RESEARCH ARTICLE

Formulation and Development of Polyherbal Ointment containing *Clerodendrum serratum, Solanum xanthocarpum*, and *Nyctanthes arbortristis* Extracts and Assessment of Anti-inflammatory Activity in Carrageenan-Induced Paw Edema Model

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ABSTRACT

In this study, our objective was to explore the phytochemical constituents of *Clerodendrum serratum* (CS), *Solanum xanthocarpum* (SX), and *Nyctanthes arbortristis* (NA) and evaluate their anti-inflammatory activity. Subsequently, we aimed to develop a polyherbal ointment formulation incorporating these extracts. The phytochemical screening of the CS extract showed carbohydrates, steroids, terpenoids, flavonoids, saponins, and tannins. Similarly, the SX extract exhibited the presence of all these constituents, along with the additional presence of alkaloids. The NA extract demonstrated the presence of alkaloids, tannins, glycosides, and flavonoids. CS roots exhibited notable inhibition of inflammation at the given doses and periods of 1 to 5 hours, surpassing the effectiveness of the standard anti-inflammatory drug, indomethacin. The polyherbal ointments were found to be greenish, non-transparent, and with acceptable pH. F3 formulation demonstrated excellent viscosity of 1200 cp, making it easy to apply on the skin. Furthermore, formulation F3 demonstrated exceptional spreadability, covering a diameter of 1.8 cm, surpassing the other formulations. Importantly, all polyherbal formulations were non-irritant when applied to the skin, as evidenced by the absence of edema or erythema upon application to rat skin. F3 formulation indicated no physicochemical variations in comparison to initial results in stability studies. Even after the 6-month stability study, the ointment formulation maintained a smooth and greenish consistency, signifying its durability and stability. These findings suggest that the developed polyherbal ointment formulation can be stored at room temperature without any additional requirements. Overall, this polyherbal ointment formulation represents a promising alternative approach to existing topical formulations for the effective treatment of inflammatory conditions.

Keywords: Inflammation, *Clerodendrum serratum*, *Solanum xanthocarpum Nyctanthes arbortristis*, Edema, Ointment, Carrageenan.

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INTRODUCTION

Natural remedies have been essential in treating and preventing human ailments throughout history. The medicinal potential of naturally occurring substances coming from prebiotic, microbial, plant, and animal sources has long piqued people's interest.[1] Initially, these ingredients were often combined with elements of witchcraft or mysticism. However, as time progressed, the efficacy of these treatments became evident, leading to the documentation and development of early herbal medicine.[2,3] In India's cultural legacy, the development of Ayurveda and the reliance on plant-based medicines are firmly rooted. As per the World Health Organization (WHO), nearly 80% of the population in underdeveloped nations depend on traditional medicine to meet their fundamental healthcare needs. Medicinal plants form the cornerstone of almost all traditional medical systems in these regions.[4] About 2000 natural medicines from diverse ancient systems and folkloric practices make up the Indian Materia Medica.[5]

In today's world, people worldwide are increasingly experiencing inflammatory conditions that often result in tissue inflammation. Therefore, the role of plant-based medicines in managing inflammation is of great interest.[6] Inflammatory conditions, including arthritis, IBD, cancer, and the prevention of organ transplant rejection, often require drug treatments,

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sometimes in combination for enhanced effects.[7] However, synthetic drugs used to treat inflammation can have undesirable side effects and are expensive to develop.[8] In contrast, plant-based medicines are believed to be safe, free from adverse effects, readily available, cost-effective, and sourced from nature. Given the undesired side effects of synthetic drugs, there is optimism about discovering bioactive compounds with anti-inflammatory properties from herbal drugs.[9]

Clerodendrum serratum (CS), also known as Bharangi or blue glory vine, is a medicinal plant that has been traditionally used in various systems of medicine for its therapeutic properties.[10] *Solanum xanthocarpum* (SX), commonly known as yellow-fruit nightshade or kantakari, is a medicinal plant that has been traditionally used in Ayurvedic medicine for its various therapeutic properties. It is believed to possess anti-inflammatory activity.[11] *Nyctanthes arbortristis* (NA), commonly known as the night-flowering jasmine or parijat, is a medicinal plant highly valued in traditional medicinal systems due to various pharmacological actions.[12] It is also known to possess anti-inflammatory activity. Research findings suggest that CS, SX, and NA extracts exhibit significant anti-inflammatory effects in various experimental models. These effects include the inhibition of inflammatory mediators and enzymes, reducing cytokines, and attenuating oxidative stress. The active constituents shows the anti-inflammatory potential of these plants have been identified as flavonoids, phenolic compounds, and alkaloids. These compounds are believed to contribute to the plant's ability to modulate inflammatory pathways and provide relief from inflammation-related conditions.[10-12]

