IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 Journal UGC CARE Listed (Group-I) Volume 11, Issu 12-, 2022

Pharmacological Action of Herbal Film-Forming Spray for Wound Healing Activity on Albino Wistar Rat

Shilpa Savata Kolhe[1], Rahulkumar Damodhar Rahane[2], Waghamode Nitin Bapurao[3], Ashwini Sopan Bansode[4], Saeed Umar Pathan[5], Aryan Ravindra Nawale[6]

[1,5,6]Vishal institute of pharmaceutical education and research ale, junnar, pune
[2]Associate professor, Matoshri Miratai Aher college of Pharmacy, ORCID- 0009-0005-2784-3483.
[3]Dattakala College of Pharmacy
[4]SGMSPM's Dnyanvilas college of pharmacy Dudulgaon, Pune.

Corresponding Author
Shilpa Savata Kolhe, Vishal institute of pharmaceutical education and research ale, junnar, pune., Orcid Id: 0000-0002-6017-9407, Email Id: kshilpu27@gmail.com

ABSTRACT

Wound healing is a complex biological process. The study focuses on *Ehretia laevis Roxb.* (Khandu chaka) For its wound healing, antimicrobial and anti-inflammatory properties. Wound healing is multifaceted process. Plant extract generally contains various bioactive compounds; methanol extract contains flavonoids tannins and napthoquinones derivatives while chloroform extract contains baurenol in addition to above. The bioactive compounds can be configured using FTIR and TLC. The film forming spray overcomes the problems associated with conventional wound healing products. Hydroxyl propyl methyl cellulose k100 (HPMC) is used as a film forming polymer and poly ethylene glycol (PEG 400) is used as plasticizer. The spray creates a flexible, waterproof and protective layer. The pharmaceutical and pharmacological evaluation of spray is performed. Pharmaceutical evaluation includes density, viscosity pH, film durability etc. pharmacological evaluation is done using albino-wister rat. Formulation 1 significantly shows more wound contraction study as compared to formulation 2 and control group. The activity of formulation is evaluated using standard cipladin. The study bridges the traditional knowledge with modern pharmaceutical innovations.

Key words- Hydroxy propyl methyl cellulose (HPMC), Film forming, Poly ethylene glycol (PEG), Bioactive.

INTRODUCTION

Wound healing is the process of repair followed by injury to the skin and proper healing of wounds is essential to prevent the systemic infection. Healing involves restoration of damaged tissue and functional status of the skin. Several medicinal plants have been used due to their wound healing and antimicrobial property, time immemorial for treatment of cuts, wounds and burns and showed promising effects some very common plants like aloe vera, azadirachtamdice, carica papaya, celosia argentea, and khandu chakka have been reported in Ayurveda, Siddha and Unani systems of medicines for their wound healing potential.[6,8] Systemic infection is due to wound, one of the most common diseases in developing countries because of poor hygienic conditions, Wounds are the physical injury that opens the skin and internal tissue which are exposed to external environment. Suitable method for healing of wound is required for the restoration of disrupted anatomical continuity and disturbed physical

functional status of the skin in other words wound is a breakage in the epithelial cell integrity by damaging the stratum corneum layer of the skin and may be accompanied by disruption of the pathological function of underlying normal tissue.[10] Healing of wounds starts with the injury and can continue for periods of time depending on the degree of wound. *Ehretia Laevis Roxb* Also called Khandu Chakka belongs family *Boraginaceae* several phytochemical and pharmacological investigators found that people are using leaves of plant mainly for wound healing, activity apart from in other conditions. So, these study emphasis on the extraction and screening and pharmacological evaluation of plant with special reference to the above-mentioned activities. Plants and their extracts have potential for the management and treatment of wounds, cuts and inflammation.[16] Ajani Vorkush and traditionally being used for wound healing, body pain & minor fractures in the form of local application by folklore in the state of Maharashtra India. *Ehretia Laevis Roxb*. contains many such chemical compounds useful for promotion of healing & repair. These chemical compounds included naphthoquinones which act as anti-bacterial and anti-fungal agent. Burenol as anti-inflammatory, and flavonoids which promotes angiogenesis these properties are Important for phenomenon of wound healing. The Phyto medicines for wound healing are not only cheap and affordable but are also safe as hyper sensitive reactions are rarely with the use of these agents. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. However, there is a need of scientific validation, standardization and safety evaluation of plants before use.

