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Wound Healing Potential of Different Extracts of Ehretia Laevis Roxb. (Khandu Chakka/Ajan Vruksha) Versus Silver Sulfadiazine in Burn Wound - Pre-Clinical Study

Research Article

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Abstract

Burns are also a leading cause of morbidity; millions who survive suffer from lifelong disability and disfigurements with resulting emotional trauma and stigma. To identify the effective extract of *Charma Vruksha* (*Ehretia Laevis* Roxb.*)* in burn wound management and to generate empirical evidence for substantiation of burn wound healing by *Kashaya Rasa* (Astringent taste) through experimental demonstration of the comparative wound healing property of *Charma Vruksha* (*Ehretia Laevis* Roxb.) this study was planned. This study is useful in broadening of therapeutic aspect of *Charma Vruksha* (*Ehretia Laevis* Roxb.) plant. This is contribution to the literature related to ayurvedic sciences. The stated side effects of silver sulfadiazine like pain, burning, itching, rash, cell death, destruction of red blood cells (in patients with G6PD deficiency), deficiency of granulocytes in the blood, low blood platelet count, low white blood cell count , can be minimized to some extent by the use of *Charma Vruksha* (*Ehretia Laevis* Roxb.) plant leaves. Wound healing was measured by histology score and wound contracture percentages. Average wound contracture percentage was 36.83 % in Crude Extract group, 33.75% in Ethanol Extract group, 34.6 % in Chloroform Extract group , 42.41% in Aqueous Extract group, as compared to 38.08 % of Silver sulfadiazine. Histological score was 16 in Crude Extract group, 15 in Ethanol Extract group,15 in Chloroform Extract group , 16 in Aqueous Extract group as compared to 18 of Silver sulfadiazine. Aqueous Extract was more effective in burn wound contracture by 11.37 % than Silver sulfadiazine Crude and Aqueous extract was more effective than ethanol and chloroform extract by 1 histological score but less effective by 2 histological score than Silver sulfadiazine. Aqueous Extract is more efficacious in burn wound contracture by **11.37 %** than Silver sulfadiazine.

Keywords: Burn Wound, *Khandu Chakka, Ajan Vruksha, Charma Vruksha* (*Ehretia Laevis Roxb.*), *Kashay Rasa* (Astringent Taste), Histology.

Introduction

In Vidarbha Region, a traditional plant species is used for wound healing, joint pain and minor fractures by local tribes. Commercial know as Khandu Chakka, latin name as *Ehretia Laevis Roxb*. and in Ayurved it is known as *Charma Vruksha* (*Ehretia Laevis* Roxb.) plant. (1-7) WHO estimates about 265 000 deaths occur each year due to burn. (8) In India around 7 million people suffer from burn injuries each year with 1.4 lakh deaths and 2.4 lakh people suffer with disability. 11th leading cause of death of children aged 1-9 years. Burns are also a leading cause of morbidity; millions who survive suffer from lifelong disability and disfigurements with resulting emotional trauma and stigma.(9)Resistance from broad-spectrum antibiotics is increasing day by day. As the antibiotics used for treatment of burn wounds are very expensive, rural tribes are unable to afford the cost of medicines. Burn wound healing is a complicated process including inflammation, proliferation, epithelisation and tissue remoulding.There is requirement to identify the effective extract of *Charma Vruksha* (*Ehretia Laveis Roxb.)* in burn wound management. The stated side effects of silver sulfadiazine like pain, burning, itching, rash, cell death, destruction of red blood cells (in patients with G6PD deficiency), deficiency of granulocytes in the blood, low blood platelet count, low white blood cell count, can be minimized to some extent by the use of *Charma Vruksha* (*Ehretia Laveis Roxb.*) plant leaves.(10) *Rasa* (Taste) of *Charma Vruksha* (*Ehretia Laevis* Roxb.) is *Kashay* (Astringent) and *Virya* (Potency) is Ushna (Hot). *Kashay rasa* (Astringent taste) is responsible for *Ropan karma* (Healing) in *Vrana*(Wound).This basic principle of ayurveda is applied and tested in burn wound healing .