Given the diverse pharmacological activities associated with the selected plants, our research aims to investigate the phytochemical constituents and assess the anti-inflammatory activity in the carrageenan-induced paw edema animal model. Additionally, we have developed a novel polyherbal topical ointment incorporating extracts from *C. serratum, S. xanthocarpum*, and *N. arbortristis*. This ointment shows potential for addressing inflammatory conditions like arthritis, rheumatism, and gout.

MATERIALS AND METHODS

Materials

C. serratum, Nyctanthus arbortristis, and *S. xanthocarpum* plants were collected from hilly regions in Akole taluka, dist. Ahmednagar. The Botanical Survey of India, Pune, carried out the authentication and identification of the samples that were gathered. Methanol, ethanol, chloroform, wool fat, hard paraffin, cetostearyl alcohol, white soft paraffin, polyethylene glycol 4000 and 400, Sorbitol mono-oleate, liquid paraffin, white beeswax, Span 60, Tween 60, methyl hydroxyl benzoate and propyl hydroxybenzoate was purchased from Bharat chemicals & Instruments.

Animal Handling and Maintenance

Albino Wistar rats (male or female) weighing 160 to 190 gm were purchased from Aarya Biotech 5th Kamalpusph Apartment Dhule Tal and Dist Dhule 424002. All animal handling was performed as per animal ethical committee guidelines of DCS'S A. R. A. College of Pharmacy, Nagaon, Dhule, Dist. Dhule, Pin-424006 (Regd. No. 1367/PO/Re/S/10/CPCSEA; Dated: 06/09/2017). All test animals were comfortably housed in typical polypropylene cages (three animals per cage), and a typical sanitary environment was maintained at 25 to 28°C up to a 12-hour light/dark cycle. They were also fed a typical pallet diet and given access to drink and food at will until the conclusion of the experiment.

Preparation of Plant Extracts

The roots of all three plants were washed & dried under shade for 7 to 8 days and stored in well-closed containers separately. The dry material was ground into a powder, and the resulting sample was then passed through filter #60. The soxhlet extraction method was used to extract the dried, powdered roots with a suitable solvent (water and methanol) at an appropriate temperature and time. The extract was filtered, and the marc was removed using the same method again until it was completely gone. The resultant extracts were employed for animal research and early phytochemical assessment.

Phytochemical Analysis of Aqueous and Methanolic Extract

Alkaloids, steroids, triterpene, tannin, glycosides, carbohydrates, flavonoids, flavonoids, saponins, proteins, and amino acids were screened from the aqueous and methanolic extract by the qualitative phytochemical study as per standard procedures.

Screening of Anti-inflammatory Activity of All the Extracts

Three groups of six albino Wistar rats each were used as a positive control, standard treatment group, and nine test group (Table 1). The test and positive control groups applied a prepared polyherbal compound of endogenous plant species topically. The standard was indomethacin. Carrageenan (0.1 mL, 1% w/v in normal saline) was injected into the sub-plantar tissue of the right hind paw in all group animals to cause edema.[13] A digital screw gauze was used to measure the linear paw circumference. Measurements of the paw circumference were taken before to edema induction and hourly for 4 hours following induction. The control group received no therapy at all. As a proportion of the volume measured immediately after the irritant injection for each animal, the rise in paw volume after 1, 2, 3, and 4 hours was determined. Table 1 below shows a comprehensive grouping of the animals.

Formulation of Polyherbal Ointment

The polyherbal ointment containing aqueous & methanolic extract fractions of the root of *C. serratum* (CS-AE and CS-ME), methanolic extract fraction of the whole plant of *S. xanthocarpum* (SX-ME), and methanolic extract fraction of bark of *N. arbortristis* (NA-ME) was developed. The formula composition of the ointment is presented in Table 2. Different batches were manufactured with different compositions as mentioned in Table 2.