Challenges with conventional wound-healing products:

1. Limited retention: require frequent reapplication as they do not stay on the wound for a prolonged period.

2. Water penetration: lack a barrier to prevent water ingress, which can hinder the healing process.

3. Need for additional covering: Gels and similar formulations often require a secondary bandage for protection.

4. Lack of protection - as conventional formulation dose not retained on skin it gets removed from skin and opens the wound which results in microbial attack and dust penetration in wound.

5. Incompliance - some topical conventional products required covering with bandage or surgical dressing it makes the incompliance.

In this study the limitations of conventional formulation can be overcome by formulating the film forming spray. The spray is consists of film forming polymer i.e. HPMC K100 along with plasticizer such as PEG 4000 along with plant extract which contains antibacterial compounds, upon actuation of valve the contents of spray get atomized the excess of solvent is evaporated followed by living a thin film of polymer. This film covers the wound prevent water penetration and dust and indirectly contribute to wound healing. The anti-bacterial constituents such as naphthoquinones prevents bacterial attack on wound along with that burenol prevent inflammation. Presence of flavonoids promotes angiogenesis and thus contribute to wound healing. The activity of formulation is evaluated by comparing it with standard cipladin.

Material and Method

Ehretia laevis Roxb, *Platyphylla Merrill*. Plant Family is *Boraginaceae* (borage). Naphthoquinone derivative, Baurenol, Ursolic acid, Gallic acid, Tannic acid, Tryptamine. Plant is known for his wound healing, antitumor activity. The plants are also useful in obesity, blood sugar, cardiovascular diseases, blood pressure, and lipids and muscle wasting. The plant has the antibacterial and antifungal property. This herbal plant has chemicals that have ability to heal the wounds the burg oil present in leaves has anti-inflammatory property a very good effect on wound healing. The plant also has thyroid uptake promotion property which will be useful for thyroid diseases. Beneficial chemicals to treat the peptic ulcer and cataract are available in this plant. This will help with the prevention of diseases.[8]

Animal Model:

Healthy adult albino Wistar rats having weight between 200-250 grams were used for the study. These rats were procured from the animal facility at Vishal Institute of Pharmaceutical Education and Research, Ale, Pune, Maharashtra, India. Upon arrival, the rats were randomly placed and assigned to different treatment groups in polypropylene cages lined with paddy husk bedding. The housing conditions maintained a temperature of 24 ±2°C by air conditioner, relative humidity between 30-70%, followed a 12-hr. light 12 hr. dark cycle. Throughout the study, the rats had access to were fed and water with standard commercial pelleted rat chow. All experimental procedures and protocols stick to the guidelines of the Institutional Animal Ethics Committee and in compliance with CPCSEA regulations.

Extraction

Preparation *of Ehretia laevis roxb* (leaves) -

Successive solvent method is utilized for the extraction of naphthoquinone derivative. The dried leaves of khandu plant are grinded in mixture to a fine powder. Which is the passed through sieve number 80. 25 gm of powder is extracted through soxhlet apparatus.[5,6]

Phytochemical Analysis

Preliminary Phytochemical Screening of Extract

Table-1: Result of phytochemical screening

Chemical test	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract
Alkaloids – Mayer's test	+	+	+	_
Phenolic tannins- Ferric chloride	_	+	+	_
Flavonoids- Lead acetate -	_	+	+	+
Reducing sugar- Benedict's test	_	_	+	+
Steroid's and triterpenoid's- Liberman's reaction	_	_	_	_
Test for quinone's Thallequine test	_	+	+	_

ISSN PRINT 2319 1775 Online 2320 7876
 Journal UGC CARE Listed (Group-I) Volume 11, Issu 12-, 2022

