



# “Formulation And Evaluation Of Analgesic Ointment By Using “Limonia Acidissima”

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**Abstract:** Despite its enormous therapeutic value in treating a variety of human ailments, *Limonia acidissima* is one of the numerous edible fruit plants that are currently underappreciated in India. The leaves, bark, roots, fruits, and seeds are all used extensively in Ayurvedic medicine as a laxative and to treat a number of ailments, including as dysentery, peptic ulcers, and chronic diarrhea. Herbal medications are utilized as ointments in addition to other dosage forms. When shear force is applied, ointments, which are semisolid dosage forms, usually behave as visco-elastic materials. The analgesic property is highlighted in this study. Creating an ointment based on its analgesic properties is the main focus of this effort. Wool fat, benzoic acid, carbopol, cetosteryl alcohol, soft paraffin (ointment base), and wood apple (drug) are the ingredients. The purpose of the evaluation tests is to evaluate the analgesic properties. Out of the five formulations {F1, F2, F3, F4, and F5}, formulation 5 with 250 mg of extract exhibits the strongest analgesic effect.

**KEY WORDS:** Ointment, Alagesic action, *Limonia acidissima*.

## 1) INTRODUCTION

### ANATOMY OF SKIN:

The human skin is the largest organ of the integumentary system and the outermost covering of the body. The skin, which is composed of up to seven layers of ectodermal tissue, shields the bones, muscles, ligaments, and internal organs. Like the skin of most other animals, the skin of pigs is strikingly similar to that of humans. Even though practically all of the human skin is covered in hair follicles, it can nonetheless appear hairless. There are two types of skin: hairy and glabrous (hairless). "Of the skin" is the literal meaning of the adjective "cutaneous." Insulation, temperature regulation, sensibility, vitamin D production, and vitamin B folate protection are among the other roles of the skin

### Epidermis

Since "epi" means "over" or "upon" in Greek, the outermost layer of skin is known as the epidermis. It forms the waterproof, protective layer that covers the body's surface and serves as an infection barrier. It is made up of stratified squamous epithelium with an underlying basal lamina. Layers beneath The stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale (also known as "stratum germinativum") are the five sublayers or strata that make up the epidermis. Blood capillaries that are attached to venules and arterioles are located beneath the epidermis. Arterial shunt vessels can bypass the network in the nose, fingers, and ears.

**Dermis** • The dermis, the layer of skin beneath the epidermis that is rich in connective tissue, shields the body from harm and stress. The dermis and epidermis are securely joined by a foundation membrane.

### **Limonia acidissima:**

Mostly found in Southeast Asia, India (especially in the states of Uttar Pradesh, Madhya Pradesh, Maharashtra, West Bengal, Chhattisgarh, and Western Himalaya), Penang Island, and Sri Lanka, *Limonia acidissima* Groff is an underutilized tree that bears tropical fruits and is a member of the Rutaceae family. The wood apple fruit is rich in tannins, glycosides, flavonoids, saponins, ascorbic acid, riboflavin, vitamin-B, and beta-carotene, along with a variety of other vitamins and minerals. The nutritional and phytochemical components that are often absent from many other fruits include amino acids, polyphenols, saponins, coumarins (including osthenol, psoralen, demethylsuberosin, bergapten, and isopimpinellin), triterpenoids, phytosterols, and tyramine derivatives. Several germs that cause human illnesses are resistant to the essential oils that are derived from wood apple fruits.

In the ancient Greek and Roman eras, it was utilized as medicine.

Analgesic action is demonstrated by *Limonia acidissima*. Because of its possible analgesic, anti-inflammatory, and antibacterial qualities, *Limonia acidissima* has been utilized historically.

A wood apple's pulp has a significant water content that might help moisturize the skin. Maintaining skin suppleness and avoiding dryness and flakiness require adequate hydration. Additionally, it has anti-inflammatory qualities that can help soothe and relax skin that is irritated.

