RESEARCH ARTICLE

Evaluation of a Newly Formulated Anti-Inflammatory Ointment from *Ficus religiosa* Root Bark

Jyothisree G[1], S. Umadevi[2*]

[1]*School of Pharmaceutical Sciences, VISTAS, Vel's University, Chennai, Tamil Nadu, India.*
[2]*Department of Pharmaceutics, School of Pharmaceutical Sciences, VISTAS, Vel's University, Chennai, Tamil Nadu, India.*

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ABSTRACT

The basis of the medical system is medicinal herbs. Herbal combinations for therapeutic alignment often include botanical components. This work aims to make an anti-inflammatory ointment from the *Ficus religiosa* root bark and test the medicine using a novel HPTLC technique. The flavonoid component from the hydroalcoholic extract was separated by column chromatography. The mobile phase used was a mixture of toluene, ethyl acetate, and methanol in the ratio 04:04:02. By using the fusion method to create a straightforward ointment from this separation, it was discovered that the formulation's physicochemical properties were all within acceptable bounds. The mobile phase for the HPTLC study included toluene, ethyl acetate, formic acid, and methanol (3:3:0.8:0.2), and the wavelength was 280 nm. Using albino mice and the Carrageenan-induced paw edema method, the anti-inflammatory effect was assessed. Rf value is discovered to be 0.86, 0.88, and 0.89 for simple ointment, fractionate, and standard quercetin, respectively. According to ICH criteria, the HPTLC method was validated, and all parameters were within acceptable ranges. The created ointment can be used in a straightforward manner to alleviate inflammation. In comparison to NSAIDS, the topical formulation showed good anti-inflammatory action in carrageenan-induced paw edema.

Keywords: *Ficus religiosa*, Ointment, HPTLC, Validation, Anti-inflammatory activity.

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INTRODUCTION

Herbal remedies are a significant part of traditional medicine. India produces the most medicinal plants overall, and its cultures are among the oldest and most diverse. Primary care physicians treat the agent using herbal remedies that contain botanical ingredients. As a result, many different plant species are used to cure a variety of diseases in traditional medicine. The beneficial effects are primarily attributable to the secondary metabolites in plants that have health-promoting properties.[1,2]

Several botanical compounds, such as flavonoids, polyphenols, tannins, terpenes, saponins, phytosteroids, and others, have been discovered in therapeutic plants. Flavonoids are plant phenolic compounds; tannins are polyphenolic substances that, depending on their content, nature, and other elements, including animal species, body composition, and diet composition, are thought to have both beneficial and harmful effects.[3] A class of polyphenolic chemicals known as flavonoids have various chemical structures and characteristics. The phenylpropanoid pathway produces the majority of phytophenols.[4]

It has been established that herbal medications are both effective and safe. The medicine's therapeutic effectiveness depends on using the proper and pure ingredients. Thus, medicinal plants and herbal products' reliability, security, and effect have emerged as critical issues that call for their botanical design[5]. A range of culinary and herbal preparations benefit from the phytochemicals found in almost all of the tree's components.[6,7]

Numerous disorders, including cancer, inflammation, diabetes, heart disease, and others, have been successfully treated with them, according to studies. Separating, recognizing, and predicting the chemical from the plant is exceedingly challenging.[8,9] Prescriptions have considerable pharmacological effects, according to a literature analysis. Herbal remedies have had their medical potential thoroughly investigated since they are simple for humans to employ. Only current technology-based examination and analysis of herbal items can make this possible.

