RESEARCH ARTICLE

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

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ABSTRACT

The important oil is *Vitex negundo* Linn. It is used in the treatment of eye diseases, toothache, inflammation, 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 plant component arrangements of *V. negundo*, are used commercially in a variety of Ayurvedic medicines and ointments for the treatment of a variety of ailments including rheumatism, arthritis, gout, cervical spondylitis, inflammatory musculoskeletal, hemorrhoids (hemorrhoids), painful wounds, painful wounds. wounds, painful sores, burns and fungal infections of the skin.

From the in vitro diffusion of drugs, we concluded that Emulgel composed of HPMC polymers can control drug secretion for a long time, which helps to prevent more fluctuation and also reduces the cost of treatment. Due to the advantages of latex for improved spreadability, adhesion, viscosity, and extrudability, this new type of drug delivery is gaining popularity, besides systemic action, the local topical utility of hydrophobic dispensers is also of interest.

Keywords: *Vitex negundo* Linn, Hemorrhoids, Cervical spondylitis.

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INTRODUCTION

Today, almost all the most prominent pharmaceutical companies, pharmacists, research and improvement companies and researchers are increasingly turning to folk remedies. Coupled with the resistance of pathogenic microbial infectious agents to antibiotics, better drug treatment against microbial infections has been studied with many medicinal plants (Verbenaceae), commonly known as nirgundi.[1-4] It is a tall, fragrant shrub, sometimes a slender tree, found in most parts of India.[5-7] The essential oil contains *Vitex negundo* Linn. It is used in the treatment of eye diseases, toothaches, irritations, leukoplakia, enlarged spleen, skin ulcers, catarrh, rheumatoid arthritis, gonorrhea and bronchitis.[7-10]

Preparations of plant parts of *V. negundo* are used commercially in various Ayurvedic medicines and ointments to treat various conditions, including rheumatic diseases, arthritis, gout, cervical spondylitis, inflammatory musculoskeletal problems, hemorrhoids (batteries), rheumatism, sprains, toothaches, wounds, burns and fungal contamination of the skin.[10,11]

MATERIALS AND METHODS

Nirgundi Oil is manufactured by 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, Methanone, Acetone Ketone, composition. Hydrogen phosphate, gum, HPMC, gellan gum, etc. was obtained from the filing department of Anuradha College of Pharmacy (Chikuri, Burdana, MS). (India) Related. List of other components used is mentioned in Table 1.[11-13]

EXPERIMENTAL

Extraction of Oil from Leaves

Manufacture of ethanolic extract of *V. negundo*. It now appears that the fresh leaves are carefully selected and washed to remove impurities. Approximately 100 gm of scintillating leaf tissue is extracted by thermal extraction using a soxhlet extraction device using 60% alcohol as the solvent. Continue extraction until the solvent in the cartridge becomes clear, then collect a few drops of solvent in the test tube at the end of the

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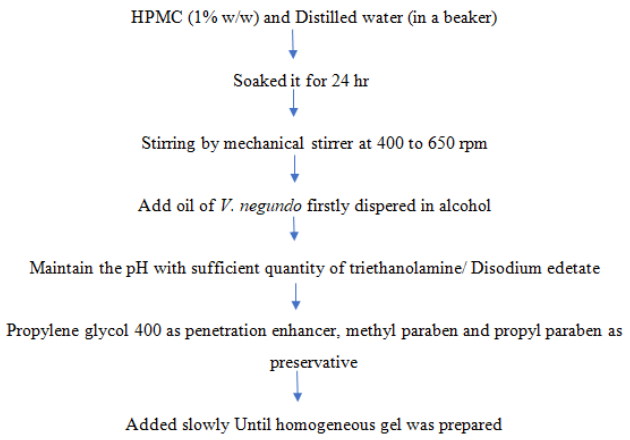
run and perform a chemical test on the solvent. After each extraction, the extract is evaporated to dryness on a rotary evaporator under a vacuum.