### **Nutritional composition and phytochemicals**

Several wood apple extracts have been found to include a variety of bioactive compounds, such as phenols, flavonoids, alkaloids, terpenoids, tannins, saponins, fat steroids, glycosides, gum mucilage, and fixed oil. These compounds are responsible for the fruit's various pharmacological characteristics. Different parts of the plant, such as its roots, fruits, bark, and leaves, have been used for centuries to treat a range of ailments, including diarrhea and dysentery. Because they contain protein, carbohydrates, vitamins, and minerals, fruits are an essential food source. Accordingly, fruits are called "protective foods" (Srivastava and Kumar, 2002). Dietary and nutritional surveys have shown that Indians are lacking in vitamins A and C, which makes fruits an essential part of a healthy diet, in addition to shortages in minerals like calcium and sodium.

#### **ANALGESIC ACTIVITY:**

**a) Writing in mice:** 0.1 ml of 1% acetic acid was delivered intraperitoneally (I.P.) into four groups of six mice each. Three groups got extract (50, 100, and 250 mg/kg.I.P.) half an hour before acetic acid, whereas one group received a vehicle. For fifteen minutes, the number of writhing motions was recorded. The proportion of writhing movement inhibition was computed.

**b) Thermal noiception** Mice were maintained at a consistent temperature of  $55 \pm 0.1$  °C on an eddy's hot plate. Before and after the extract was administered, the duration of time spent jumping or licking the paws was noted. There were four groups of mice. The other group was given extract at doses of 50, 100, and 250 mg/kg I.p. half an hour before being placed on the hot plate, whereas the control group was given the vehicle. Subjects with significant joint range loss and associated chronic pain who took a spoonful of dried apple peels every day for 12 weeks reported improvements in their necks, shoulders, backs, and hips as well as a decrease in pain scores.

## **2) Methodology**

**Plant selection:** Choose ripe, fresh *Limonia acidissima*

**Cleaning and Drying:** To get rid of toxins, moisturw.and grime, clean and dry thr fruits

### **Method of extraction;**

**Maceration method:** The maceration process produced the extract. 200ml of ethanol was mixed with 100g of powder and stirred for two days. In order to obtain residue, the ethanolic extract was finally collected and concentrated. The extract was kept in a cool, dark location in an airtight container.

**Procedure:****Procedure for preparation of herbal ointment:**

First, make the ointment base by precisely weighing the grated soft paraffin that will be placed in the evaporating dish over the water bath. Once the soft paraffin has melted, add the remaining components and stir gently to help melt and combine them evenly. Then, let the ointment base cool. Make the herbal ointment by combining precisely weighed Limonia acidissima extract with the ointment base using the fusion method. This creates a smooth paste that is two or three times the weight of the base. Gradually add more base until the ointment is homogenous, and then transfer it to an appropriate container.

**FORMULATION:****Table: OINTMENT BASE:**

Ingredients	Quantity	Uses
Wool fat	10g	Moisturiser
Cetosteryl alcohol	5g	Thickening agent
Soft paraffin	10g	Skin protectant
Carbapol	5g	Spreading agent
Benzoic acid	0.5g	Preservative

**FORMULATION TABLE:**

Ingredients	F1	F2	F3	F4	F5
Wood apple	50mg	100mg	150mg	200mg	250mg
Wool fat	10g	10g	10g	10g	10g
Cetosteryl alcohol	5g	5g	5g	5g	5g
Soft paraffin	10g	10g	10g	10g	10g
Carbapol	5g	5g	5g	5g	5g
Benzoic acid	0.5g	0.5g	0.5g	0.5g	0.5g

➤ **Evaluation Test:**

- Spreadability test
- Washability test
- Irritancy test
- Viscosity

**Test for solubility:** Identification test of pH: To find out the pH of limonia acidissima ointment in practical basethe electron can dip in the solution of limonia acidissima. The solution was prepared by one gram of weighed formulation was dispersed in 100ml od diluted tween 80

**Loss on drying:**

**Procedure:**

1)Weigh about 15g of the powdered drug into a weighed flat and thin porcelain dish. 2)Dry in the oven at 100°C and 105°C until two consecutive weighing do not differ by more than 0.5mg

3)Cool in a desiccators and weigh. The loss in weight is usually recorded as moisture. → **Identification test ointment:**

