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FORMULATION AND EVALUATION OF TOPICAL HERBAL GEL FORMULATION FROM AQUEOUS EXTRACT OF VITEX NEGUNDO LEAVES

SWAMI A[1*], RAUT D[1], KADAM S[1], MHATRE D[1], GADHAVE R[1], OZARDE Y[1] AND MEHTA P[2]

1: School of Health Sciences and Technology, Dr. Vishwanath Karad MIT World Peace University, Pune

2: Navsahyadri Institute of Pharmacy, Pune

***Corresponding Author: Dr. Arti Swami; E Mail: arti.swami@mitwpu.edu.in**

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ABSTRACT

Inflammation is a defense mechanism against any tissue injury. Along with various dosage forms, gels are used topically for inflammation. Herbal gels are preferred due to fewer side effects as compared to synthetic ones. Various non-aqueous herbal gels are available in the market, and the extraction from leaves is done using an organic solvent. This gel is made from an aqueous extract of *Vitex negundo,* family Lamiaceae. Extraction of leaves of *Vitex negundo* is done using the decoction technique. Phytoconstituents present in nirgundi leaves are alkaloids, phenolic compounds, carbohydrates, and saponins. *Vitex negundo* has anti-inflammatory action along with anti-ulcer, antioxidant, antinociceptive, and hepatoprotective. The gel is prepared using the base Carbopol 940. Four batches were prepared. Parameters for the evaluation of aqueous herbal gel are visual inspection (particulate matter), pH (pH meter), spreadability (spreadability apparatus), extrudability, viscosity (Brookfield viscometer), and diffusion study (Franz diffusion cell).

Keywords: *Vitex negundo*, aqueous extract, diffusion, anti-inflammatory, herbal gel

1. INTRODUCTION

Inflammation is a defense mechanism against injuries where living tissues show a response that accumulates blood cells and plasma fluid **[1]**. For enhancement of phagocytic activity, phagocytic cells mediate inflammation through mediators such as pro-inflammatory cytokines and by producing Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). The professional phagocytic cells are neutrophils, they are mainly contributing towards inflammation and also for the production of RNS and ROS. For pathogens to be eliminated the superoxide anion and NO are important, the mechanism is oxygen-dependent killing **[2]**. Excessive production of these ions may lead to tissue damage. Although it is said to be a defense mechanism, it can induce or aggravate many diseases. Therefore, these pathologies can be treated using anti-inflammatory agents.

The herbal gel can be used for topical inflammation. Various herbal anti-inflammatory gels are available in the market but this gel is made from aqueous extract of *Vitex negundo. Vitex negundo* mainly shows anti-inflammatory activity. Chrysophenol D, flavonoids like casticin, luteolin-7-glucoside, and iridoid glycoside; vitexin and chrysosplenol, hydrocotylene and nishindine are the major constituents of this plant. It also contains aucubin, eurostoside, and monoterpenes agnuside. The pharmacological activities shown by these constituents are anti-inflammatory, anti-ulcer, antinociceptive, antioxidant, free radical scavenging, hepatoprotective, and many more **[3], [4]**. An essential oil present in the leaves of *Vitex negundo* which contains β-caryophyllene, viridiflorol 4-terpeniol, anti-inflammatory and analgesic effect produced by these constituents **[5]**.

Scientific Classification [4]:

Table 1: Scientific Classification of Vitex negundo

Kingdom	**Plantae**
Sub Kingdom	**Tracheobionta**
Super Division	**Spermatophyta**
Division	**Magnoliophyta**
Class	**Magnoliopsida**
Sub Class	**Asteridea**
Order	**Lamilales**
Family	**Lamiaceae**
Genus	**Vitex linn**
Species	***Vitex negundo* Linn.**

2. MATERIALS AND METHODS:

Fresh plant twigs with leaves were authenticated by Maharashtra Association for the cultivation of Science Agharkar Research Institute, Pune, followed by the preparation of gel. Chemicals required for preparation and evaluation of gel are carbopol- 940,

propylene glycol, triethanolamine, and reagents for phytochemical testing were of AR grade and acquired from Research Lab Fine Chemicals.

2.1 Method of extraction: Aqueous extraction of leaves of *Vitex negundo* in 50 ml water using a heating mantle for 2 hours at 50°C was carried out by decoction technique. The mixture has been filtered and the filtrate was placed in a Hot air oven at 50°C to obtain a dry extract. The dry extract was scraped to get a dry powder of *Vitex negundo* **(Figure 1)**.