Table 1: Details of animal grouping, treatment, and number of animals

Sr. No	*Group*	*Treatment*	*Animal*
1	I (Positive control)	Vehicle (1% Sol^n)	6
2	II (Std treatment)	Indomethacin (10 mg/kg)	6
3	III (Test)	Aqueous extract (CS-AE) (200 mg/kg)	6
4		Methanolic extract (CS-ME) (200 mg/kg)	6
5		Chloroform extract (CS-CE) (200 mg/kg)	6
6		Aqueous extract (SX-AE) (200 mg/kg)	6
7		Methanolic extract (SX-ME) (200 mg/kg)	6
8		Chloroform extract (SX-CE) (200 mg/kg)	6
9		Aqueous extract (NA-AE) (200 mg/kg)	6
10		Methanolic extract (NA-ME) (200 mg/kg)	6
11		Chloroform extract (NA-CE) (200 mg/kg)	6

F1 (Hydrocarbon base)

All ingredients as per formula composition were weighed accurately and waxy materials were melted in a beaker under continuous stirring. Weighed quantity of extracts was added to the above-molten mass under continuous stirring and allowed to cool under stirring. The product was transferred to a wide-mouth bottle after cooling.

F2 (Water soluble base) and F3 (Water miscible base)

All ingredients were weighed and melted in a beaker under continuous stirring. A weighed quantity of extracts was added to the above-molten mass under continuous stirring and allowed to cool under stirring. The product was transferred to a wide-mouth bottle after cooling.

F4 (Absorption base)

Cetosteryl alcohol, white beeswax, span 60, and propylparaben were melted in a beaker in a water bath. Tween 60 and methyl hydroxybenzoate were dissolved in distilled water in a separate tumbler and heated in a water bath. The hot aqueous phase and weighed quantity of extracts were added to the hot oil phase with constant stirring until cold. The prepared formulation was filled with mouth bottles and kept for analysis in the future.

Characterization of Polyherbal Formulation

Physical appearance and clarity

Developed polyherbal formulations were assessed for clarity and appearance through a visual examination conducted against a black-and-white background.[14, 15]

pH of the formulation

The polyherbal formulations ' pH was assessed using a digital pH metre. In 100 mL of pure water were used to dissolve a weighed amount of the formulation, which was then let to stand for around two hours. Next, the pH was determined.[14, 15]

Determination of spreadability

The sample excess was placed between two glass slides and squeezed for 5 minutes with a weight of 1000 grams to obtain a consistent thickness to determine its spreadability. The pan was given a weight boost of 240 grams. Recorded was the

Table 2: Formula composition of the polyherbal ointment

Sr. No.	*Ingredients*	*F1 (Hydrocarbon base) gm*	*F2 (Water soluble base) gm*	*F3 (Water miscible base) gm*	*F4 (Absorption base) gm*
1	Wool fat	-	-	-	1.5
2	Hard paraffin	0.9	-	-	1.5
3	Cetostearyl alcohol	1.5	-	4..5	1.5
4	White soft paraffin	23	-	-	19.5
5	PEG 4000	-	10	-	-
6	PEG 400	-	14	-	-
7	White bees wax	0.6	-	0.75	-
8	Span 60	-	-	2.25	-
9	Tween 60	-	-	1.5	-
10	Methyl hydroxyl benzoate	-	-	0.006	-
11	Propyl hydroxybenzoate	-	-	0.006	-
12	Purified water	-	-	16.988	-
13	CS-AE	1	1.5	1	1.5
14	CS-ME	1	1.5	1	1.5
15	SX-ME	1	1.5	1	1.5
16	NA-ME	1	1.5	1	1.5

Table 3: Test performed and phytochemicals present in the extract

Sr. No.	*Chemical constituent*	*Chemical test*	*C. serratum*	*S. xanthocarpum*	*N. arbortristis*
			Methanol extract		
1	Alkaloids	Dragendorff Mayers	-	+	+ +
2	Steroids	Salkowaski Liebermann-burchard	+	+	- -
3	Triterpene	Vanillin-sulphuric acid	+	+	-
4	Tannin	Ferric chloride Dilute nitric acid	+	+	+ +
5	Glycoside	Keller-killani	-	-	+
6	Carbohydrate	Molish Fehling's	+	+	- -
7	Flavonoid	Shinoda Lead acetate	+	+	+ +
8	Saponins	Foam formation	+	+	-
9	Protein	Biuret Millon's	-	-	- -
10	Amino acid	Ninhydrin	-	-	-

Table 4: Comparative anti-inflammatory effects of test extracts on rat paw edema brought on by carrageenan