**Colour and odour:** Physical parameters like colour and odour were examined by visual examination.



**Consistency:** It is smooth and doesn't appear green.

**Flow characteristics:**

Density of bulk

The tapped density

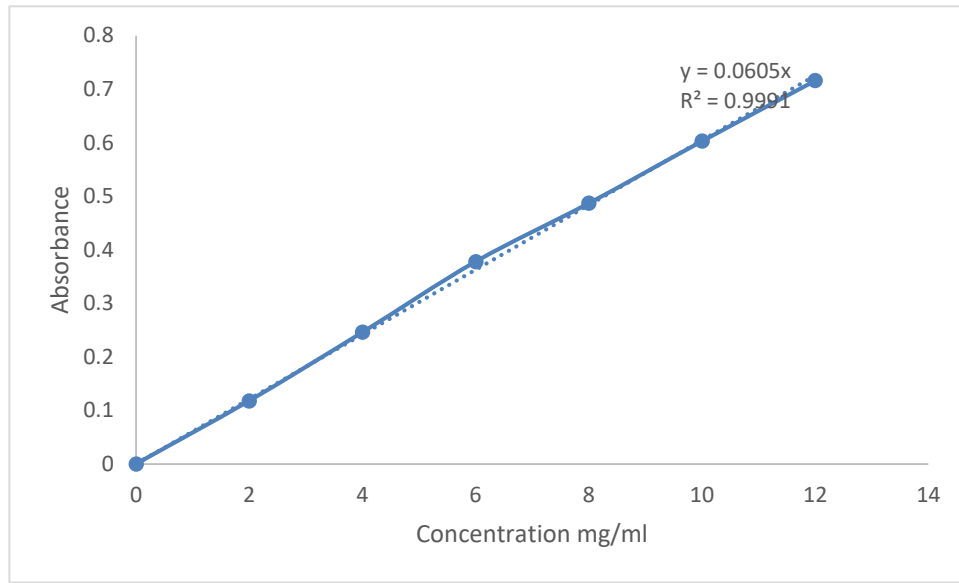
Curve of Limonia acidissima

**FTIR Research:** Fourier Theanalytical method of transform infrared spectroscopy, sometimes referred to as FTIR analysis or FTIR spectroscopy, is used to identify organic, polymeric, and occasionally inorganic materials. Infrared light is used in the FTIR analysis procedure to scan test materials and examine their chemical characteristics.

