RESEARCH ARTICLE

Development of the Formulation and Evaluation of the Anti-inflammatory Activity of *Vitex negundo* Gel and Latex

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ABSTRACT

Essential oils include *Vitex negundo* Linn. It is used in the treatment of eye diseases, toothache, redness, white spots, splenomegaly, skin ulcers, catarrh, rheumatoid arthritis, gonorrhea and bronchitis. It is also used as a tonic, insect repellent, emulsifier, menstrual aid, antibacterial, antipyretic and antihistamine. Preparations from plant parts of *V. negundo* treat a variety of ailments, including rheumatic diseases, arthritis, gout, cervicitis, inflammatory diseases of the musculoskeletal system, hemorrhoids (thousands), rheumatic pains, sprains and toothaches.

It has been used commercially for miles in various Ayurvedic remedies and ointments to treat wounds, burns, and fungal skin infections. We concluded from in vitro drug delivery studies that latex composed of HPMC polymers facilitates controlled release of drugs over long periods of time, avoids further fluctuations, and reduces treatment costs. This new class of drug delivery is gaining popularity due to the spreadability, adhesion, viscosity and extrudability properties of emulgel, which makes topical application of hydrophobic pills desirable for every near and systemic effect.

Keywords: Anti-inflammatory, *Vitex negundo*, Paw edema.

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INTRODUCTION

Today, all the most eminent pharmaceutical companies, pharmacists, developers and researchers are increasingly turning their attention to human drugs, better drugs in the direction of microbial infections and the display of antibiotic resistance and sales of pathogenic microbial infections. many medicinal plants (Verbenaceae) are commonly called Nirgundi. It is a tall, fragrant shrub and sometimes a slender tree found in most parts of India. The essential oil is composed of *Vitex negundo* Linn. It is used to treat eye diseases, toothache, infections, leukoplakia, splenomegaly, skin ulcers, catarrh, rheumatoid arthritis, gonorrhea and bronchitis. It is also used as a tonic, insect repellent, galactagogue, menstrual agent, antibacterial, antipyretic and antihistamine.

The oil obtained from it is suitable for the sinuses and scrofula. Its extract also showed anticancer effects against Ehrlich's ascites tumor cells.

Preparations of plant parts of *V. negundo* treat a variety of ailments, including rheumatism, arthritis, gout, inflammation of the cervix, inflammation of the musculoskeletal system, hemorrhoids (cuticles), rheumatic pain, sprains and toothache. It is widely used commercially in various Ayurvedic medicines and ointments for wounds, burns and fungal skin infections.

MATERIALS AND METHODS

Nirgundi Oil is prepared from Anuradha College of Pharmacy Chikuri, Burdana and Tween 20 and 80, Span 20 and 80, macrogol 200, 400, 600 and 800, propylene glycol, poloxamer 188 and 407, methanol, ethanol, methanol, hydrogen phosphate, gum, HPMC, Gellan gum, Ens. Available from the Anuradha College of Pharmacy Relief Department (Chikuri, Burdana, MS). (India) Related.

EXPERIMENTAL

Extraction of Oil from Leaves

Instructions for *V. negundo* ethanol extract. The glitter sheets were carefully selected and washed in the current study to remove impurities. Approximately 100 g of fresh leaf tissue

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is extracted by thermal extraction using a soxhlet extraction apparatus using 60% alcohol as the solvent. Extraction continues until the solvent in the cannula becomes clear, then a few drops of solvent collect in the control tube when the cycle is complete and a solvent chemical check is complete. After each extraction, the extract is evaporated to dryness on a rotary evaporator under vacuum.