Groups	*Drug doses*	*Percentage of inflammation at a time (in hours)*					
			1	*2*	*3*	*4*	*5*
I	Carrageenan Control	1% Sol^{n}	34.17 ± 1.6^{c}	81.83 ± 1.2^{c}	113.3 ± 2.12^{c}	130.0 ± 1.87^{c}	146.7 ± 1.82^{c}
II	Indomethacin	10 mg/kg	12.55 ± 1.66^{c}	19.11 ± 1.05^{c}	27.17 ± 1.88^{c}	32.65 ± 2.13^{c}	30.15 ± 1.97^{c}
III	CS-AE	200 mg/kg	26.50 ± 1.32^{c}	38.45 ± 1.1^{c}	68.34 ± 2.75^{c}	67.01 ± 2.75^{c}	55.65 ± 2.55^{c}
IV	CS-ME	200 mg/kg	19.68 ± 1.31^{c}	32.00 ± 2.43^{c}	63.67 ± 1.77^{c}	58.75 ± 2.37^{c}	46.51 ± 2.772^{c}
V	CS-CE	200 mg/kg	36.00 ± 1.44^{c}	78.63 ± 1.75^{c}	108.5 ± 1.38^{c}	127 ± 2.39^{c}	139.8 ± 3.20^{c}
VI	SX-AE	200 mg/kg	37.88 ± 1.44^{c}	77.42 ± 2.0^{c}	101.8 ± 3.80^{c}	128.7 ± 3.50^{c}	126.7 ± 2.80^{c}
VII	SX-ME	200 mg/kg	35.12 ± 1.66^{c}	80.43 ± 1.20^{c}	115.3 ± 2.12^{c}	130.0 ± 1.87^{c}	142.7 ± 1.72^{c}
VIII	SX-CE	200 mg/kg	34.37 ± 1.67^{c}	81.88 ± 1.21^{c}	111.3 ± 2.12^{c}	128.0 ± 1.87^{c}	141.7 ± 1.82^{c}
IX	NA-AE	200 mg/kg	33.17 ± 1.60^{c}	78.73 ± 1.24^{c}	108.3 ± 2.12^{c}	136.0 ± 1.87^{c}	147.9 ± 1.82^{c}
X	NA-ME	200 mg/kg	21.87 ± 1.60^{c}	39.83 ± 1.20^{c}	75.3 ± 2.12^{c}	78.00 ± 1.87^{c}	81.70 ± 1.82^{c}
XI	NA-CE	200 mg/kg	34.17 ± 1.61^{c}	83.83 ± 1.20^{c}	113.3 ± 2.12^{c}	124.0 ± 1.87^{c}	139.7 ± 1.82^{c}

Each value is the mean ± S.E. for 6 rats (n=6), a P < 0.05; b P < 0.01; c P < 0.001 compared with control.

duration it took for the upper glass slide to detach from the lower plate, demonstrating the spreadability (s).[14,15]

S = m * l / t

Were,

m = weight tide to upper slide

l = length moved on the glass slide

t = time taken

Determination of viscosity

Using a Brookfield digital viscometer, the produced formulations' viscosity (measured in cps) was determined in a semi-solid state. A temperature of 30 ± 1°C was used for the measurements.[14,15]

Primary skin irritancy studies

Four groups of six rats each were created for the rats. On the dorsal region of each rat, a 4 cm^2 area was shaved and sterilized with surgical spirit. Formulations F1, F2, F3, and F4 were administered in specific amounts to the targeted areas. After application, the test areas underwent 48 hours of erythema and edema monitoring.[16]

Stability study

The polyherbal formulations were packed in collapsible tubes, and accelerated stability studies were carried out following the guidelines outlined in ICH Q1AR2. The tubes were stored at temperatures of 20 to 25°C and 40 ± 2°C for 6 months. During

Table 5: Physicochemical properties of the ointment

S. No	*Parameter*	*F1*	*F2*	*F3*	*F4*
1	Appearance	Greenish	Greenish	Greenish	Greenish
2	Texture	Slightly rough	Slightly rough	Smooth	Slightly rough
2	Clarity	Not clear	Not clear	Not clear	Not clear
3	pH	5.0	5.2	5.3	5.1
4	Viscosity (CP)	740	815	1200	790
5	Spreadability (Cm)	0.7	1.0	1.8	1.3
6	Skin irritation	No edema, erythema			