### 3)RESULTS AND DISCUSSION

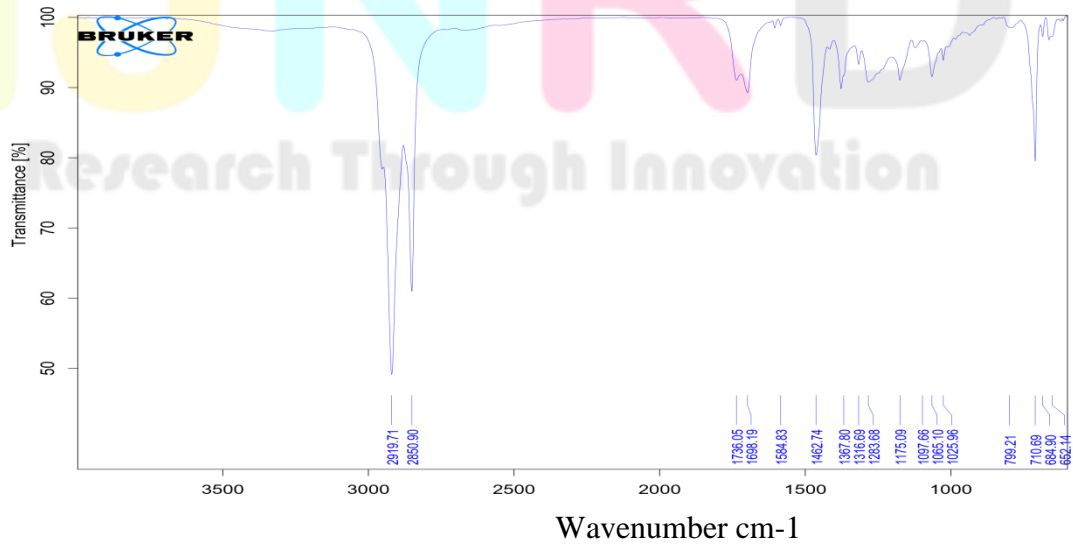
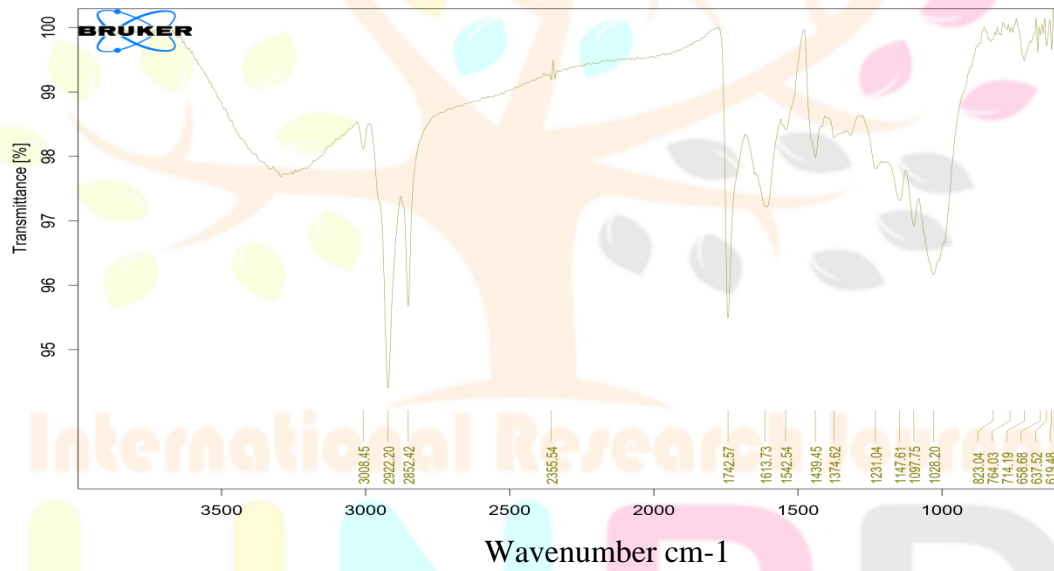
**TABLE: CALIBRATION CURVE OF DIFFERENT FORMULATIONS**

Concentration mg/ml	Absorbance
0	0
2	0.118
4	0.246
6	0.378
8	0.487
10	0.603
12	0.716



**Fig.no.18:** Calibration curve of *Limonia acidissima* concentration versus absorbance

**FTIR STUDIES**



**DISCUSSION:**

Wood apples are compatible with excipients (wood fat, cetosteryl alcohol, soft paraffin, carbapol, and benzoic acid), according to the FTIR studies mentioned above.