Additionally, part of the extract was saved for the initial phytochemical screening to discover many parts of the plant, and the extract's relaxation was used to formulate the gel base.[14-18]

Formulation and Development of Gel

During formulation, four different batches of leaf extract gels were generated using different concentrations of gelling agent, for a total of four batches. In this case, use a gelling agent of the HPMC K 100 M type. The gelling agent is used as follows. HPMC K 100M (1 and 1.5% concentrations). The gel formulation was perfected through trial and error.[19,20]

Preparation of Gel by using Cold Method[21]



In-vitro Drug Release Studies

All 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 36°C. G3.[22]

Optimization[22]

Optimize batches for pH, viscosity, dispersion and extrusion by testing and verifying the physical evaluation of all formulated batches. 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

The physical and chemical compatibility of water-insoluble tablets with oils, wetting agents and co-surfactants should be considered when selecting oils, wetting agents and co-surfactants. Physical compatibility includes fragment segregation and color alternation for the wetting agent-drug response under study. In most cases, chemical compatibility is considered the chemical stability of the drug with oils, surfactants, and co-surfactants. Oils, surfactants and co-surfactants were considered most effective for further development if they were physically and chemically compatible with the reactants. Compatibility studies of pharmaceutical excipients should be considered after the study.[23]

Formulation of Emulgel

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

In this process, surfactant and co-surfactant are combined in a fixed weight ratio. *H. Emulgel* has a three:1 formula. A portion of each wetting agent/surfactant combination (S_{mix}) was then mixed with oil in bottles at room temperature. The tablets are then delivered in these blends of oils.

In-vitro Drug Release Studies

Studies were performed by the use of modified vertical franz diffusion cell (1.44 cm^2 effective diffusion environment and 15.5 mL cell span). The system is applied to a 0.55 µm nylon membrane (pre-soaked in phosphate-buffered saline, pH 6. 4 for 24 hours). Embedded between the donor and acceptor compartments where Franz diffusion moves. A phosphate buffer pH 6.4 + ethanol (80:20) was taken as the medium for dissolution.

Anti-arthritic Evaluation

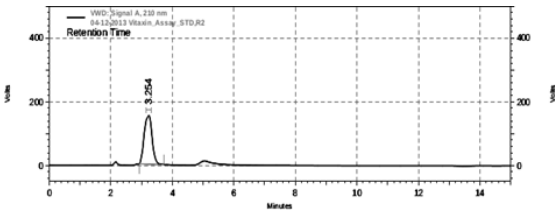
Viewing is authorized by the CPCSEA (Committee on Animal Research Administration and Oversight; Ref. ARTI/CPCSDA/0048-2017) Institutional Ethics Committee (Ref. 732/PA/a/CPCCEA/IAEX/EXP-48). ICR small Mice (male or female) were housed in cages with free of polypropylene with an access to water and food for 1 week at 24 ± 2**°C.**

Before the treatment, they made him fast for 19 hours and allowed him to drink water as much as he wanted. Selected flowers have been recognized for their pharmacological interest. Methanol extracts of the 4 plants are recommended for pharmacological evaluations rather than suggested toxicity.

Grouping of Mice

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

- -ve manage- handled with most effective automobile (1% gum acacia in H_2O)
- + ve manipulate- handled with the preferred drug in 1% of gum acacia (in H_2O).
- 250 mg/Kg meth. extract of *L. aspera* in 1% of gum acacia (in H_2O).



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).

Table 1: List of chemicals

S. No	*Compound Name*	*Manufacture and Supplier*
1.	DRUG	
2.	Nirgundi oil	ACP Chikhli, Buldana, (India)
3.	OIL	
4.	Nirgundi oil	ACP Chikhli, Buldana, (India)
5.	SURFACTANT	
6.	Tween-20 and 80,Span-20	ACP Chikhli, Buldana, (India)
7.	Poloxamer-188 and 407	
8.	CO-SURFACTANT	
9.	Propylene Glycol	
10.	Polyethylene Glycol-200,400,600	ACP Chikhli, Buldana, (India)
11.	Ethanol	
12.	GUM	
13.	HPMC	
14.	Gellan Gum	ACP Chikhli, Buldana, (India)
15.	Xanthan Gum	
16.	Alginate	
17.	SOLVENT	
18.	Distilled Water	ACP Chikhli, Buldana, (India)
19.	Ethanol, Methanol, Acetone, Acetonitrile	ACP Chikhli, Buldana, (India)
20.	OTHER MATERIAL	
21.	Nacl, Cacl2, kcl	
22.	Disodium Hydrogen Phosphate	ACP Chikhli, Buldana, (India)
23.	Sodium Dihydrogen Phosphate	
24	Sodium Lactate,Citric Acid	