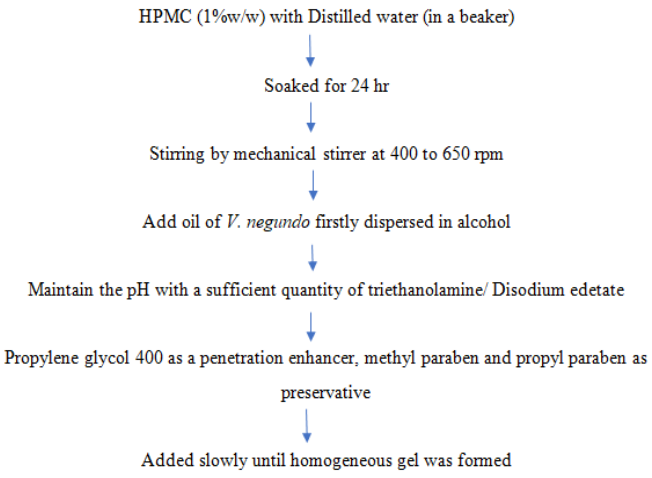
Additionally, part of the extract was saved for initial phytochemical screening to detect various botanical constituents and the final extract was used to formulate a batch of gels.

Formulation and Development of Gel

During formulation, 4 different batches of leaf extract gels were generated using different concentrations of gelling agents, for a total of four batches. In this case, use a HPMC K 100 M type gelling agent. The gelling agent is used as follows. HPMC K 100M (1 and 1.5% concentrations) The gel formulation was perfected through trial and error.

And list the completed configuration here. All groups are formed according to the test plan.

Preparation of Gel by using the cold method



In-vitro Drug Release Studies

All gel formulations were subjected to *in-vitro* diffusion studies. The use of a Franz diffusion cell setup pursued this study. HPMC and oil concentrations varied in all formulations. G2 at 36°C. G3.

The G4 formulation is stable but not very thick, while the G1 formulation has the best viscosity and consistency.

Optimization

Optimize batches for each batch formulation's pH, viscosity, dispersion, and extrusion through testing and physical evaluation. By checking the assessment parameters for all groups.

Preparation of Emulgel

For the preparation of Emugel various surfactants and co-surfactants were used for solubility studies are listed in Table 2.

Drug-excipients Compatibility Study

Water insoluble tablets' physical and chemical compatibility with oils, wetting agents and co-surfactants should adopt the selection method of internal oils, wetting agents and co-surfactants. Physical compatibility includes observed phase separation and color exchange of surfactant solutions. In most cases, chemical compatibility is considered the chemical balance of the drug with oils, surfactants, and co-surfactants. Further improvements will be considered only when the oil, surfactant, and co-surfactant are physically and chemically well-suited to the drug. After the study, a drug compatibility study should be considered.

Formulation of Emulgel

For the preparation of emugel concentration surfactants and co-surfactants are used which is described in Table 3.

In this process, a surfactant is mixed with a co-surfactant in a fixed weight ratio. H. Emulgel is formulated in a 3:1 ratio. A portion of each surfactant/cosurfactant mixture (S_{mix}) was then mixed with the oil in a bottle at room temperature. The drug is then added to these oil mixtures.

In each phase diagram, the oil to mixture ratios are 9:1, 8:2, 6:3, 6:4, 5:5, 4:6, 3:6, 2:8, and 1:9. (% of /v) changes. Distilled water was added dropwise to each oil mixture with vigorous stirring. Add the appropriate concentration HPMC gelling agent in the formula mentioned above. After equilibration, visually inspect the sample and determine if it is a clear emulsifier, lotion or gel.

In-vitro Drug Release Studies

In-vitro drug release studies were performed using a modified vertical Franz diffusion cell (effective diffusion area 1.44 cm^2, cell volume 15.5 mL). Apply the formulation to a 0.45 µm nylon membrane (soaked in phosphate buffer, pH 6) at 2°C. Place the cells in a water bath. A magnetic stirrer maintains the composition, stirring the solution continuously at 50 rpm using magnetic beads. Samples (1.0 mL each) were taken at appropriate time intervals, diluted appropriately and analyzed for drug content using a 321 nm UV-vis spectrophotometer.

Anti-inflammatory Evaluation

The study was approved by the Institutional Ethics Committee of CPCSEA (Animal Experimentation Control and Review Board; Ref. ATI/CPCSEA/0046-2013) (ref. 722/PO/a/ CPCSEA/ IAEC/EXP-46). It's done. House an ICR mouse (male or female) in a polypropylene cage at 24 ± 2°C and leave food and water ad libitum for 1 week.

