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Research Article

WOUND HEALING ACTIVITY OF VARIOUS FRACTIONS FROM AN EXTRACT OF *EHRETIA LAEVIS ROXB.* (KHANDU CHAKKA) LEAVES IN ANIMAL MODEL

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ABSTRACT

Phytochemical studies are for drug discovery and very important in the discovery of new plant natural products which have commercial values and human benefits. The problem of wound treatment is getting complicated and expensive due to increasing old age population, chronic diseases, diabetes, cancer, autoimmune diseases and heavy uses of antibiotics. *Ehretia Laevis Roxb.* have been traditionally being used for wound care, pain management by local application in the state of Maharashtra India. An objective of this study is to know the wound repairing efficacy of various fractions of ethanolic extract of *E. Laevis* in an animal model.

E. Laevis is also known as Ajan Vruksha and commercially known as Khandu Chakka. Ethanol extraction was done of leaves powder. n-Butanol, n-Hexane, distilled water and chloroform solvent were selected for fractionation. Weighed quantities of E. Laevis leaves fractions 5% was mixed together with simple ointment. The study of wound in animal model was done by marking the wound area on a transparent paper on 4th, 8th 12th and 16th day, using a millimeter-scale graph paper. The % of wound contraction was calculated. Control groups showed least rate of wound healing (66.77±0.28 %). Faster rate of healing was seen in groups treated with 5% w/w ointments of CFEL. **WFEL** 5% treated group was 68.36±0.30 %, **BFEL** 5% treated group had 71.86±0.30% and **HFEL** 5% treated groups showed 66.80±0.17 %. Reference standard heals the wound at the rate of 96.35±0.16% which is almost equivalent to the groups treated with 5% w/w ointments of CFEL i.e. 95.41±0.11.

Keywords: *Ehretia Laevis Roxb.*, Povidone Iodine, Khandu Chakka, Ajan Vruksha, Wound healing, Fractions.

1. INTRODUCTION

Phytochemistry is the science of phytochemicals present in the plant. Plants synthesize phytochemicals for their functions and protection. The different compounds found in plants can be divided into four groups i.e alkaloids, phenylpropanoids, terpenoids and polyketides. Phytochemicals studied for drug discovery and very important in the discovery of new plant natural products which have commercial values and human benefits. In phytochemistry various techniques are used like extraction from plants and then isolation and then structural elucidation of herbal products, Along with this, many chromatography methods like Medium pressure liquid chromatography (MPLC), High Performance Liquid Chromatography **(**HPLC) and Liquid Chromato-graphy-Mass Spectrometry (LC-MS) are being used. Herbal plants are rich resources of useful phytochemicals, ayurvedic medicines and mother generation of many modern medicines. These herbal and folklore medicines are always motivations for continuous research in the benefits of human being in cost effective manners.

Socioeconomic challenges related to wound management results in structured approach in wound care and research. The international wound treatment market was USD 19.83 billion in year 2020 and is expected to hike at a compound annual growth rate of 4.1% from year 2021 to year 2028 [1]. The problem of wound treatment is getting complicated and expensive due to increasing old age population, chronic diseases, diabetes, cancer, autoimmune diseases and heavy uses of antibiotics. Road accidents, burns are causing financial burden for wound care. For wound healing various tools are being used like negative pressure devices, sutures, staples, antibiotics, bandages, dressing pads, Collagenase-based enzymatic

wound debridement products, and enzymatic wound debridement products. Since ancient times the herbal plants are known for their abilities in wound healing and for prevention of infection without untoward effects.

Likewise *E. Laevis* have been traditionally being used for wound care, pain management by local application in the state of Maharashtra India. *E. Laevis* contains lots of chemicals useful for wound healing. Objectives of this study are to know the wound repairing efficacy of various fractions of ethanolic extract of *E. Laevis* in an animal model. A wound forms due to discontinuation of skin by any means. Wound healing takes place in three stages: inflammation, proliferation, and remodeling. *E. Laevis* is an Indian herbal plant and it belongs to the Boraginaceae family. It is highly valued herbal plant but rare in the Maharashtra state of India. It also has a religious importance among people of Maharashtra state of India. It is growing luxuriantly at Alandi near the Dnyaneshwar temple Maharashtra. It is called as Ajan Vruksha after taking Sanjivan Samadhi by sant Dnyaneshwar maharaj under it. It is a highly valued plant cited in the literature of Nath Sampradaya. *E. Laevis* is commercially known as Khandu Chakka .Folklore had faith about plenty of it's medicinal healing properties [2].

