was detached by a depilator cream before the wounding. A longitudinal paravertebral incision 2 mm deep and 6 cm long was created by a sterile scalpel throughout the cutaneous muscle and skin on the dorsal surface. The wounds were blocked with surgical sutures at a gap of 1 cm. Topically the extracts were applied one time daily, starting from the first day for 10 days. The sutures were taken out on the 8$^{th}$ day and the tensile strength was measured of the healed wound on the 10$^{th}$ day, with the 'Continuous Water Pouring Technique' (11).

**Measurement of tensile strength:**
The animals were anesthetized and moved to the operating table. Two forceps were tightly utilized 3 mm far from the limit of wound confronting each other on inverse side of the cut injury. One of the forceps was settled on stands, while the other was associated with an openly suspended lightweight plastic of volume 1000 ml through a string keep running over to a pulley. Water was permitted to stream persistently from the repository gradually and relentlessly into the holder. The minute the injury simply opened up, the water stream was captured and the volume of water gathered in the holder (roughly equivalent to its weight) was noted as tensile strength (12).

## RESULT & DISCUSSION:

**Excision wounds:**
Conventional medications are forever a superior option for the wound healing principle since non-toxicity, their extensive availability, their effectiveness as crude preparations and zero side effects. About one quarter of all conventional drugs in exercise are for the management of wounds and skin diseases, in comparison of merely 2-4% of contemporary medicines. Data about therapeutic plants disturbing different stages of the wound healing procedure, for instance coagulation, fibroplasia, inflammation, epithelization, collagenation and wound contraction are plentiful in the technical literature. The outcome of the excision wound healing technique discovered that every group of animals treated with the plant extracts showed improved wound contraction constantly from 2nd day to 16th day or till the day they were completely healed. The mean proportion of wound closing area was considered on the 4, 6, 8, 11, 14, and 16th post wounding days. Every reading is established to be statistically important and similar with the control. However on the 16$^{th}$ post wounding day, animals of control group showed 80.56% of healing (which may

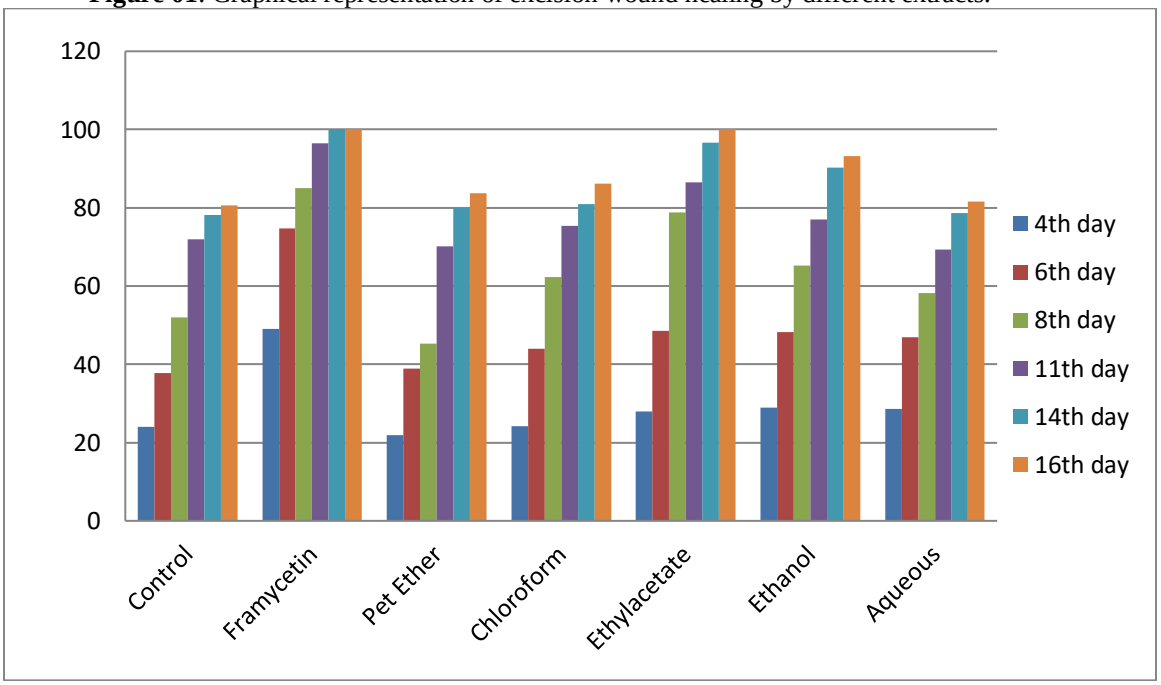
be due to self immunity of the animals). Whereas ointment of Petroleum ether extract showed 83.78% of healing. On the other hand ointment of chloroform, ethyl acetate, ethanol & aqueous extract showed 86.10%, 100%, 93.27% & 81.53% respectively.