Table 2: List of the oil, Co-surfactant, and surfactant used for the 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

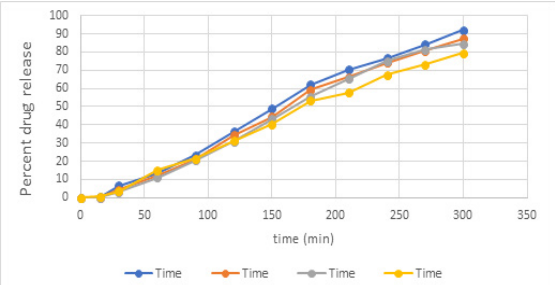


Figure 2: *In-vitro* drug-release studies for gel formulation.

- 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).

Table 3: Various concentrations of Co-surfactant and surfactant opted for stock S_{mix} ratio

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

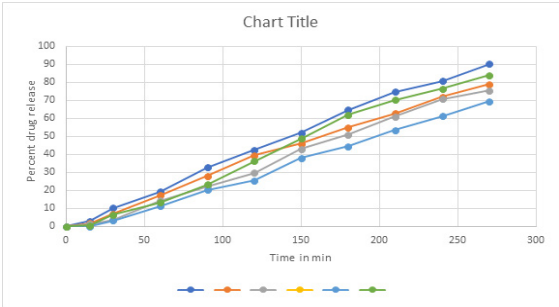


Figure 3: *In-vitro* drug release studies of emulgel formulation.

- 250 mg/Kg meth. extract of *M. alba* in 1% gum acacia (H_2O).
- 500 mg/kg meth. extract of *M. alba* in 1% gum acacia (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 water Xylene induced ear edema.

Table 4: Extraction of oil from Nirgundi leaves

Solvent	*Plant part*	*Colour*	*%Yield*	*Alkal-oids*	*Glyco-Sides*	*Carbo-hydrates*	*Tannins % phenolic compounds*	*Flav-noids*
Ethanol	leaf	grey	0.0486	+	-	-	+	+
methanol	leaf	blackish grey	0.0791	-	+	-	+	+

Table 5: Preparation of Gel by using cold method

Ingredient	*G1*	*G2*	*G3*	*G4*
Nirgundi oil (mL)	1	2	3	4
HPMC (gms)	0.250	0.250	0.300	0.400
Propylene glycol 400 (mL)	2.50	2.50	2.50	2.5
Methyl paraben (gms)	0.150	0.150	0.150	0.150
Propyl paraben (gms)	0.150	0.150	0.150	0.150
Water (mL)	3	3	3	3
Triethanolamine	q.s.	q.s.	q.s.	q.s.

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

S. No.	*Time (min)*	*Percent 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	0.62 ± 0.02
3	30	6.55 ± 0.01	4.352 ± 0.01	3.17 ± 0.10	3.37 ± 0.03
4	60	13.24 ± 0.01	12.04 ± 0.78	11.09 ± 0.30	15.19 ± 0.04
5	90	23.15 ± 0.02	21.23 ± 0.56	20.31 ± 0.23	21.60 ± 0.05
6	120	36.34 ± 0.02	34.28 ± 0.02	31.10 ± 0.20	31.23 ± 0.48
7	150	48.76 ± 0.04	44.51 ± 0.78	43.28 ± 0.01	40.53 ± 0.63
8	180	62.12 ± 0.05	59.42 ± 0.54	55.54 ± 0.21	53.62 ± 0.20
9	210	70.23 ± 0.01	66.67 ± 0.68	65.50 ± 0.10	57.48 ± 0.98
10	240	76.69 ± 0.01	74.31 ± 0.51	75.27 ± 0.30	67.30 ± 0.58
11	270	84.08 ± 0.01	80.89 ± 0.20	81.59 ± 0.25	73.15 ± 0.01
12	300	92.16 ± 0.01	87.56 ± 0.14	84.64 ± 0.01	79.67 ± 0.03