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This is contribution to the subjects of ayurveda. (11-12) This study is useful in broadening of therapeutic aspect of *Charma Vruksha* (*Ehretia Laevis* Roxb.) plant. This is contribution to the literature related to ayurvedic sciences. Burn wound is common clinical problem. For the management of burn wound silver sulfadiazine ointment is available as standard treatment. Present study was planned to generate empirical evidence for substantiation of burn wound healing by Astringent taste through experimental demonstration of the comparative wound healing property of *Charma Vruksha* (*Ehretia Laevis Roxb*.) *"Dravasya-Rasa-Virya-Vipak-Prabhav-Siddhant"* is described in Ayurved Samhita. Concept of *Rasa* (Taste) in context with *kashaya rasa* (Astringent taste) as wound healing needs to be demonstrated through experimental study to generate the empirical evidence.

Materials and Methods

Ethical Statement: The study is approved by institutional animal ethical committee of DMIMS(DU) Wardha(MS) India. Ref.no. DMIMS9 (DU)/IAEC/ 2019-20-01 and institutional ethical committee of MGACH & RC, Salod (HI), Wardha(MS), India. Ref.no. MGACH & RC/IEC/July-2020/87.

Steps involved in the selection of the plant.

Ehretia laevis roxb., plant and leaves were authenticated by, The foundation for revitalizing local health traditions. Voucher no. FRLH Col. No. 122021

- Study type: -Pre-Clinical Comparative Interventional Study
- Sample Size- 30 Albino Rabbits
- Healthy albino rabbits were selected from DMIHER Sawangi(M), Wardha, animal house. Then they were divided into 5 groups of 6 in each groups

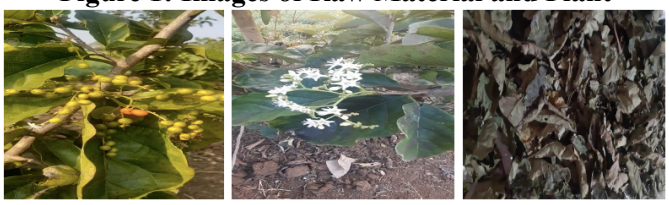
Assessment was done by :- (13)

1. Amount of granulation tissue (profound -1, moderate – 2, scanty-3, absent-4)
2. Inflammatory infiltrate (plenty -1, moderate – 2, a few- 3)
3. Collagen fiber orientation (vertical -1, mixed -2, horizontal-3)
4. Pattern of collagen (reticular -1, mixed-2, fascicle-3)
5. Amount of early collagen (profound-1, moderate -2, minimal-3, absent-4)
6. Amount of mature collagen (Profound-1, Moderate -2, Minimal-3)

Extractions

Crude extract of *Ehretia Laevis Roxb.* done in mortal and pestle, Ethanolic extract of Ehretia Laevis Roxb., Chloroform extract of *Ehretia Laevis Roxb*., Aqueous extract of *Ehretia Laevis Roxb*. done by Soxhlet apparatus, Silver Sulfadiazine ointment was purchased from pharmacy manufactured by Dr. Reddy's Laboratories, Inc (RX).

Figure 1: Images of Raw Material and Plant



The fresh leaves of identified plant *Charma Vruksha (Ehretia Laveis Roxb.)* collected from farm of Wardha district. The leaves of plant are shade dried. After the leaves are completely dried, they are powdered separately and preserved in an airtight container.