**Table 01:** Effect of different extracts of Vitex trifolia on percent (%) wound closing (excision wound model).

| Group | Treatment (Extract/ Standard) | Percentage (%) wound closure | | | | | | Time of epithelialization (Number of days) |
|---|---|---|---|---|---|---|---|---|
| | | 4th day | 6th day | 8th day | 11th day | 14th day | 16th day | |
| I | Control | 23.96 ±1.12 | 37.84 ±1.85 | 52.04±1.71 | 71.88±2.32 | 78.24 ±1.18 | 80.56 ±1.03 | 24.16 ± 0.71 |
| II | Framycetin | 49.03 ±2.07* | 74.78±3.45** | 85.02±1.26** | 96.46±1.32** | 100±00** | — | 13.7± 1.52** |
| III | Pet Ether | 21.97 ±1.78 | 38.98 ±1.98 | 45.30 ±3.18 | 70.11 ±1.57 | 80.04 ±1.12 | 83.78 ±2.09 | 20.8 ± 1.23 |
| IV | Chloroform | 24.22 ±1.01 | 43.97±1.82 | 62.31±1. 38 | 75.42±2.42 | 80.95± 1.56 | 86.10±1.45 | 19.7 ± 1.02* |
| V | Ethyl acetate | 27.98 ±1.59 | 48.50±1.68* | 78.84±2.01* | 86.54 ±1.09** | 96.65±2.14** | 100±00** | 16.43±0.89** |
| VI | Ethanol | 28.96 ±1.65 | 48.22±1.92** | 65.18±2.38* | 76.95 ±1.11* | 90.22±1.88** | 93.27 ±1.52 | 18.6 ± 0.17* |
| VII | Aqueous | 28.68 ±2.31 | 46.88±1.83 | 58.26 ±1.65 | 69.37±1.01** | 78.70 ±3.25 | 81.53 ±2.87* | 22.18±1.48 |

Values are shown as mean ± S.E. ($n$ = 6). All columns are significant using ANOVA.
*P < 0.005, **P < 0.001 when compared to control; Dunnet's $t$-test.

**Figure 01**: Graphical representation of excision wound healing by different extracts.




**Incision Wound model:**
The preparations containing 10% ointment of the crude extract exhibited major increase (p< 0.01) in wound tensile strength as compared with the control group. The extracts were topically used one time daily, beginning from the first day for up to 10 days. On the 8th day the sutures were cut and on the 10th day the tensile strength was measured of the healed

wound, with the 'Continuous Water Pouring Technique' **(11).**The outcome of the incision wound healing model showed that the breaking strength was established to be greater in ethyl acetate extract treated group of *Vitex trifolia* 479.85 ± 12.92** g and it was similarly potent to standard drug Framycetin group 494.42 ± 15.82 g (Table 02). In incision wound model every test sample revealed to have major wound healing activity. The best activity was noted for ethyl acetate extract with 479.85 ± 12.92 g reading followed by ethanolic extract with 377.97 ± 17.92 followed by Chloroform extract 347.57 ± 14.03 g and the least activity was shown by aqueous extract treated group with a reading of 332.41 ± 13.21 g.

**Table 2:** Effect of different extracts of *Vitex trifolia* on tensile strength (incision wound model).

| Group | Treatment (Extract) | Tensile strength (g) |
|---|---|---|
| I | Control | 328.21 ± 16.20 |
| II | Framycetin | 494.42 ± 15.82** |
| III | Pet ether | 335.93 ± 15. 46 |
| IV | Chloroform | 347.57 ± 14.03 |
| V | Ethyl Acetate | 479.85 ± 12.92** |
| VI | Ethanol | 377.97 ± 17.92* |
| VII | Aqueous | 332.41 ± 13.21 |

Values are shown as mean ± S.E. ($n$ = 6). All columns are significant using ANOVA.
*P < 0.005, **P < 0.001 when compared to control; Dunnet's $t$-test.