FTIR Spectrum

FTIR spectroscopy was used to identify functional groups based on peak values in FT-IR, chloroform and methanolic extract of leaves of plant *Ehretia Laevis Roxb*. Were subjected to FT-IR analysis and the functional groups of the constituents were separated based on their peaks. The results obtained indicated the presence of the following functional groups viz., free alcohol; Inter and intra-molecular bonded alcohols, aromatic compounds, imines or oximes, alkyl ether, aliphatic amines, alkynes and isothiocyanate stretching. [17]

The FT-IR spectra of leaves of plants *Ehretia Laevis Roxb* As in figures 1,2, and 3 Absorption bands, can be obtained by the plot of transmittance vs wave number (cm-1). In figure 1 shows the spectra of Chloroform extract (F1) of *Ehretia Laevis Roxb*. The peak at 3428.02 cm-1 shows the presence of alcohol, phenol (O-H stretch, H-bonded). The peak at 2830.98 cm-1 refers to the presence of alkanes (C-H stretch). The peak at 1690-1640 cm-1 corresponds the imines or oxime. The peak at 1440-1395 cm-1 corresponds the OH group (bending).

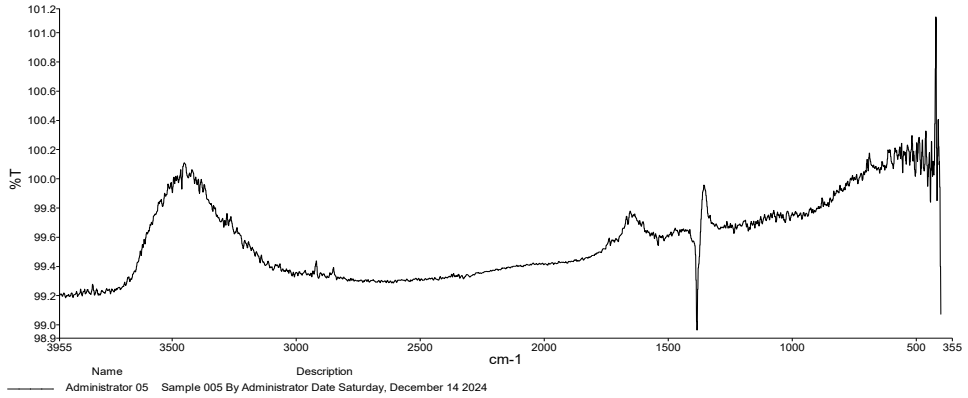

Fig 1:FTIR of Chloroform extract (F1) of *Ehretia Laevis Roxb*

In figure 2 shows the spectra of Hydroxy propyl methyl cellulose K100 (Film forming polymer) The broad peak at 3500-3200, 2976.79, 2695, 1740.42 represents the presence of functional groups such as alcohols, phenols (O-H stretch, H-bonded), carboxylic acids (O-H stretch), amines salt (N-H), alkyl (C-H stretch), anhydride or acid halide(C=O stretch) respectively.

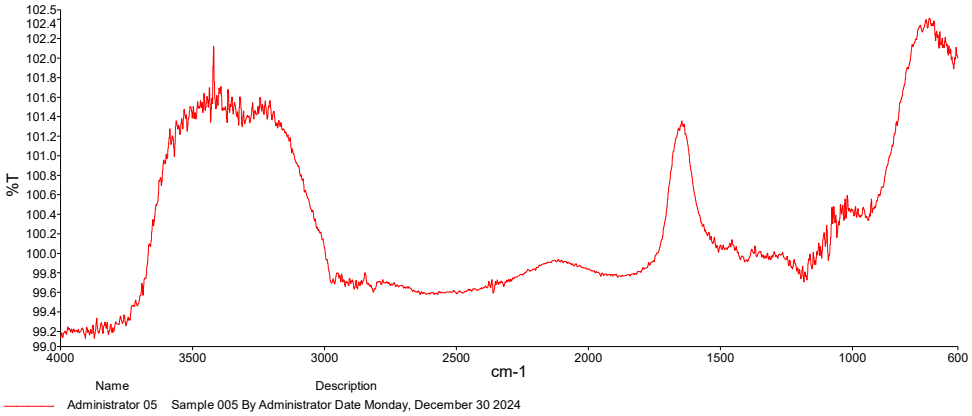

Fig 2: FTIR of Hydroxy propyl methyl cellulose K100

In figure 3 shows the spectra of methanolic extract of leaves (F2) of plant. The broad peak at 3500-3200, 1740.42,1500,1476 . represents the presence of functional groups such as alcohols. Phenols (O-H stretch, H-bonded), imines , aromatic vibration, alkyl ether respectively.