1.1. Drug

Plant Description

Following is the binomial nomenclature for the plant in the botany

Botanical name

Ehretia Laevis Roxb.

Classification: Its Kingdom is Plantae, Division is Tracheophyta, Class is Magnoliopsida, Order is Boraginales, Family is Boraginaceae, Genus is Ehretia, Species is Ehretia laevis (Roxb).

Fig. 1: Flowers of *E. Laevis*.

Fig. 2: Fruits of *Ehretia Laevis Roxb.*

1.2. Medicinal uses

Plant has many chemical compounds having useful medicinal properties including wound healing [3]. Plant is used in many medicinal conditions like wound healing, pain relief and minor fracture [4]. It has a very good potential of wound healing by crude extract of leaves [5]. Also it is used as blood coagulation [6]. It shows antioxidant and hepatoprotective activity against paracetamol induced acute hepatotoxicity in wistar rats [7]. Leaves paste of this plant is also very effective on varicose vein ulcers by local application [8]. Leaves powder is very effective on shoulder pain management when given internally [9]. *E. Laevis* leaves paste enhances granulation, collagenisation and re epithelisation as compared to Phenytoin application in wound of animal [10].

1.3. Chemical compounds useful for wound healing

E. Laevis contains many useful compounds responsible for wound healing like, naphthoquinone derivative responsible for antimicrobial activity. Ursolic acid responsible for antioxidant property, minerals are responsible for enhancement of immune system and shows antioxidant and antiviral activity. Gallic acid is responsible for antiviral activity. Tannic acid is responsible for antimicrobial activity. Rutin is responsible for antimicrobial activity, enhances immunity and wound healing. Ascorbic acid is responsible for immunity booster. Phytol is antioxidant and responsible for enhancement of immunity. α and β amyrin responsible for antimicrobial activity. Piperazine, betulin, betulinic acid, lupeol, β-sitosterol

shows antimicrobial activity. Cysteine is responsible for wound healing. Histidine shows antioxidant activity. Hydroxy proline promotes collagen synthesis. Lysine is responsible for formation of antibodies. 12,15-Octadecadienoic acid, methyl ester shows antimicrobial activity. Benzoic acid acts as a antiseptic. Ethyl Iso-allocholate shows antimicrobial activity. Arachidonic acid promotes wound healing [3].

1.4. Antimicrobial activity of *E. Laevis*

Crude and water extracts of *E. Laevis*. shows better antimicrobial activity in S. aureus than E. coli. Crude and Water extracts of *E. Laevis*. shows better antimicrobial activity on S. aureus than ethanolic extract. Water extract was best amongst three extracts [11]. Ethanolic extract of *E. Laevis* shows antimicrobial activity against *S. aureus* and *Pseudomonas aeruginosa*. No antimicrobial activity of Chloroform extract up to 1000µg/mL against both the organism [12] Acetone and isopropanol extracts of *E. Laevis* plants shows antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* and there was no activity against *Pseudomonas aeruginosa* [13]. Acetone extracts of *E. Laevis* shows greater antimicrobial activity than M. pubescens extracts at low concentrations against human salivary microflora [14] Aqueous, methanol and chloroform extract of *E. Laevis* shows remarkable antimicrobial activity on *S. aureus*, *B. subtilis*, *P. aeruginosa* up to 50 (µg/ml) [15].

2. MATERIAL AND METHODS

2.1. Collection of plant

Plant material was collected from Dhaga forest of Wardha district (Maharashtra), India in the month of September**.** Authentication **of** herbarium species was done from Foundation for Revitalisation of Local Health Traditions, Banglore, India.

2.2. Extraction of leaves of *E. Laevis* leaves

Freshly collected leaves were washed by using distilled water and ethanol. Washed leaves were shade dried under aseptic condition for 6 to 7 days. As lowering the size of particle increases the surface. The dried leaves were powdered up to the size smaller than 0.5mm. Finely powdered sample was placed in a "thimble", and thimble was placed inside the chamber of the Soxhlet. Ethanol was then boiled in the flask to vaporize and vapours then condensed into the condenser. When it reaches up to the siphon arm, the condensed solvent dropped back into the RB flask of Soxhlet apparatus and then same process was done till complete solvent from the RB flasks get condensed. Then the solvent was evaporated from an extract at room temperature [16].