Currently, the design and quality control of many herbal products has proven to benefit from the publication of various experiments, especially when it is difficult to identify all the

**Author for Correspondence: umadevi.sps@velsuniv.ac.in*

active ingredients included in natural products.[10] Among other chromatographic techniques, thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) have shown to be effective analytical tools for herbal products. The HPTLC method is often used for identifying and quantitatively estimating herbal remedies due to its flexibility, dependability, efficiency, and cost-effectiveness.[11] It is also possible to evaluate many plant extracts or herbal preparations simultaneously. The HPTLC method can be validated for its accuracy, precision, specificity, linearity, etc., by using the formulation of quercetin.[12]

The simplest separation method, HPTLC, offers superior resolution and material prediction in less time, given the right material. HPTLC has evolved as the preferred analytical instrument for the fingerprinting and quantification of transcripts in herbal medicine due to its ease of use, sensitivity, accuracy, and usability.[13,14] In this study, a straightforward HPTLC approach for the identification of chemicals present in novel formulations has been developed, refined, and validated. To prevent inflammatory disease, we formulated and analyzed Arishta from *Ficus religiosa* root bark extracts.[15]

MATERIALS AND METHODS

Ficus plant roots were gathered at Kozhikode, Kerala, India. Dr. A. K. Pradeep, Associate Professor, Department of Botany, University of Calicut identified the plant; the test number is 88488. The root bark is finely powdered after being carefully washed with tap water, dried in the shade, and ground. For future research, keep in an airtight container. Standard procedures were used to conduct testing on the macroscopic and physicochemical levels.

Gather the root, clean it, and remove any foreign objects that may have become lodged. Then, let the roots air dry for two weeks in the shade. The raw medication should be ground into a powder and kept in an airtight container for future usage. Weigh 50 g of raw medication accurately, add it to the soxhlet extractor's thimble, and use 250 mL of ethanol: water (30:70) by weight to extract it for 72 hours. Weight distillation was used to concentrate the extract, and the solvent was subsequently evaporated to produce a residue.

The extracts' phytochemical make-up was analyzed using conventional methods. The extract was then separated using column chromatography, with the mobile phase consisting of a 4:4:2 ratio of toluene, ethyl acetate, and methanol. All fractions were subjected to TLC analysis, and fractions with the same Rf value were pooled and concentrated.

Ointment Formulation

Hard paraffin and cetearyl alcohol were first melted together to make ointment in a water bath. While continually stirring, add the remaining ingredients. Using the fusion process, the ficus root bark that was separated in the second step is added to the molten base and cooled by constant stirring until it hardens. The ointment was formulated as per IP procedure (Table 1).

Appearance

The ointment's color, scent, and composition all affect how it looks.

Table 1: Formulation of ointment

Ingredients	*Quantity*
Ficus religiosa isolate	500 mg
Hard paraffin	5 gm
Cetostearyl alcohol	5 gm
Wool fat	5 gm
White soft paraffin	85 gm

pH

A digital pH meter was used to calculate the solution's pH. The ointment was made with 100 mL of distilled water and applied after two hours. Three different measurements were used to test the answer, and the mean value was calculated.

Viscosity

A Brookfield viscometer was used to measure the viscosity of the produced ointment. Each formulation's results were examined three times.

Spreadability

Maximum samples were placed on two glass slides, and the spreadability was assessed by compressing the slides to a consistent thickness over time using a particular weight. The length of time needed to separate the two slides was meticulously timed. The spreadability increases with the time it takes to separate the two slides.

The formula for calculating spreadability is $S = (L \times M) \div T$.

S stands for spreadability.

M is the top-mounted weight, and L is the slide's length. T is the time needed to remove the slider.

Extrudability

The extrudability test measures the force necessary to extrude material from a shrinkable tube. The mixture was contained in a tube-shaped container that collapsed. This investigation calculated the amount of ointment that emerged from the tube when a particular load was established. The formula for the ointment's extrudability was determined as follows

Extrudability = (amount of ointment extruded from the tube ÷ the total amount of ointment in the tube) x 100

Diffusion analysis

Agar, the nutritive medium, was prepared to study diffusion. Put some ointment and a plate of water in the center. It is noted how long it takes the ointment to spread (60 minutes later).

Loss on drying

To calculate loss on drying, 1 g of ointment was placed in a petri plate set over a water bath and then dried until the weight stabilized.