Before the treatment, they fasted him for 19 hours and gave him free access to water. Selected plants known for their pharmacological activity. Pharmacological evaluations have been reported using methanolic extracts of the four plants with no reported toxicity.

Grouping of Mice

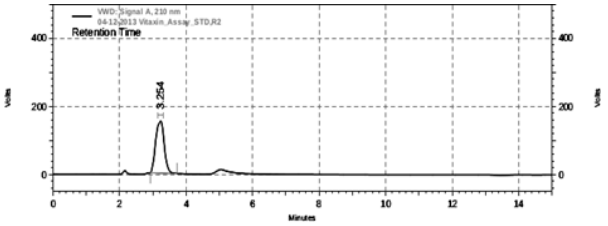
All the mice were divided equally into 10 groups of 6 each, as follows:

Table: 1 List of chemical

S. No	*Compound Name*	*Manufacture and Supplier*
	Drug	
	Nirgundi oil	ACP Chikhli, Buldana, (India)
	Oil	
	Nirgundi oil	ACP Chikhli, Buldana, (India)
	Surfactant	
	Tween-20 and 80, Span-20 Poloxamer-188 and 407	ACP Chikhli, Buldana, (India)
	Co-Surfactant	
	Propylene glycol Polyethylene glycol-200,400,600 Ethanol	ACP Chikhli, Buldana, (India)
	Gum	
	HPMC Gellan gum Xanthan gum Alginate	ACP Chikhli, Buldana, (India)
	Solvent	
	Distill water Ethanol, methanol, acetone, acetonitrile	ACP Chikhli, Buldana, (India) ACP Chikhli, Buldana, (India)
	Other Material	
	NaCl, $CaCl_2$, KCl Disodium hydrogen phosphate Sodium dihydrogen phosphate Sodium lactate, Citric acid	ACP Chikhli, Buldana, (India)

Table 2: List of oil, surfactant, and co-surfactant used for solubility study

S. No.	*Vehicles*	*S. No.*	*Vehicles*
1	Nirgundi oil	5	Propylene glycol
2	Isopropyl myristate	6	Polyethylene glycol-600
3	Tween-80	6	Span-20
4	Tween-20	8	Ethanol



VWD: Signal A, 210 nm Results

Pk #	Name	Retention Time	Area	Area %
1	Vitexin	3.254	45541252	100.00

Figure 1: HPLC Chromatogram of Vitexin having mobile phase (methanol : water ; 70:30)

- - ve control – i.e., treated with only vehicle (1% gum acacia in H_2O)
- + ve control – i.e., treated with standard drug in 1% gum acacia (H_2O).
- 250 mg/Kg meth. extract of *L. aspera* in 1% of gum acacia (in H_2O).
- 500 mg/kg meth. extract of *L. aspera* in 1% of gum acacia (in H_2O).
- 250 mg/Kg meth. extract of *L. nodiflora* in 1% of gum acacia (in H_2O).
- 500 mg/kg meth. extract of *L. nodiflora* in 1% of gum acacia (in H_2O).
- 250 mg/Kg meth. extract of *M. alba* in 1% gum acacia (H_2O).
- 500 mg/kg meth. extract of *M. alba* in 1% of gum acacia (in H_2O).
- 250 mg/Kg meth. extract of *N. indicum* in 1% gum acacia (H_2O).
- 500 mg/kg meth. extract of *N. indicum* in 1% of gum acacia in H_2O xylene-induced ear edema, were used.