2.3. Fractionation of extract

For fractionation of an extract the separating funnel technique was used. Solvent selected for fractionation were n-Butanol, n-Hexane, distilled water and chloroform. The ethanol extract of leaf of *E. Laevis*. was completely dissolved and 250ml of hydroalcoholic extract was prepared. The hydroalcoholic fraction was transferred into a separation funnel, shook, and settled. Then 50mL of *n*-hexane was added into separation funnel, shook and was allowed to settle. Then the aqueous layer was separated from the opening of separation funnel. The left *n*-hexane fraction from separation funnel was poured in a clean glass petridish 50 ml of *n*-hexane was again poured into the separation funnel and same procedure was done using *n*-hexane till no extract appears to pass in *n*-hexane fraction. Same procedure was done for *n*-Butanol and chloroform in order to get *n*-Butanol and chloroform fractions resp. Left fraction into the separation funnel was collected as a water fraction [17].

2.4. Chemicals

Povidone iodine ointment (Cipla GX), Ethanol (Merck), n-Hexane (Merck), n-Butanol (Merck), Chloroform (Merck), Cosmo silky hair remover cream (Olina professional cosmetics Pvt. Ltd. Delhi).

2.5. Wound healing activity

2.5.1. Experimental Animals

Wistar rats (150-200g) were procured from animal house of IPER, Wardha. Rats were kept in proper cages in proper hygienic conditions and fed with standard pellet diet (VRK Nutritional Solution, Sangli) and water ad libitum. All Wistar rats were maintained under proper conditions, Room temperature was 26±3°C, relative humidity was 45-55% and light was 12:12 h-dark cycle. Proper hygienic conditions were maintained to avoid infection in cages while experiment in animal office. Animal studies had an approval of IAEC (IPER/IAEC/2018-19/03) [18].

2.5.2. Preparation of Ointment

The ointment was prepared by adding weighed quantities of hard paraffin (3%), white bee's wax (2%), white soft paraffin (90%), cetosteryl alcohol (5%) and melted. Weighed quantities of E. Laevis leaves fractions

5% was mixed together with simple ointment by using spatula and ointment slab. Then the prepared ointments were kept in refrigerator for application on wounds [19].

2.5.3. Experimental Design

The rats were marked and then separated into 6 groups and in each group there were 6 rats. The Groups were made as per follows and treated accordingly.

Group I (Control): Applied topically by simple ointment 0.5 g.

Group II: (Standard): Applied topically by Povidone Iodine ointment 0.5 g, 5% w/w.

Group III: Applied with water fraction of ethanolic extract of *E. Laevis* **(WFEL)** 5% w/w ointment 0.5 g, locally.

Group IV: Applied with n-Butanol fraction of ethanolic extract of *E. Laevis* **(BFEL)** 5% w/w ointment 0.5 g, locally.

Group V: Applied with chloroform fraction ethanolic extract of *E. Laevis* **(CFEL)** 5% w/w ointment 0.5 g, locally.

Group VI: Applied with n-Hexene fraction ethanolic extract of E. Laevis **(HFEL)** 5% w/w ointment 0.5 g, locally.

2.5.4. Excision Wound Model

Excision wound model was used. The rats were anesthetized by diethyl ether. Hairs from dorsal thoracic region were removed by application of a hair removing cream. Hair removing cream was purchased from medical store. (cosmo silky). Approximate 500 mm^2 area was marked by an indelible ink and rubber seal. Then it was cleaned by normal saline water and skin was excised and circular wound was created by cutting throughout the marked area through the skin. The wounded rats were kept in different cages. The prepared ointments of different fractions (5%) and the reference drug Povidone-iodine ointment was applied locally once in a day till the wound was completely recovered. The study of wound was done by marking the wound area on a transparent paper on 4^{th}, 8^{th} 12^{th} and 16^{th} day, using a millimetre-scale graph paper.

The % of wound contraction was calculated by given formula.