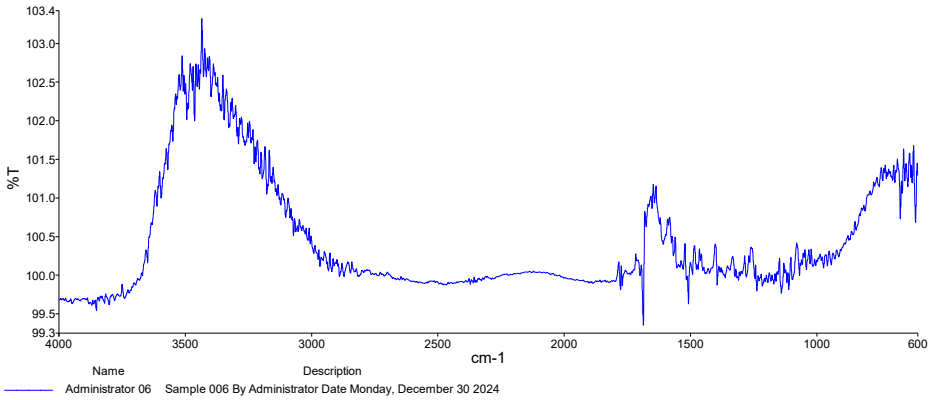

Fig 3: FTIR of methanolic extract of Leaves (F2) of plant *Ehretia Laevis Roxb*

From FTIR spectrum, we can confirm the presence of functional components in the leaves and extract them. The pharmacological action of therapeutically active constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds or secondary metabolites of plant.

TLC Analysis-

Mobile Phase - Toluene: Ethyl alcohol (7:3)						
Detection						
No. of Spots		At 253 nm	At Day Light	At 366 nm	Rf Value	Rf Value of standard
Extract (Chloroform)	1	Green	Green	Green	0.17	0.16
	2	Green	Green	Green	0.28	0.22
	3	Green	Green	Green	0.38	0.28
	4	Yellow	Yellow	Orange	0.51	-
	5	Yellow	Yellow	Orange	0.70	-
	6	Green	Green	Green	0.76	-
	7	Green	Green	Green	0.83	-
	8	Green	Green	Green	0.92	0.81

Mobile Phase - Toluene: Ethylalcohol (9:1)						
Detection						
No. of Spots		At 253 nm	At Day Light	At 366 nm	Rf Value	Rf Value of standard
Extract (Ethylalcohol)	1	Dark green	Dark green	Dark green	0.25	0.3
	2	Green	Green	Green	0.28	0.4
	3	Yellow	Yellow	Orange	0.38	0.6
	4	Green	Green	Green	0.44	-
	5	Yellow	Yellow	Orange	0.80	0.8
	6	Green	Green	Green	0.92	-

Table-2: result of TLC of chloroform extract and methanolic extract

Fig 4: TLC of chloroform extract

Fig 5: TLC of methanolic extract

Procedure For Preparation of Film Forming Spray

Different concentration of polymer in methanol and water was studied after trial-and-error method following quantities are decided for formulation.

Part 1 - Initially vehicle is prepared by dissolving HPMC K100, 1gm in 20 ml of methanol, mixed uniformly, 1 ml of polyethylene glycol 400 is added and homogenously mixed using mechanical stirrer.

Part 2- Herbal spray can be prepared by dissolving extract Chloroform (0.5 ml)\ methanol(1.5 ml) in 10ml of methanol .

Part 3- Finally, part 1 and part 2 is mixed with mechanical stirrer and stir at 900 rpm to form a clear solution, final volume is adjusted with distilled water up to 50ml.