Non-irritant test

Apply a formulated herbal ointment to human skin and watch what happens.

Accelerated stability study

A three-month accelerated stability test was conducted on herbal medication that was prepared as it was packaged at a

temperature of 40 ± 2°C and relative humidity of 70 ± 5%. Samples are taken every 30 days, and their physical properties, pH, viscosity, dispersion, and extrusion are all checked.

Analytical Evaluation of Ointment using HPTLC

For the chromatography, 8.0 × 10.0 cm TLC plates with a 0.20 mm layer of silica gel 60 F254 (Merck, Darmstadt, Germany) were employed. Use the CAMAG Linomat 5 sample applicator, which has a 100 µL volume syringe, to apply the samples in bands that are 8 mm broad and separated by 11.3 mm. Use a fixed application rate of 150 nLs^{-1}. Extract 5.25 g of the produced ointment into a container with 10 mL of methanol to create the sample concentration of 100 g/mL. Use 10 µL of the sample after that. Toluene, ethyl acetate, formic acid, and methanol (3:3:0.8:0.2v/v) were used as the mobile phase. Densitometric scanning in absorption mode at 280 nm was performed using the CAMAG TLC Scanner-III in conjunction with the Win Cats software. The design's precision, LoD, LoQ, robustness, specificity, and other attributes have all been confirmed to meet ICH standards.

Pharmacological Anti-Inflammatory Activity Study

In-vivo anti-inflammatory study

We utilized male or female albino wistar rats weighing 150–200 g. With six animals each, they were split into three groups: growth, negative control, and positive control. Group 2 (standard drug) received indomethacin (0.2 gm p.o.), while group 1 (control group) received normal saline as a treatment. The test sample (0.2 g orally) is in group 3. Animals were given free access to water and were fasted for 24 hours before the experiment.[16]

Edema was created by injecting 0.1 mL of 1% w/v carrageenan in saline into the rat's right hind leg plantar side an hour before each experiment. Utilizing the index finger, lightly brush the planting region of the chest paw 50 times before applying 0.2 g of ointment. The mice in the control group established the proper baseline. As a benchmark, 1% indomethacin ointment, 0.2 gm, was employed. One hour was spent giving the medication or a placebo. Prior to administering Carrageenan. Use digital calipers to gauge paw volume at 1, 2, and 3 hour intervals right after injection. The amount of pain is indicated by the difference in thickness between the left and right paws, and the percentage of inhibition was calculated.[17-19]

Figure 1: Formulated herbal ointment

RESULT AND DISCUSSION

The Moraceae family, which includes *Ficus religiosa*, is found in India's tropical and subtropical regions. The plant is considered to have different therapeutic benefits in various parts.

This study aimed to create and assess a herbal product using the banyan tree's root extract. To determine the phytochemical characteristics of banyan tree roots, the soxhlet extraction method was used to extract the roots using a hydroalcoholic solvent.

Production and assessment of the cream

The ointment was made using the fusion method, which combined the base of the ointment and the herbal medicine essence for stable storage (Figure 1).

Examining the physical and chemical qualities yielded positive results in terms of spreadability, extrudability, washability, solubility, and loss on drying, as shown in Table 2. A three-month stability analysis of the formulation was also conducted at 40 ± 2°C and 70 ± 5% relative humidity. Diffusion studies and irritating effects remain unchanged, and the results were shown in Table 3.

This investigation aimed to enhance the concurrent HPTLC analysis of secondary metabolites in a brand-new banyan root bark ointment. The presence of the components in charge of the bioactivity was confirmed by figuring out the Rf values using HPTLC with detection at 280 nm. Simple topical ointments, separated fractions, and quercetin as a reference

Table 2: Physiochemical parameters of ointment

Parameters	*Inference*
Color	Pale yellow
Odor	Pleasant
Consistence	Semisolid
pH	6.2 ± 0.8
Viscosity	1110 ± 20
Spreadability	7.41 ± 20 sec
Extrudability	Good
Loss on drying	30%
Diffusion study	0.7 cm
Non-irritancy	Non-irritant