% of wound contraction = (Wound area on day "0"- n (wound area on days)/(Wound area on day "0")×100 [20]

Where, n = no of days (4^{th}, 8^{th} 12^{th} and 16^{th} days).

2.6. Statistical analysis

Statistical analysis was done by ANOVA test and then followed by Dunnett's t comparison test. The values were expressed as mean±SEM and $p< 0.05$ as considered as significance.

3. RESULTS AND DISCUSSION

3.1. Wound healing study

Contraction of wound of different groups till the 16^{th} day was calculated and recorded in Table 1 and Fig. 1. Control groups showed least rate of wound healing (66.77±0.28 %). Faster rate of healing was seen in groups treated with 5% w/w ointments of CFEL. WFEL 5% treated group was 68.36±0.30 %, BFEL 5% treated group had 71.86±0.30 % and HFEL 5% treated groups showed 66.80±0.17 %. Reference standard heals the wound at the rate of 96.35±0.16% which is almost equivalent to the groups treated with 5% w/w ointments of CFEL i.e. 95.41±0.11.

Process of wound healing represented in Fig. 4, Fig. 5 and Fig. 6.

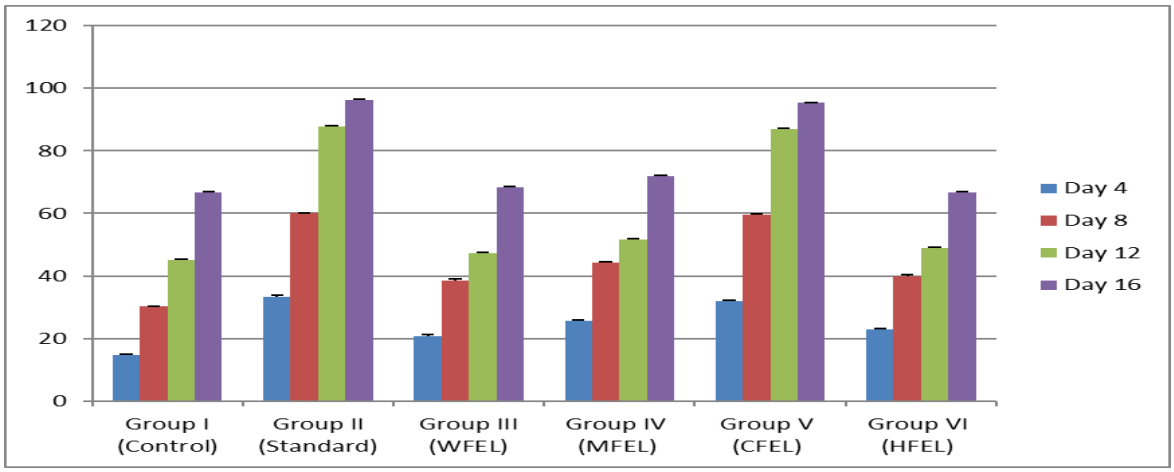

Fig. 3: Percentage of wound healing

Table 1: Wound healing Activity

Treatments	4th day	8th day	12th day	16th day
Group I (Control)	14.78±0.19	30.20±0.20	44.98±0.36	66.77±0.28
Group II (Standard)	33.44±0.39	60.04±0.22	87.73±0.41	96.35±0.16
Group III (WFEL 5% w/w)	20.86±0.48	38.63±0.32	47.29±0.23	68.36±0.30
Group IV (BFEL 5% w/w)	25.67±0.23	44.18±0.31	51.74±0.24	71.86±0.30
Group V (CFEL 5% w/w)	32.03±0.26	59.71±0.07	86.88±0.35	95.41±0.11
Group VI (HFEL 5% w/w)	22.88±0.44	39.99±0.38	48.86±0.26	66.80±0.17