Figure 2

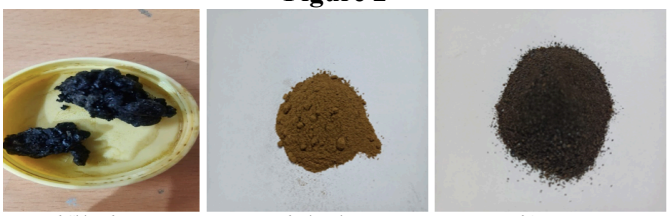


Image of Chloroform Extract Image of Ethanol Extract Image of Aqueous Extract

Ointments are prepared by homogeneously mixing of all following ingredients as per mentioned weight

Table 1: Preparation of ointment

Sr no	Percentage	Weight
1	w/w Extract 50%	w/w 5gm extract
2	w/w Petroleum Jelly 40%	w/w 4gm petroleum jelly
3	w/w White wax 1%	w/w 0.1 gm white wax
4	w/w Steric acid 1%	w/w 0.1 gm Steric acid
5	w/w Sesame oil 8%	w/w 0.8 ml Sesame oil
		w/w 10 gm ointment

Figure 3

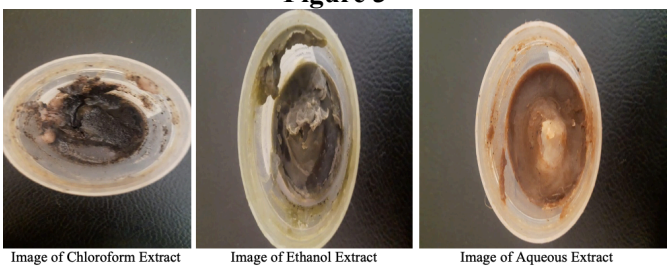


Image of Chloroform Extract Image of Ethanol Extract Image of Aqueous Extract

Image of Chloroform Extract, Image of Ethanol Extract, Image of Aqueous Extract ointments

Standardization of Ointment

Table 2: Standardization of Chloroform Extract of *Ehretia Laevis Roxb.* ointment

1	Color	Greenish
2	Odor	Characteristics
3	Loss of drying at 105 degree	0.53
4	Refractive	1.462
5	Rancidity	Absent
6	Iodine value	14.82
7	Peroxide value	8.63
8	pH	5.6

Table 3: Standardization of Ethanol Extract of *Ehretia Laevis* Roxb. ointment

1	Color	Faint Greenish
2	Odor	Characteristic
3	Loss of drying at 105 degree Celsius	0.62%
4	Refractive index	1.464
5	Rancidity	Absent
6	Iodine value	17.28%
7	Peroxide value	15.82%
8	pH	5.3

Table 4: Standardization of Water Extract of *Ehretia Laevis* Roxb. ointment

1	Color	Faint Greenish
2	Odor	Characteristics
3	Loss of drying at 105 degree Celsius	0.72
4	Refractive index	1.463
5	Rancidity	Absent
6	Iodine value	10.47
7	Peroxide value	7.63
8	pH	5.7

Animal Study

Animals were anesthetized before creating wound. The skin shaving done by trimmer, disinfection will be done 70% alcohol and then skin injected with 1 ml of Lignocaine HCl (2%, 100 mg/5 ml) for local anaesthesia. The rabbits were anesthetized with xylazine (5 mg/kg) by administered intramuscularly. The animals were prepared for burn injury to create the wound. The 3rd degree burn injury done by hot coin measuring near about 2cmX2cm after infiltration of lignocain 2% locally. The burn sites at the thoraco-lumbar region prepared by removing hairs by clipper and applying Povidine iodine. After formation of burn wound, treatment started with treatment protocol in each group under same managerial and environmental condition. Albino Rabbits placed in cage & with standard pellet diet and water. They were placed in air conditioned room having 12 hours of day light and dark cycles. The animal experiments performed as per approval of Institutional Ethics Committee & institutional animal ethical committee. Wound was measured by following formula on every second day.

SAI – SACi /SAI x 100 = _____% reduction

(*SAI= surface area (Length width) on the day burn injury, SAC = surface area currently) (14). Daily dressing was done in all aseptic precaution. Near about one gram of ointment of various paste applied in respective groups two times a day for 30 days. Before application of paste, the edges of the wound to be sampled by forceps biopsy.