Lastly the pH is adjusted using sodium citrate and citric acid.[9,14]

Formulation Table

Sr,no	Ingredients	F1	F2	Use
1	HPMCK100	1 gm	1 gm	Film forming polymer
2	PEG 400	1ml	1ml	Plasticizer
3	Extract	0.5ml	1.5 ml	API
4	Methanol	30 ml	30 ml	Co solvent
5	Water	Upto50ml	Upto50ml	solvent
6	Sodium citrate	qs	qs	pH modifier

Table-3: Formulation Table

Evaluation of Spray

Physicochemical characteristics spray formulations:

1) Color and appearance-

Physical parameters like color, appearance and odor were examined by visual examination a yellow-colored turbid solution was observed , having characteristic odor.

2) Density-

gravity bottles are utilized to determine the density of the formulation. First the density of water was determined and then formulation density was calculated.

3) Viscosity-

The measurement of viscosity of the spray formulation was done using Brookfield viscometer. The spindle no.63 is deep in spray formulation and rotated at 30,60 rotation per min. At each speed the corresponding dial reading was noted. The viscosity of gel was calculated by using formula.

Viscosity = dial reading x spindle factor

4) pH-

The pH of spray formulations was determined by using digital pH meter. The pH meter is calibrated using distilled water to pH 7. The electrode is deep in 50 ml distilled water. The measurement of pH of each formulation was done in triplicate and average values are determined.

Pharmacological Evaluation

The animals weighing between 200 to 250 gm are selected for study, animals are divided into 4 groups as follows -

A. Excision wound model-

Hairs were eliminated from the thoracic area of central region of the rats. animals were wounded under mild chloroform anesthesia by using partial aseptic techniques. skin is removed fully. An area was cut out using sterile bled to create a wound having approximately 2 cm in size. The wound was wiped with a cotton swab that had been dipped in povidone iodine. The two experimental formulations and cipladine were tested by applying to the wound twice a day for two weeks beginning on the measurement of wound contraction was done on the initial day of injury for a duration of 2 weeks with a gap of 2-3 days between each interval.

Table-4: Animal grouping

Groups	Treatment	No. of animals
Group I	Untreated(Control)	1
Group II	Standard (Cipladin)	1
Group III	Formulation I	2
Group IV	Formulation II	2

B. Statistical analysis-

The results of study were reported as the mean plus or minus the standard error of the mean (SEM)

C. Measurement of Wound Contraction-

In the excision wound model wound area was measured by tracing the wound with the help of transparent Paper or sheet and mark on millimeter-based graph paper on days 0, 3, 5, 8 and 11 for all groups. Wound contraction was measured every 3rd day until complete wound heals. Wound healing can be represented as percentage of healing wound area. Percentage of wound

contraction was calculated by taking the initial size of the wound as 100% and using following formula:

$$\% \text{ wound contraction} = \frac{(\text{Initial wound area} - \text{Specific day wound area})}{\text{Initial wound area}} \times 100$$

Control day-1

Control day-5

Control day-8

Standard day- 5

Standard day- 8

Control day-11

Standard day- 11

Formulation- I Day-1

Formulation- II Day-1

Formulation- I Day-8

Formulation- II Day-8

Formulation- I Day-11

Fig 6: Wound contraction study

Formulation- II Day-11

Result

1) Results of preliminary phytochemical analysis-

The preliminary phytochemical screening of *Ehretia laevis Roxb*. leaf extracts were performed using different solvents as, petroleum ether, chloroform, methanol, and water. The results give the presence of various bioactive compounds, as summarized below: chloroform extract showed significant presence of alkaloids, phenolic tannins, flavonoids, and napthoquinones. Methanol extract contained alkaloids, phenolic tannins, flavonoids, reducing sugars, and napthoquinones. Water extract displayed a limited presence of flavonoids and reducing sugars. Petroleum ether extract: No significant phytochemicals were detected except steroids and triterpenoids. The study highlights the potential of chloroform and methanol extracts of *Ehretia laevis Roxb*. as sources of bioactive compounds like alkaloids, flavonoids, and napthoquinones. which are known to contribute to wound-healing and antimicrobial properties.