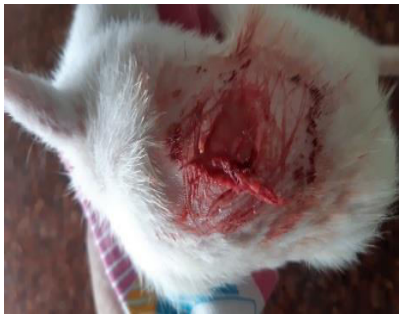

Fig. 4: Fresh wound

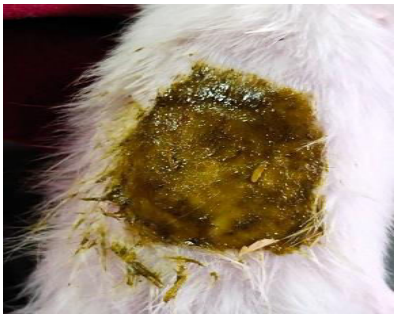

Fig. 5: Treatment

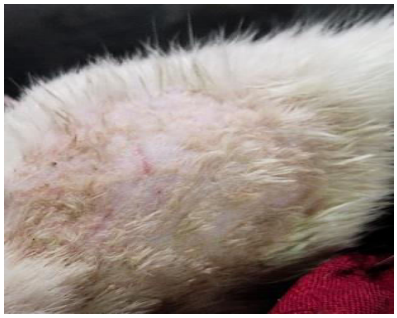

Fig. 6: Healed Wound

4. CONCLUSION

This study concluded that wound healing property of chloroform fraction of ethanol extract of E. Laevis shows effective wound healing property like Povidon Iodine ointment in animal model.

Further studies shall focus on compound isolation from chloroform fraction of ethanolic extract of E. Laevis and to study their wound healing activity.

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Conflict of interest

Nil

6. REFERENCES

1. Grand View Research, Wound Care Market Size, Share Analysis Report, 2021-2028 https://www.grandviewresearch.com/industry-analysis/wound-care-market accessed on 1.8.2021
2. Thakre R, Bhutada S, Chouragade B, Khobragde P, Ketaki H. *Int J Ayurveda Pharma Res*, 2016; **4(7):** 68-73.
3. Thakre R, Harne K, Tekade P, Parve S. *Int. j. res. pharm. sci*, 2020; **11(1)**:224-233
4. Thakre R, Bhake A, Tekade P, Harne K, Borkar P. Eur. J. Mol. Clin. Med**,** 2020;**7(2)**:1901-10.
5. Thakre R, Bhutada S, Chouragade B, Khobragade P, Harne K. *Int. J. Ayurveda Res.,* 2016; **7(suppl 4)**:54-57.
6. Bande D, Murarkar K. *Int. J. Curr. Res*, 2018; **7(6)**:2220-1.
7. Rangnathrao TS, Shanmugasundaram P. *Res J Pharm Technol,* 2019; **12(12)**:6143-6148.
8. Thakre R, Borkar PS, Harne K, Tekade P. *Indian J. Forensic Med. Toxicol*, 2021; **15(2)** :703-707.
9. Thakre R, Meghe A, Thakre K, Tekade P. *Indian J. Forensic Med. Toxicol*., 2021; **15(2)**:708-712.
10. Thakre R, Bhake A, Tekade P, Harne K, Borkar PS. *Indian J. Forensic Med. Toxicol*, 2021; **15(2)** :713-721
11. Thakre R, Khobragade P, Harne K. *World J. Pharm. Life Sci*, 2018; **4(7)**:112-116.
12. Thakre R, Harne K. *International Interdisciplinary Research Journal*, 2019; **6(10)**:7-9.
13. Kshama M, Diksha B, Anita C. *World J. Pharm. Res*, 2018; **7(12)**: 691- 697.
14. Mutha M, Deshpande RR, Patil V, Shep S, Deshpande NR, Torne R. *Asian J. Pharm. Clin. Res*, 2015; **8**:217-9.
15. Jyothirmai N, Nagaraju B, Kumar JS. *Int. J. Pharm. Sci. Res*, 2016; **8(8):**715-720.
16. Azwanida NN. *Med Aromat Plants*, 2015; **4(196)**:2167-2412.
17. Abubakar AR, Haque M. *J Pharm Bioallied Sci.*, 2020; **12(1)**:1-10.
18. Chaturvedi AP, Kumar M, Tripathi YB. *Int. Wound J.,* 2013; **10(6):**675-82.
19. Roy K, Chakraborty M, Theengh A. *Int. J. Pharm. Sci. Res,* 2021; **12(1)**:554-558.
20. Murthy S, Gautam MK, Goel S, Purohit V, Sharma H, Goel RK. *Biomed Res. Int***.,** 2013; **(6):**972028.