Figure 4: Photomicrograph of Histology of Rabbit Burn Wound Healing by Application of Chloroform Extract of *Ehretia Laevis* Roxb. on day-15 and histological score

Histological Parameters	Results
Amount of granulation tissue	Scanty
Inflammatory infiltrate	Few
Collagen fiber orientation	Mixed
Pattern of collagen	Fascicle
Amount of early collagen	Minimal
Amount of mature collagen	Profound

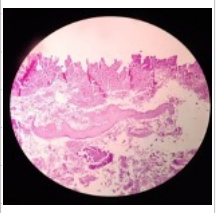


Figure 5: Photomicrograph of Histology of Rabbit Burn Wound Healing by Application of Crude Extract of *Ehretia Laevis* Roxb. on day-15 and histological score

Histological Parameters	Results
Amount of granulation tissue	Absent
Inflammatory infiltrate	Few
Collagen fiber orientation	Mixed
Pattern of collagen	Fascicle
Amount of early collagen	Minimal
Amount of mature collagen	Profound

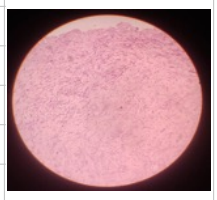


Figure 6: Photomicrograph of Histology of Rabbit Burn Wound Healing by Application of Ethanolic Extract of *Ehretia Laevis Roxb.* on day-15 and Histological score

Histological Parameters	Results
Amount of granulation tissue	Scanty
Inflammatory infiltrate	Few
Collagen fiber orientation	Mixed
Pattern of collagen	Mixed
Amount of early collagen	Minimal
Amount of mature collagen	Moderate

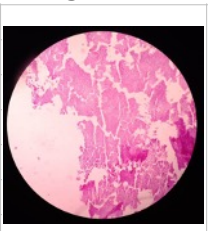


Figure 7: Photomicrograph of Histology of Rabbit Burn Wound Healing by Application of Silver Sulfadiazine on day-15

Histological Parameters	Results
Amount of granulation tissue	Absent
Inflammatory infiltrate	Few
Collagen fiber orientation	Horizontal
Pattern of collagen	Fascicle
Amount of early collagen	Absent
Amount of mature collagen	Profound

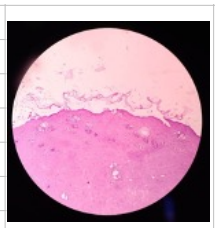
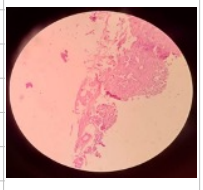


Figure 8: Photomicrograph of Histology of Rabbit Burn Wound Healing by Application of Aqueous Extract of *Ehretia Laevis* Roxb. on day-15

Histological Parameters	Results
Amount of granulation tissue	Absent
Inflammatory infiltrate	Few
Collagen fiber orientation	Mixed
Pattern of collagen	Fascicle
Amount of early collagen	Minimal
Amount of mature collagen	Profound



Statistical analysis

Table 5: Statistical Analysis of Histological Score of Various Extracts

Sr no	Groups	Standard score	Results of Histology score	z-value
1	Crude extract of *Ehretia Laevis Roxb.*	20	16	8.94,S
2	Ethanolic extract of *Ehretia Laevis Roxb.*	20	15	7.74,S
3	Chloroform extraction of *Ehretia Laevis Roxb.*	20	15	7.74,S
4	Aqueous Extract of *Ehretia Laevis Roxb.*	20	16	8.94,S
5	Silver sulfadiazine Ointment of *Ehretia Laevis Roxb.*	20	18	13.41,S
z-test for single proportion, If z>1.96, result is significant				