2) Results of FTIR spectrum analysis-

The FTIR spectral analysis of the chloroform and methanolic extracts of *Ehretia Laevis Roxb*. leaves show the presence of various functional groups, indicating the presence of bioactive

constituents. The FTIR analysis confirmed the presence of functional groups such as alcohols, phenols, alkynes, imines, isothiocyanates, and carbonyl compounds in the extracts. These functional groups indicate the presence of bioactive compounds like flavonoids, tannins, alkaloids, and other secondary metabolites, which has predominant action on wound-healing.

3) Results of thin layer chromatography (TLC) analysis-

The TLC analysis of *Ehretia Laevis Roxb*. leaf extracts were performed using two mobile phases: Toluene: Ethyl Alcohol (7:3) and Toluene: Ethyl Alcohol (9:1). The results give the distinct spots, indicating the presence of various phytoconstituents. The TLC analysis confirmed the presence of multiple phytochemicals in *Ehretia Laevis Roxb*. extracts, as evidenced by distinct spots at various Rf values. Green spots indicate the presence of flavonoids, while yellow-orange spots suggest phenolic compounds or quinones. The diversity in Rf values highlights the complex nature of bioactive components within the plant extracts.

Treatment	Day- 3	Day- 5	Day- 8	Day- 11
Group I(Control)	14.5±1.55	35±2.41	40±3.16	55±3.53
Group II(Standard)	19±1.77	60±3.16	70±3.41	95±3.97
Group III-F1	13.5±1.50	35±2.41	73.5±3.5	83±3.76
Group IV-F2	12±1.41	24.5±2.02	69.5±3.40	75±3.53

4) Result of physicochemical parameter of spray-

Parameters	pH	Density	Viscosity	Evaporation time
F1	5.84	1.23 m/v	120 cp	19 sec
F2	6.07	2.21 m/v	200 cp	23 sec

Table-5: Evaluation of spray

4) Wound contraction studies-

The results of wound healing studies were above in photographs (Figure 6) From wound area contraction data, statistically it is concluded that the formulation 1 Group produced more wound contraction as compared with the other tested formulations 2 and wound contraction increases as the day passes. The standard treated group has maximum wound contraction while control treated group has lower rate of wound healing.

Conclusion and Discussion

The study highlights the significance of *Ehretia Laevis Roxb*.(Khandu Chakka) In wound healing by formulating a novel film forming spray (bandage spray). Wound healing is multifaceted process. Plant extract generally contains various bioactive compounds, methanol extract contains flavonoids tannins and napthoquinones derivatives while chloroform extract contains burenol in addition to above. The film- forming spray overcomes the different problems of conventional wound healing product.

Table-6: Result of wound contraction studies

It includes formulation of protective layer, waterproof barrier over wound, antimicrobial. action. The thin transparent film prevents the dust on wound and indirectly promotes the wound healing. The effect of formulation on wound healing is evaluated using albino-wister rat. The standard formulation (Cipladin), shows the optimum wound contraction. Formulation 1 shows more wound contraction than formulation 2 and control group. It may due to the presence of

baurenol in chloroform extract which is absent in methanolic extract. The control group shows most delayed wound contraction.

Overall, this research underscores the traditional knowledge with modern pharmaceutical techniques.