Table 3: Accelerated stability studies

Properties	*Storage condition*			
	40 ± 2°C and 70 ± 5% RH			
	1st day	*30th day*	*60th day*	*90th day*
Color	yellow	yellow	Yellow	yellow
Appearance	Homog enous	Homog enous	Homog enous	Homog enous
pH	6.2			± 0.8 6.2 ± 0.6 6.2 ± 0.4 6.2 ± 0.2
Spreadability	7.4	7.4	7.4	7.4

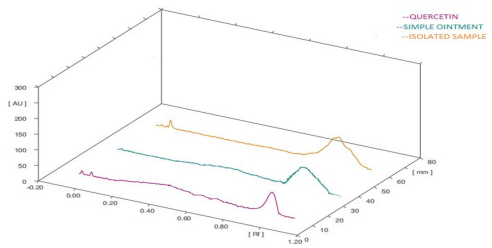

Figure 2: HPTLC densitogram

Table 4: Validation parameters

Method property	*Quercetin*
R_f	0.89
Accuracy study	0.4137
Precision–intraday	0.049
Precision–interday	0.047
Correlation coefficient value	0.997
Calibration range [ng]	200–750
LoD	3.75 ng/mL
LoQ	90 ng/mL
Specificity	Specific
Robustness	Robust

Table 5: Mean of difference in paw thickness

Groups	*15 minute*	*30 minute*	*60 minute*	*120 minute*	*180 minute*
Negative control	1.56 ± 0.01	1.57 ± 0.10	1.31 ± 0.04	1.31 ± 0.03	1.19 ± 0.01
Positive control	1.56 ± 0.04	1.29 ± 0.01	0.61 ± 0.03	0.23 ± 0.04	0.1 ± 0.01
Formulation	1.34 ± 0.18	1.25 ± 0.09	1.07 ± 0.03	0.91 ± 0.02	0.61 ± 0.02

Table 6: Percentage inhibition of paw edema

Groups	*15 minute*	*30 minute*	*60 minute*	*120 minute*	*180 minute*
Negative control	--	--	--	--	--
Positive control (%)	0.077	18.23	52.26	83.21	92.54
Formulation (%)	13.83	20.19	49.65	66.61	68.94

marker for flavonoids were used as samples to evaluate topical formulations.

We can conclude that there was only one significant component in the solution with an Rf value of almost 0.88, which corresponds to the standard quercetin exponent (Rf value was 0.89) since the simple ointment had only one characteristic peak with an Rf value of 0.86. There were no other points on the HPTLC plate (Figure 2). The HPTLC procedure has been approved in accordance with ICH standards. Here, we checked many factors, including accuracy and precision, LoD, LoQ, requirements, robustness, etc. In Table 4, information displayed the values within the acceptable ranges.

Evaluation against Inflammation

The preparation's ability to reduce inflammation was tested using carrageenan-induced edema, and the findings are displayed in Tables 5 and 6. After being treated with the formulation, the animals' feet were measured at various points. Calculating the difference in paw thickness, it was discovered that paw thickness decreased over time. Using one-way ANOVA, mean differences between paws were computed. At 60 minutes, the group that received regular medication treatment (the positive control) had significantly improved. All comparisons were done against a carrageenan-free group or the negative control group.

CONCLUSION

This prepared ointment might be utilized as a media for the medicinal characteristics as a straightforward dosage form because *Ficus religiosa* has been used for its numerous therapeutic properties, such as anti-inflammatory, anti-oxidant, anti-diabetic, and other capabilities since ancient times. A new HPTLC finger printing method was also created to estimate the formulation of ointment. According to ICH criteria, the suggested HPTLC method was validated and confirmed to be precise and focused. Pharmacological research was conducted to determine the prepared ointment's medicinal activity. Results from an *in-vivo* investigation showed that the prepared ointment has better anti-inflammatory action than NSAIDs already on the market.

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