Figure 9: Graphical Presentation of Histological Score of Various Extracts

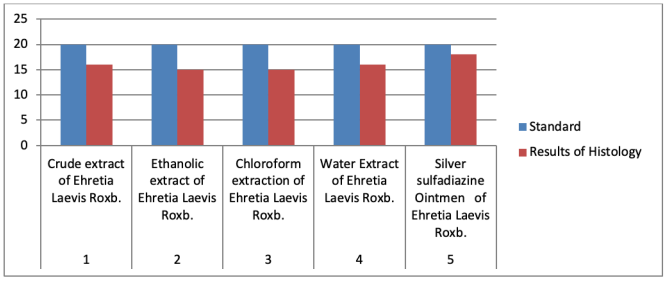


Table 6: Comparison of wound contracture in five groups. Descriptive Statistics

	F-value	p-value
Day 5	3.74	0.016,S
Day 7	23.36	0.0001,S
Day 9	22.02	0.0001,S
Day 11	26.65	0.0001,S
Day 13	26.42	0.0001,S
Day 15	16.59	0.0001,S

Table 7: Comparison of wound contracture in five groups - Descriptive Statistics

Sr no	Groups	Wound Healing Average percentage
1	Crude extract of *Ehretia Laevis Roxb.*	36.83%
2	Ethanolic extract of *Ehretia Laevis Roxb.*	33.75%
3	Chloroform extraction of *Ehretia Laevis Roxb.*	34.6%
4	Aqueous Extract of *Ehretia Laevis Roxb.*	42.41%
5	Silver sulfadiazine Ointment	38.08%

Figure 10: Graphical presentation of wound contracture percentage in various groups

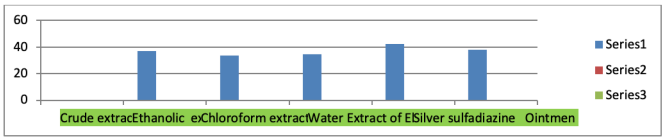


Figure 11: Images of Wound Contractures at various days

Study, Observations and Results

Average wound contracture percentage was 36.83 % in Crude Extract group, 33.75% in Ethanol Extract group, 34.6 % in Chloroform Extract group , 42.41% in Aqueous Extract group, as compared to 38.08 % of Silver sulfadiazine.

Histological score was 16 in Crude Extract group, 15 in Ethanol Extract group,15 in Chloroform Extract group , 16 in Aqueous Extract group as compared to 18 of Silver sulfadiazine.

Aqueous Extract is more effective in burn wound contracture by 11.37 % than Silver sulfadiazine Crude and Aqueous extract are more effective than ethanol and chloroform extract by 1 histological score but less effective by 2 histological score than Silver sulfadiazine.

Discussion

Ehretia Laevis Roxb. is effective in burn wound healing. Phytochemicals present in Ehretia Laevis Roxb. might promote burn wound healing by antimicrobial, anti-inflammatory, antioxidant, collagen synthesis stimulation, cell proliferative and angiogenic effect.

Ehretia Laevis Roxb. has good antimicrobial effect. There was no local infection in any burn wound of any animal. Ehretia Laevis Roxb. has many chemical compounds, which has antimicrobial effect like Naphthoquinone derivatives, minerals, Gallic acid, Tannic acid, Rutin, Vitamin C -ascorbic acid, Decanoic acids, α & β amyrin, Piperazine, Betulin & Betulinic acid, Lupeol, β-sitosterol, 12,15-Octadecadienoic acid, methyl ester; Benzoic acid, Ethyl Iso-allocholate. (15)

Aqueous extract is most effective in E. coli. and S. aureus than crude, ethanol extract. Ethanolic extract is effective against S. aureus at 100 µg /ml and ineffective against Pseudomonas aeruginosa at the same concentration. Chloroform extract did not show antimicrobial activity up to 1000 µg /ml against Staphylococcus Aureus gram positive and Pseudomonas Aeruginosa gram negative species. (16,17)

Ehretia Laevis Roxb. has good antioxidant activity (18) which is helpful in burn wound healing without any complication. *Ehretia Laevis Roxb.* heals burn wound very fast without any complications like sepsis, inflammation and pain. Ehretia Laevis Roxb. contains various compounds which has antioxidant activity like Ursolic acid, Phytol, β-sitosterol, Cysteine, Histidine. Ehretia Laevis Roxb. has very good antioxidant activity (15)