**Evaluation tests:**

Irritability test

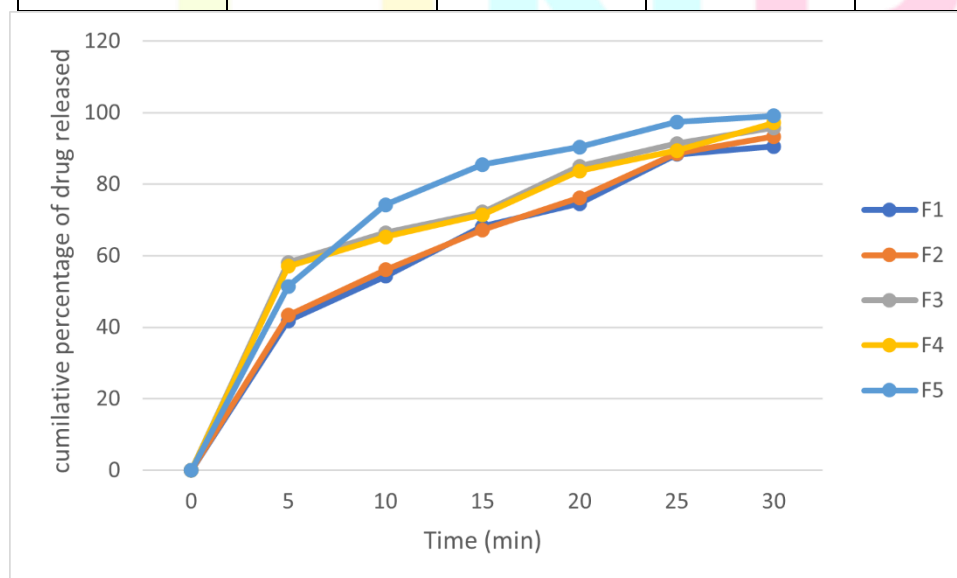
Tests for washability

Reproducibility

evaluation

**DIFFSIBILITY STUDIES OF LIMONIA ACIDISSIMAON DIFFERENT FORMULATION**

TIME (MIN)	F1	F2	F3	F4	F5
0	0	0	0	0	0
5	41.78±1.17	43.35±1.48	58.08±1.38	57.02±1.39	51.38±1.28
10	54.25±1.25	56.06±1.18	66.42±1.67	65.23±1.25	74.20±1.16
15	68.17±1.37	67.24±1.37	72.16±1.25	71.45±1.76	85.45±1.47
20	74.58±1.49	76.15±1.25	85.02±1.78	83.69±1.57	90.37±1.52
25	88.34±1.25	88.54±1.16	91.34±1.26	89.42±1.35	97.41±1.49
30	90.50±1.18	93.35±1.43	95.74±1.45	97.17±1.42	99.12±1.75



## CONCLUSION

- The potential of *Limonia acidissima* pulp powder as a topical preparation for analgesic action is demonstrated in this study. Additionally, it has neuroprotective, anti-inflammatory, anti-diabetic, antibacterial, and antifungal properties.  
The herbal ointment showed notable analgesic effects, indicating that it may be used to treat pain. Pre-formulation studies were conducted to assess the analgesic ointment. Five formulations (F1, F2, F3, F4, F5) of the ointment (50 mg, 100 mg, 150 mg, 200 mg, and 250 mg) were made using the excipients wool fat, ceto steryl alcohol, benzoic acid, carbopol, and soft paraffin.

*Limonia acidissima* pulp powder was used to make a herbal analgesic ointment, which was then evaluated for a number of physical characteristics.

- The ointment is next tested for physical criteria, including drug content, diffusibility studies, viscosity, solubility, PH, loss upon drying, identification tests (color, odor, and consistency), spreadability, washability, and irritancy.  
According to the diffusibility study and tests mentioned above, 99% of the medication was released in 30 minutes. Thus, it can be said that out of the five formulations, the F5 formulation exhibits the highest level of analgesic activity.

## 10. REFERENCES

1. S. Pandey, G. Satpathy, R.K. Gupta, Evaluation of nutritional, phytochemical, antioxidant and antibacterial activity of exotic fruit " *Limonia acidissima*", J. Pharmacogn. Phytochem. 3 (2) (2014).
2. N. Singhania, P. Kajla, S. Bishnoi, A. Barmanray, Ronak, R. Development and storage studies of wood apple (*Limonia acidissima*) chutney, Int. J. Chem. Stud. 8 (1) (2020) 2473–2476.
3. S. Murakonda, G. Patel, M. Dwivedi, Characterization of engineering properties and modeling mass and fruit fraction of wood apple (*Limonia acidissima*) fruit for post-harvest processing, Journal of the Saudi Society of Agricultural Sciences 21 (4) (2022) 267–277.
4. S.P. Kerkar, S.S.S.A. Patil, A. Dabade, S.K. Sonawane, *Limonia acidissima*: versatile and nutritional fruit of India, Int. J. Fruit Sci. 20 (sup2) (2020) S405–S413.
5. S. Lamani, K.A. Anu-Appaiah, H.N. Murthy, Y.H. Dewir, J.J. Rikisahedew, Analysis of free sugars, organic acids, and fatty acids of wood apple (*Limonia acidissima* L.) fruit pulp, Horticulturae 8 (1) (2022) 67