Table 6: Comparative stability study results of F3 formulation

Sr. No	*Parameter*	*F3 Polyherbal formulation*		
		Initial	*20-25°C/6M*	*40 ± 2°C/6M*
1	Visual Appearance	Greenish	Greenish	Greenish
2	Texture	Smooth	Smooth	Smooth
2	Clarity	Not clear	Not clear	Not clear
3	pH	5.3	5.5	5.4
4	Viscosity (CP)	1200	1110	1175
5	Spredability (Cm)	1.8	1.6	1.7
6	Skin irritation	No edema, erythema	No edema, erythema	No edema, erythema

this period, the gels were assessed for clarity, pH, consistency, and overall formulation stability was determined.

RESULTS AND DISCUSSION

Phytochemical Screening of *C. serratum*, *S. xanthocarpum,* and *N. arbortristis* Extract

The phytochemical screening of the *C. serratum* extract showed the presence of carbohydrates, steroids and terpenoids, flavonoids, saponins, and tannins. While *S. xanthocarpum* also showed all these constituents, alkaloid is also present in the extract. *N. arbortristis* extract also showed the presence of alkaloids, tannins, glycosides, and flavonoids. The details of the test performed and the phytochemicals present in the extract are presented in Table 3.

Anti-inflammatory Activities of the Extracts

In comparison to the normal untreated animals, the carrageenan-induced inflammation in the control group was significantly reduced. *C. serratum* L. Roots exhibited notable inhibition of inflammation at the given doses and periods of 1, 2, 3, 4, and 5 hours (Table 4), surpassing the effectiveness of the standard anti-inflammatory drug, indomethacin. Particularly, a dose-dependent anti-inflammatory activity was observed with CS at a dosage of 200 mg/kg. Indomethacin at a dose of 10 mg/kg demonstrated significant inhibition of inflammation across all time intervals. This shows that the inhibition of the cyclooxygenase enzyme and consequent reduction of prostaglandin production may be responsible for the methanolic extract's anti-inflammatory effects.[17] Serotonin and/or histamine inhibition may have contributed to the early-study considerable reduction of paw edoema.[17] However, the diminishing inhibition of paw edema at +5 hours indicates the cessation of the drug's effects during that time (Table 4). These findings highlight the potent anti-rheumatic properties of CS roots.

Evaluation of Polyherbal Formulation

The developed polyherbal formulation underwent physicochemical analysis; the findings are shown in Table 5. All the polyherbal formulations exhibited a greenish color and non-transparency due to the presence of plant extracts. Among the formulations, F3 stood out as it possessed a smooth texture attributed to the inclusion of white beeswax, Span 60, and Tween 60.

The other formulations had a slightly rough texture. The pH of the polyherbal formulations ranged from 5.0 to 5.3, indicating good compatibility with the skin and the absence of irritation.[19] Notably, the F3 formulation demonstrated excellent viscosity of 1200 cp, making it easy to apply on the skin. Additionally, F3 exhibited remarkable spreadability, covering a diameter of 1.8 cm, surpassing the other formulations. This high spreadability and minimal spreading time are vital for effective topical drug delivery systems and patient adherence.[20] Importantly, all the polyherbal formulations were found to be non-irritating to the skin, as no signs of edema or erythema were observed after application on rat skin.[21]

Stability Study of Polyherbal Formulation

The stability of the polyherbal ointment was assessed over 6 months at temperatures of 20 to 25°C and 40 ± 2°C. The comparative stability study results are presented in Table 6.

When compared to the preliminary findings, the stability experiments on the optimized formulation (F3) showed no appreciable changes in consistency, pH, or clarity. After the 6-month stability study, the ointment formulation maintained a smooth and greenish consistency. These observations indicate that the developed polyherbal ointment formulation is durable and stable, allowing for room temperature storage without additional storage requirements.

CONCLUSION

The current study concludes the successful development of a polyherbal ointment formulation to effectively treat inflammatory conditions. The anti-inflammatory activity of *C. serratum (CS), S. xanthocarpum (SX),* and *N. arbortristis (NA)* plants is attributed to their active constituents, including flavonoids, phenolic compounds, and alkaloids. These compounds are believed to play a role in modulating inflammatory pathways and providing relief from inflammation-related conditions. The polyherbal ointment formulation also exhibited favorable physicochemical properties necessary for a topical dosage form. It demonstrated stability over 6 months and can be conveniently stored at room temperature without any additional storage requirements.

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