Xylene-induced Ear Edema Method

This is a "subacute" model of inflammation. Indomethacin (10 mg/kg) was used as standard drug. Here are the protocols I used:

Table 3: Different concentrations of co-surfactant and surfactant, S_{mix} ratio

S. No.	*Volume of surfactant (mL)*	*Volume of co-surfactant (mL)*	*Ratio of S_{mix}*
1	65	25	3:1

Table 4: Extraction of oil from Nirgundi leaves

Solvent	*Plant part*	*Color*	*%yield*	*Alkal-oids*	*Glyco iss sides*	*Carbo/hydrates*	*Tannins %phenolic compounds*	*Flav noids*
Ethanol	leaf	grey	0.0456	+	-	-	+	+
Methan ol	leaf	blackish grey	0.0763	-	+	-	+	+

Table 5: Preparation of Gel by using cold method

Ingredient	*G1*	*G2*	*G3*	*G4*
Nirgundi oil (mL)	1	2	3	4
HPMC (gm)	0.25	0.25	0.30	0.40
Propylene glycol 400 (mL)	2.5	2.5	2.5	2.5
Methyl Paraben (gm)	0.15	0.15	0.15	0.15
Propyl Paraben (gm)	0.15	0.15	0.15	0.15
Water (mL)	3	3	3	3
Triethanolamine	QS	QS	QS	QS

Table 6: *In-vitro* Drug Release of gel formulation

S. No.	*Time (min)*	*%drug release, Mean ± SD, n=3*			
		G1	*G2*	*G3*	*G4*
1	0	0	0	0	0
2	15	0.43 ± 0.03	0.00	0.00	0.61 ± 0.02
3	30	6.55 ± 0.01	4.352 ± 0.01	3.26 ± 0.10	3.45 ± 0.03
4	60	13.22 ± 0.01	12.04 ± 0.78	11.07 ± 0.30	15.26 ± 0.04
5	90	23.16 ± 0.02	21.15 ± 0.56	20.21 ± 0.23	21.52 ± 0.05
6	120	36.37 ± 0.02	34.23 ± 0.02	31.05 ± 0.20	31.45 ± 0.48
7	150	48.78 ± 0.04	44.42 ± 0.78	43.28 ± 0.01	40.43 ± 0.63
8	180	62.12 ± 0.05	59.44 ± 0.54	55.54 ± 0.21	53.22 ± 0.20
9	210	70.26 ± 0.01	66.27 ± 0.68	65.32 ± 0.10	57.68 ± 0.98
10	240	76.64 ± 0.01	74.12 ± 0.51	75.16 ± 0.30	67.70 ± 0.58
11	270	84.18 ± 0.01	80.73 ± 0.20	81.44 ± 0.25	73.25 ± 0.01
12	300	92.13 ± 0.01	87.47 ± 0.14	84.45 ± 0.01	79.67 ± 0.03

Table 7: Composition of Nirgundi oil, Tween 80, PEG 400 and distilled water at 3:1

Smix ratio of Emulgel formulation

S. No.	*Ratio (O:S) **	*The volume of different components in the formulation*			*composition*	*Observation*
		Oil (mL)	*S mix (mL)*	*Water (mL)*	*HPMC*	
1	1:9	0.25	2.25	1.4	0.25	Emulgel
2	2:8	0.5	2	2.5	0.25	Emulgel
3	3:7	0.75	1.75	3.5	0.25	Emulgel
4	4:6	1	1.50	5	0.25	Emulgel
5	5:5	1.25	1.25	5.5	0.25	Emulgel
6	6:4	1.50	1	-	-	NO
7	7:3	1.75	0.75	-	-	NO
8	8:2	2	0.5	-	-	NO
9	9:1	2.25	0.25	-	-	NO

*O: S:-oil: S_{mix} ratio (S_{mix} ratio:-surfactant: co-surfactant)

- Mice were dosed orally in each group.
- One hour later, each animal received 30 µL of xylene using a micropipette anteriorly and posteriorly to the left ear. Use the right ear as a control.
- Ear thickness was measured at 1, 2, 3 and 4 hours intervals with a micrometer gauge.
- Percent ear edema was calculated using the following formula

$$\%\text{of E.A.} = \frac{\text{Thickness of L.E. - thickness of R.E.} \times 100}{\text{Thickness of R.E.}}$$