Ehretia Laevis Roxb. promotes Immune response. Ehretia Laevis Roxb. has immunity booster like Minerals such as Na, NH3, Fe, Mn, K, P, Zn, Cu, Si, Mg, Ca; Tannic acid, Rutin, Vitamin C -ascorbic acid, Phytol, β-sitosterol;5,8,11,14-Eicosatetraynoic acid-(Arachidonic acid is an omega-6 fatty acid) (15)

Ehretia Laevis Roxb. has anti-inflammatory, pain relief (19-20) and antinociceptive activities which are very helpful in burn wound healing. Ehretia Laevis Roxb. contains various compounds like Naphthoquinone derivative, Baurenol, Ursolic acid, Rutin, Phytol, α & β amyrin, Betulin & Betulinic acid, Lupeol, β-sitosterol, Histidine, Hexadecanoic acid, Benzoic acid which has anti-inflammatory , pain relief and antinociceptive activity. Also its pain relief and anti-inflammatory activities are proved in various studies. (15)

Ehretia Laevis Roxb. promotes collagen synthesis. *Ehretia Laevis Roxb*. contains chemical compounds like Hydroxy proline, which promotes collagen which maintains the structure and strength of connective tissue like bones, cartilage, blood vessels & skin (15)

Ehretia Laevis Roxb. has angiogenic effect. (21) Ehretia Laevis Roxb. contains chemical compounds like β-sitosterol, which has angiogenic effect, which is very useful for wound healing. (15)

Ehretia Laevis Roxb. promotes wound healing. (22-24) Ehretia Laevis Roxb. contains chemical compounds like Rutin, Cysteine; 5,8,11,14-Eicosatetraynoic acid-(Arachidonic acid is an omega-6 fatty acid), which promotes wound healing. (15)

Ehretia Laevis Roxb. has Ayurvedic fundamental principles which are useful in burn wound healing. *Ehretia Laevis* Roxb. i.e *Charma Vruksha* (*Ehretia Laevis* Roxb.) has *Kashay Rasa* (Astringent taste) . It's probable mode of action is based on *Kashaya Rasa*, i.e., the astringent taste is effective in the inflammatory phase (absorbs excess moisture) and proliferative phase (stimulates wound contracture) but not in the remodeling phase.

Overuse of antibiotics, pain killers can be avoided by using *Ehretia Laevis Roxb.* in burn wounds. Hence side effects of overuse of antibiotics, pain killers can be avoided. *Ehretia Laevis Roxb.* has very good research proven antimicrobial and analgesic activity and also contains various chemical compounds which have antimicrobial and analgesic activities. Hence untoward effects of antibiotics, pain killers can be avoided. (15) Aqueous extract is found most effective among all extract. Useful chemical compounds or active ingredients for wound healing in burn wound present in Aqueous extract of *Ehretia Laevis Roxb.* as compared to crude, ethanol, chloroform extracts. Also in antimicrobial studies of *Ehretia Laevis Roxb.* Aqueous extract is more efficient than crude, ethanol and chloroform extracts. Astringent taste is found efficacious in burn wound healing. Empirical data generated. Astringent taste is responsible for burn wound healing through generation of empirical data. Tannins are considered as Astringent (26), Tannins are water soluble compounds (27).

Conclusion

Aqueous extract of *Ehretia laevis Roxb.* was found more efficacious than other extracts. Empirical data is generated to substantiate the basis principle of Astringent taste in regards to wound healing of extracts of *Charma Vruksha* (*Ehretia Laevis* Roxb.) in burn wound healing. Aqueous extract of *Charma Vruksha* (*Ehretia Laevis* Roxb.) can be further fractionated to identify active constituent for burn wound healing in animal model

Safety Study and Clinical Trials of Aqueous extract of *Charma Vruksha* (*Ehretia Laevis* Roxb.) should be done in future.

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