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

S_{mix} ratio of Emulgel formulation						
S. No.	*Ratio (O:S)**	*Volume of different components in formulation*			*Composition*	*Observation*
		Oil (mL)	*S_{mix} (mL)*	*Water (mL)*	*HPMC*	
1	1:9	0.250	2.250	1.40	0.250	Emulgel
2	2:8	0.50	2	2.50	0.250	Emulgel
3	3:7	0.750	1.750	3.50	0.250	Emulgel
4	4:6	1	1.500	5	0.250	Emulgel
5	5:5	1.250	1.250	5.50	0.250	Emulgel
6	6:4	1.500	1	-	-	NO
7	7:3	1.750	0.750	-	-	NO
8	8:2	2	0.50	-	-	NO
9	9:1	2.250	0.250	-	-	NO

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

Formalin- induced Paw Edema/Arthritis

This is by far the "acute" version of inflammation. Aspirin (10 mg/Kg) is used as medicine. The protocol used is as follows.

- Control/Motor drugs/Compounds orally administered to mice.
- Measure number of legs and joint diameter after 24 hours. After 30 minutes, mice again received drug/test compound.
- After 30 minutes, inject 20 µL of freshly prepared 2% formalin (FA) into the right hind paw.
- Measure paw volume with lab setup after 1, 2, 3, and 4 hours.

Table 8: Drug-Release Studies of the emulgel formulation (*In-vitro*)

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	1.76 ± 0.02	1.31 ± 0.10	0	0.52 ± 0.03
	30	10.24 ± 0.02	7.15 ± 0.02	3.80 ± 0.12	3.22 ± 0.04	6.67 ± 0.01
	60	19.63 ± 0.01	17.37 ± 0.20	14.45 ± 0.12	11.24 ± 0.02	13.35 ± 0.01
	90	32.64 ± 0.01	28.21 ± 0.01	22.54 ± 0.10	20.28 ± 0.03	23.28 ± 0.02
	120	42.70 ± 0.12	39.31 ± 0.01	29.57 ± 0.01	25.49 ± 0.05	36.46 ± 0.02
	150	51.49 ± 0.21	46.11 ± 0.10	43.22 ± 0.02	37.82 ± 0.01	48.57 ± 0.04
	180	64.74 ± 0.14	55.21 ± 0.02	51.21 ± 0.01	44.66 ± 0.01	62.23 ± 0.05
	210	74.59 ± 0.15	62.71 ± 0.03	60.77 ± 0.02	53.63 ± 0.02	70.39 ± 0.01
	240	80.29 ± 0.01	71.54 ± 0.12	70.88 ± 0.03	61.30 ± 0.02	76.45 ± 0.01
	270	90.63 ± 0.01	79.10 ± 0.48	75.68 ± 0.01	69.78 ± 0.03	84.18 ± 0.01
	300	96.75 ± 0.01	90.68 ± 0.40	81.40 ± 0.01	76.65 ± 0.02	92.34 ± 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	99.44 ± 0.02	92.24 ± 0.01
EG1	5.01	42500	30.41 ± 03	-	103.52 ± 0.01	96.65 ± 0.01

Table 10: Anti-arthritic activity for the methanolic extracts (Formaldehyde method)

Treatment type	*Doseage (mg/kg)*	*Joint diameter (mm)*		
		8th Day	*9th Day*	*10th Day*
Control	-	0.3865 ± 0.02384	0.365 ± 0.00545	0.345 ± 0.0263
Aspirin	10	0.2475 ± 0.0135*	0.2335 ± 0.01281***	0.225 ± 0.01433***
V. negundo gel	250	0.3525 ± 0.0025**	0.285 ± 0.00269*	0.235 ± 0.0129**
V. negundo gel	500	0.224 ± 0.0129*	0.19 ± 0.03289***	0.1525 ± 0.02494***
V. negundo emugel	250	0.34 ± 0.03636*	0.3345 ± 0.0025**	0.3375 ± 0.02584***
V. negundo emugel	500	0.2 ± 0.02141***	0.176 ± 0.01543**	0.1635 ± 0.0135***

Values given are in mean ± SE, n = 6, *p< 0.05, **p< 0.01, ***p< 0.001 vs. control.