E.A. = Ear Edema; L.E. = Left year; R.E. = Right ear

Table 8: *In-vitro* drug release studies of emulgel formulation

S. No.	*Time (min)*	*Percent drug release, Mean ± SD, n=3*				
		EG1	*EG2*	*EG3*	*EG4*	*EG5*
	0	0	0	0	0	0
	15	2.63 ± 0.01	1.75 ± 0.02	1.22 ± 0.10	0	0.41 ± 0.03
	30	10.15 ± 0.02	7.02 ± 0.02	3.71 ± 0.12	3.212 ± 0.04	6.56 ± 0.01
	60	19.21 ± 0.01	17.26 ± 0.20	14.24 ± 0.12	11.24 ± 0.02	13.35 ± 0.01
	90	32.68 ± 0.01	28.12 ± 0.01	22.35 ± 0.10	20.17 ± 0.03	23.12 ± 0.02
	120	42.51 ± 0.12	39.43 ± 0.01	29.76 ± 0.01	25.55 ± 0.05	36.26 ± 0.02
	150	51.89 ± 0.21	46.08 ± 0.10	43.13 ± 0.02	37.93 ± 0.01	48.57 ± 0.04
	180	64.76 ± 0.14	55.21 ± 0.02	51.21 ± 0.01	44.45 ± 0.01	62.63 ± 0.05
	210	74.85 ± 0.15	62.86 ± 0.03	60.96 ± 0.02	53.56 ± 0.02	70.26 ± 0.01
	240	80.85 ± 0.01	71.95 ± 0.12	70.75 ± 0.03	61.50 ± 0.02	76.66 ± 0.01
	270	90.45 ± 0.01	79.02 ± 0.48	75.47 ± 0.01	69.65 ± 0.03	84.18 ± 0.01
	300	96.54 ± 0.01	90.76 ± 0.40	81.92 ± 0.01	76.34 ± 0.02	92.15 ± 0.01

Table 9: Comparison of Gel and Emulgel formulation

Parameter	*pH*	*Viscosity*	*Spreadability*	*Extrudability*	*Drug content*	*In- diffusion study*
G1	5.12	42600	27	81.11	99.54 ± 0.02	92.54 ± 0.01
EG1	5.01	42500	30.21 ± 03	-	103.72 ± 0.01	96.75 ± 0.01

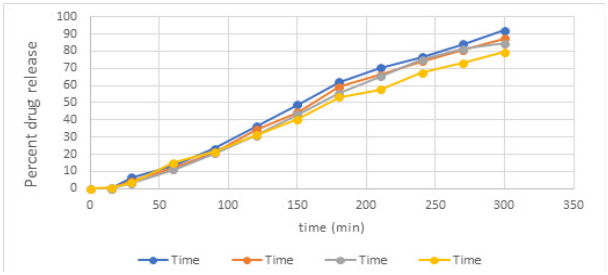


Figure 2: *In-vitro* drug release of gel formulation

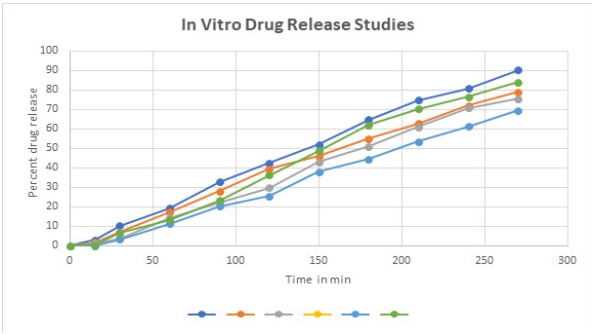


Figure 3: *In-vitro* drug release studies of emulgel formulation

Formalin-induced Paw Edema/Arthritis

Is an "acute" inflammation model. Aspirin (10 mg/kg) was used as standard. Here are the protocols I used:

- Mice are orally dosed with drug/test compound/vehicle.
- After 24 hours, paw volumes and joint diameters were measured, 30 minutes later, mice were rechallenged with test drug/compound.