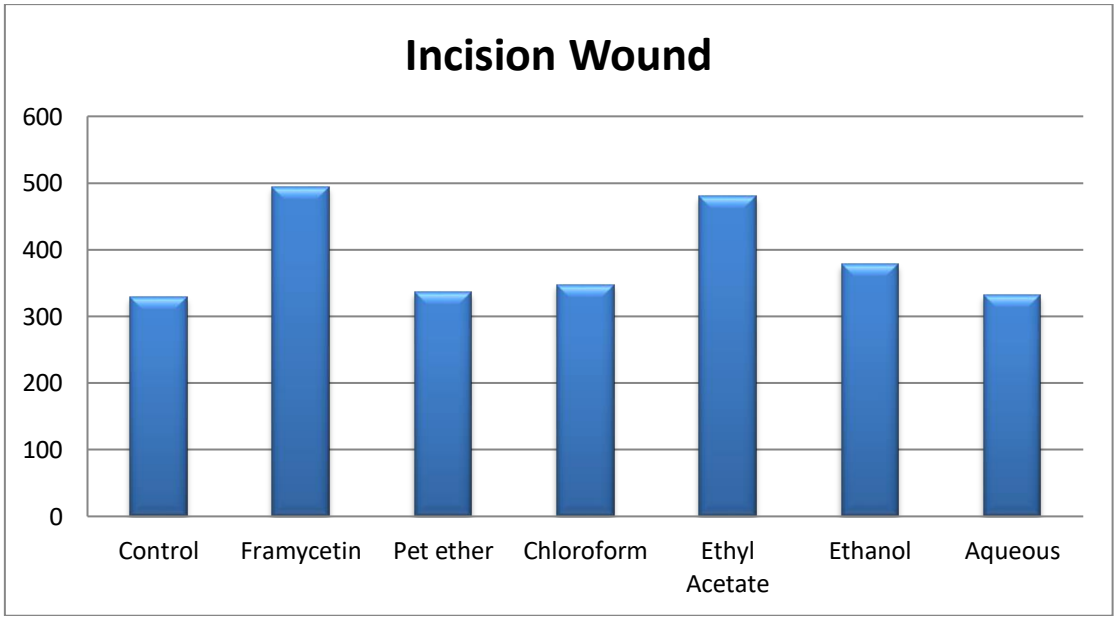



**Figure 02:** Graphical representation of incision wound healing by test extracts.

## CONCLUSION:

The ethyl acetate fraction showed best activity both in incision and excision model, the epithelization period was 16.43±0.89** in excision wound model which was recorded highest among all the fractions and was comparable with the standard drug. In the incision wound model it showed activity almost near to the standard drug with a reading of 479.85± 12.92**.

**REFERENCE:**


1. Kerstein MD. The scientific basis of healing. Adv Wound Care,1997 10(3), 30-36.
2. Kane D.Chronic wound healing and chronic wound management. Chronic Wound Care, A Clinical Source Bookfor Healthcare Professionals, 2001, (3), 7-17.
3. Mariyam Roqaiya, Wajeeha Begum and Sana Fatima Majeedi: A review on unani traditional uses of sambhalu in females as well as phytochemical and pharmacological properties: International journal of pharmaceutical Science & research 2016; Vol. 7(1): 31-41.
4. S. Thenmozhi, K. Vibha, M. Dhanalakshmi, K. Manjuladevi, Sumeet Diwedi and U. Subasini. Evaluation of Anthelmintic activity of Vitex trifoliaLinn. leaves against Pheretima posthuma. International Journal of Pharmaceutical & Biological Archives 2013; 4(5): 878 -880.
5. Aweng E.R., Nur Hanisah, Mohd Nawi M.A., Nurhanan Murni Y. and Shamsul M. Antioxidant Activity and Phenolic Compounds of Vitex Trifolia Var, Simplicifolia Associated with Anticancer. ISCA Journal of Biological Sciences. (2012); 1(3): 65-68
6. Dumian odimegwn, Emmanuel C Ibezim & C. O. Esiomone; Wound healing and antibacterial activities of the extract of Dissotis theifolia (Melastomataceae) stem formulated in a simple Ointment base; Journal of Medicinal Plant; 2(1), Jan 2008; 011-016.
7. Nayak, S., Nalabothu P., Sandiford S., Bhogadi V., and A. Adogwa, "Evaluation of wound healing activity of Allamanda cathartica. L. and Laurus nobilis. L. extracts on rats. BMC Complementary and Alternative Medicine. 2006; 6(12): 12-18.
8. Nithya V, Brinda P, Anand K V. Wound healing activity of Leonotis nepetaefolia R.Br., in wistar albino rats. Asian Journal of Pharmaceutical and Clinical Research 2011; 4(2): 23-26.
9. Madhavan, R., Gnyawali, D. R., & He, J. 2004. Two's a company, three's is a crowd? Triads in cooperative-competitive networks. Academy of Management Journal, 47: 918-927.
10. Wesley, J.J, Christina A.J.M, Chidambaranathan N, Ravikumar K (2009). Wound healing activity of the leaves of Tribulus terrestris (linn) aqueous extract in rats. Journal of Pharmacy Research 2(5):841–843.
11. Lee, K.H, Tong T.G. Mechanism of action of retinyl compounds on wound healing II. Effect of active retinyl derivative on granuloma formation. J Pharm Sci 1970; 59: 1195– 1197.
12. Kaliappan Ilango & Vellapandian Chitra; Wound Healing and Anti-oxidant Activities of the Fruit Pulp of Limonia Acidissima Linn (Rutaceae) in Rats; Tropical Journal of Pharmaceutical Research 9(3); 2010 103-107.