- Calculate the percent paw edema using the following system.

%PE = PV 1 hour after FA injection - PV 1 hour before injection FA1 Hours injection FA x 100 PV.

PE = paw oedema; FA = formaldehyde; PV = volume of the paw.

- Duration of administration extended to 10 days.
- On day 3, 20 **µ**L of formalin was administered to the right hind paw.
- The diameter of the joint of the right hind paw was measured on days 8, 9 and 10, 30 minutes after administration.

Complete edema/arthritis induced by Freund's adjuvant in rats

This is an immunogenicity model. Diclofenac (15 mg/kg) was used as standard. Below is the protocol used.

- Reference recordings of joint diameter and paw volume with a micrometer screw gauge
- Medication/vehicle administered orally
- Subplantar *injection of 0.1* mL *CFA (0.*Mycobacterium butyrate 05% w/v (in mineral oil) on left hind paw of all rats
- Paw volume was measured at 1, 2, 3, and 4-hours *intervals*
- All groups retreated with vehicle/drug 12 days
- On days 7, 14, and 21, 30 minutes after vehicle/drug administration, measure joint diameter of injected paw again.

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 is shown in Figure 1.

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 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.

Table 11: Anti-arthritic activity of methanolic extracts (CFA method)

Treatment Type	*Doseage (mg/kg)*	*Joint diameter (mm)*		
		7th Day	*14th Day*	*21st Day*
Control	-	0.40267 ± 0.01562	0.40267 ± 0.01839	0.39567 ± 0.01656
Diclofenac	10	0.11567 ± 0.00843***	0.075 ± 0.01332***	0.06677 ± 0.01274**
V. negundo gel	250	0.33267 ± 0.02588*	0.29233 ± 0.00432**	0.285 ± 0.00816**
V. negundo gel	500	0.26267 ± 0.04542*	0.24657 ± 0.02789**	0.23567 ± 0.03332**
V. negundo emugel	250	0.33233 ± 0.00221*	0.29157 ± 0.00443***	0.25 ± 0.01391***
V. negundo emugel	500	0.2 ± 0.02436***	0.16567 ± 0.02208***	0.14267 ± 0.01547***

Values are mean ± SE, n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 vs. control.

Formulation of Emulgel

Formulation of 3:1 S_{mix} ratio Emulgel

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

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

The *in-vitro* release profiles of nirgendi oil from various emulsified formulations are demonstrated above. Each of its Emulgel formulations has been shown to exhibit advanced drug delivery compared to conventional gels formulated according to the USP. It was organized, with 55, 67% drug release at 6 o'clock. For Nirgundi-Emulgel formulations, drug introduction can be ranked in the following descending order: EG1 > EG2 > EG3 > EG4 > EG5, after 6 hours of drug introduction phase..

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. Comparison of optimized gel and latex formulations. Emulgel showed better drug content (103.62 ± 0.01) compared to freezing (99.64 ± 0.02). In-vitro 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-arthritic activity for the methanolic extracts for Formaldehyde method are shown in Table 10, and results for the Anti-arthritic activity of methanolic extracts using CFA method are shown in Table 11.

SUMMARY AND CONCLUSION

Extensive investigation has concluded that topical gels made from HPMC polymers have excellent stretchability, extrudability, and bioadhesive strength. Excellent for making topical preparations. Emulgel (EG1) has a higher swelling index (96.67%) compared to the other properties. From the results, it can also be inferred that the emugel formulation of its *V. negundo* showed better anti-arthritic activity than the gel formulation.

Emulgel showed higher potency (103.62 ± 0.01) compared to gel (99.64%). *In-vitro* drug release for 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 maximum drug release at 6 hours compared to the gel formulation.

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