Thirty minutes later, 20 µL of freshly prepared 2% formalin (FA) was injected into the right hindpaw.

- Paw volume was measured at 1, 2, 3, and 4 hours using a laboratory-built rig (Figure 3).
- Paw edema percentage was calculated using the following formula.

% P.E. = P.V. after 1-hour of F.A. injection- P.V. before 1-hour of F.A. injection x 100 P.V. before 1h of the F.A. injection

P.E. = Paw edema; F.A. = Formaldehyde; P.V. =Paw Volume

RESULTS AND DISCUSSION

Firstly, the leaves of Nirgundi were extracted for the oil using various solvents shown in Table 4.

HPLC Chromatograph of Vitexin

HPLC Chromatogram of vitexin having mobile phase (methanol : water ; 70:30).

Preparation of Gel by using Cold Method

The gel was prepared using the cold method. Various components were used at different concentrations for different formulations as shown in Table 5.

In-vitro Drug Release Studies

In vitro drug release studies are shown in Table 6, Figure 2.

All the gel formulations have been subjected to *in-vitro* diffusion studies. This study was performed using a Franz diffusion cell setup. HPMC and oil concentrations varied in all formulations. G2 at 37°C. G3.

The G4 formula is stable, but not very consistent. However, the G1 formula has the best viscosity and consistency. The release rate of preparation G1 was 92.14%, while the release rate of preparation G2 was 87.48% and the release rate of preparation G3 was 84, 35 and G4 79.57%. Therefore, the G1 formulation was chosen for further study.

Formulation of Emulgel

Formulation of 3:1 S_{mix} ratio Emulgel
(Table 7).

In-vitro Drug Release Studies (Table 8), (Figure 3)

The *in-vitro* release profiles of nirgendi oil from various emulsified formulations are shown above. All of its Emulgel formulations have been observed to exhibit superior drug release compared to standard gels formulated according to USP. Prepared and had 55.67% drug release at 6 hours. For Nirgundi-Emulgel formulations, drug release can be ranked in descending order: EG1 > EG2 > EG3 > EG4 > EG5, levels of drug release after 6 hours. 96.55, 90.78, 81.90, 76.35, 92.14%, but drug release occurred from emulsion-based formulations.

Comparison of Gel and Emulgel Formulation (Table 9)

Gels and latexes were optimized based on the above data by evaluating various parameters. In this gel formulation, G1 exhibited good pH, viscosity, spreadability, extrudability, drug loading, and drug release *in-vitro*. In this latex formulation, lot EG1 exhibited good pH, diffusion coefficient, good swelling index, viscosity, bioadhesive force, drug content and in vitro drug release. Comparison of optimized gel and latex formulations. Emulgel showed superior drug content (103.62 ± 0.01) compared to freezing (99.64 ± 0.02). *In-vitro* drug release from HPMC-based latex has been shown (96.55 ± 0.01) and the *in-vitro* drug release of the HPMC-based gel (92.14 ± 0.01) showed the maximum drug release at 6 hours compared to the gel formulation. Emulgel provides maximum therapeutic effect in the shortest time compared to HPMC-based gel formulations. Results of anti-inflammatory activity for the methanolic extracts for formaldehyde method, and results for the anti-inflammatory activity of methanolic extracts using CFA method.

SUMMARY AND CONCLUSION

A thorough investigation concluded that topical gels made from HPMC polymers possess excellent extensibility, extrudability, and bio-adhesive strength. Excellent for making topical preparations. Emulgel (EG1) refers to topical gels made of natural polymers that swell more easily with a higher swelling index (96.67%) compared to other properties.

Emulgel shows superior potency (103.62 ± 0.01) compared to Gel (99.64%). The *in-vitro* drug release of HPMC-based emulgel shows (96.55 ± 0.01) and the *in-vitro* drug release of HPMC-based gel (92.14 ± 0.01) shows the maximum drug release at 6 hours compared to the gel formulation. From the results it can also be concluded that *V. negundo*'s Emugel formulation showed a better anti-inflammatory effect than the gel formulation.

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