Reference

1. Mohamed B.Saad W.c khan, Kan Weng Chin, Basma Yahya Alashwal, & Arun Gupta. (2020). Development of wound healing spray from keratin protein. Maejo International Journal of Energy and Environmental Communication, 2(2), 43–49.
2. Kolhe S. et al., formulation and evaluation of poly herbal ointment, Americal Journal Of Pharma Tech Research, 2017; 7(6).
3. Wayal, V., Nagasree, K., & Vishwanath, B. A. (2021). Design, development and evaluation of silk-based film forming spray for wound healing. Journal of Drug Delivery and Therapeutics, 11(3-s), 15–18
4. Dr. K.R. Khandelwal; Practical Pharmacognosy Technniqes And Experiments; Nirali Prakashan, 2011.
5. Thakre, R., Borkar, P., & Harne, K. (2023). Wound healing potential of different extracts of Ehretia Laevis Roxb. (Khandu Chakka/Ajan Vruksha) versus silver sulfadiazine in burn wound - Pre-clinical study. International Journal of Ayurvedic Medicine, 14(4), 939–944.
6. Sharma, P., Shri, R., Ntie-Kang, F., & Kumar, S. (2021). Phytochemical and ethnopharmacological perspectives of Ehretia laevis. Molecules, 26(12), 3489.
7. Kolhe SS, Shinde K, Jori R, Gadhave MV, Jadhav SL, Gaikwad DD, Evaluation of polyherbal ointment for wound healing activity in Wistar rats, Journal of Drug Delivery and Therapeutics. 2018; 8(6-s):26-31.
8. Wadkar, S., Buchude, A., Wavhal, P., Wayal, Y., Walunj, A., Bhapkar, N., & Kumbhar, U. (n.d.). Pharmacological action of polyherbal gel for wound healing activity on albino Wistar rat. International Journal of Pharmaceutical Sciences.
9. Ranade, S., Bajaj, A., Londhe, V., Babul, N., & Kao, D. (2017). Fabrication of topical metered dose film-forming sprays for pain management. European Journal of Pharmaceutical Sciences, 96, 301–308.
10. Kokate C.K., Gokhale S.B., Purohit S.P. A textbook of Pharmacognosy, Nirali Prakashan, 34th edition;2013; 9:117
11. Thakre, R., Harne, K., Tekade, P., & Parve, S. (n.d.). Role of Ajan Vruksha/Khandu Chakka plant (Ehretia Laevis Roxb.) in COVID-19 pandemic. International Journal of Research in Pharmaceutical Sciences.
12. Khadsinge, P. R., & Sahasrabuddhe, S. H. (n.d.). Various roles of Ehretia Laevis Roxb: A review article. Department of Cosmetic Technology, Lady Amritabai Daga College & Smt. Ratanidevi Purohit College, Nagpur.
13. Patil, S. B., Naikwade, N. S., Kondawar, M. S., Magdum, C. S., & Awale, V. B. (n.d.). Traditional uses of plants for wound healing in the Sangli district, Maharashtra. Appasaheb Birnale College of Pharmacy, Sangli.

14. Sayyad Jaanali Mahammadjabir, Shilpa Kolhe, Dhananjay Deshmukh et al. Evalution of Wound Healing Activity of Polyherbal Gel Formultion. Inventi Impact Ethnopharmacology,2015;(1):13-20.
15. Badwaik, C. B., Lade, U. B., Agarwal, T., Barsagade, P., Nandgave, M., & Gaddamwar, N. (2022). Formulation and evaluation of herbal face cream. International Journal of Pharmaceutical Research and Applications, 7(1), 955–960.
16. Suryavanshi, G. M., Hingane, L. D., Khade, P. B., & Korde, A. B. (Year). Formulation and evaluation of topical antifungal herbal spray. International Journal of Pharmaceutical Research and Applications,
17. Kasabe, A., & Shende, V. (2024). Fourier transform infrared spectroscopy as a versatile analytical tool for studies of unexplored plant Ehretia laevis Roxb. Journal of Emerging Technologies and Innovative Research (JETIR).
18. 93. Jain, A.K.; Thanki, K.; Jain, S. Co-encapsulation of tamoxifen and quercetin in polymeric nanoparticles: Implications on oral bioavailability, antitumor efficacy, and drug induced toxicity. Mol. Pharm. 2013, 10, 3459–3474.
19. 94. Miean, K.H.; Mohamed, S. Flavonoid (myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants. J. Agric. Food Chem. 2001, 49, 3106–3112.
20. Somerset, S.M.; Johannot, L. Dietary flavonoid source in Australian adults. Nutr. Cancer 2008, 60, 442–449. Jain, A.K.; Thanki, K.; Jain, S. Co-encapsulation of tamoxifen and quercetin in polymeric nanoparticles: Implications on oral bioavailability, antitumor efficacy, and drug induced toxicity. Mol. Pharm. 2013, 10, 3459–3474