2.2 Phytochemical screening of extract: The presence of various phytoconstituents was evaluated in the aqueous extract using the following tests **(Table 2)**:

Figure 1: Extraction method

Table 1: Phytochemical screening of the extract

Phytoconstituents	Tests	Results
Alkaloids	Dragendroff's reagents test	Positive
Flavonoids	Shinoda test	Negative
Phenolic	With FeCl3	Positive
Carbohydrates	Molisch's test Fehling's test	Positive
Steroids and Terpenoids	Salkowski reaction	Negative
Carotenoids	Sulphuric acid, Hydrochloric acid	Negative
Tannins	Test with lead acetate	Negative
Saponins	Froth test	Positive
Coumarins	With ammonia	Negative
Anthraquinone	Borntrager's test	Negative
Glucoside	Modified Borntrager's test	Negative

Figure 2: Phytoconstituent Screening Test

2.3 Preparation of gel base:

Carbopol-940 was added gradually by stirring in 60ml of water to avoid agglomerates. It is continuously stirred for 1 hour. 10ml of disodium edetate and 2ml of triethanolamine were added and stirred for 10 minutes. The mixture of 5 ml of propylene glycol and 12 ml of water was prepared and stirred for 10 minutes. The propylene glycol solution is added to the Carbopol solution and stirred for 10 minutes to get a clear, uniform gel base.

2.4 Nirgundi gel formulation:

Four batches of gel formulations were prepared each containing 5g dried powder of *Vitex negundo* and Carbopol 940 polymer concentration ranging from 1 to 2.5%. Carbopol is added as a gelling agent in the extract as it is bio-adhesive, non-biodegradable, irritation-free, biocompatible, and non-absorbable in the body Propylene glycol is said to be one of the best permeability enhancers. Sufficient quantity of methyl paraben and propyl paraben were added as preservatives. The pH of the gel is adjusted using disodium edetate and triethanolamine.

Sr. No.	Name of ingredients	Quantity taken			
		B1	B2	B3	B4
1	Carbopol 940	1%	1.5%	2%	2.5%
2	Leaf powder	5gm	5gm	5gm	5gm
3	Propylene glycol	5ml	5ml	5ml	5ml
4	Methyl paraben	q.s	q.s	q.s	q.s
5	Propyl paraben	q.s	q.s	q.s	q.s
6	Disodium edetate	10ml	10ml	10ml	10ml
7	Triethanolamine	2ml	2ml	2ml	2ml

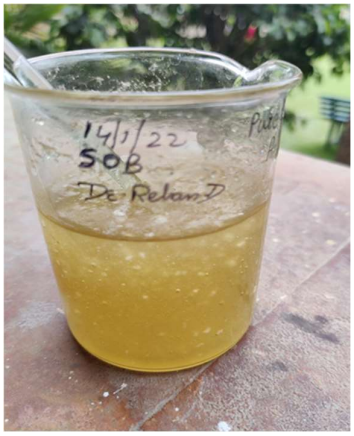

Figure 2: Nirgundi Gel Formulation

Figure 3: Nirgundi Gel Container

2.5 Evaluation Parameters:

Visual Inspection **[6], [7]**:

Visual examination was done to assess the manufactured gels' physical characteristics and uniformity. The results are shown in **Table 3**.

pH measurements **[6], [7]**:

A digital pH metre was used to measure the pH of herbal gel. The results are shown in **Table 4**.

Viscosity **[6], [7]**:

A Brookfield viscometer was used to measure the produced gel's viscosity at a temperature of 25 °C and a spindle speed of 10 rpm. At 25ºC viscosity of gels was found to be in centipoises. The results are shown in **Table 4**.

Extrudability [6], [7], [8]:

The gel compositions were put inside the collapsible aluminium tubes with conventional caps, and the ends were crimped shut to seal them. The weights of the tube were recorded. Clamps were used to hold the tubes in place between two glass slides. After applying 500 grams to the slides, the cap was removed. The volume of the extruded gel was collected and weighed. The estimated extruded gel % was evaluated based on the following standard values: Excellent (Extrudability above 90%), good (Extrudability above 80%), and fair (Extrudability above 70%). The results are shown in **Table 4**.

Spreadability [6], [7], [8]:

The spreadability apparatus consists of a wooden block with a pulley mounted to one end. This method was used to evaluate spreadability based on the gel's slide and drag qualities. Approximately 2.5g of the drug was placed on the bottom slide, and another slide containing a hook was placed above it. For five minutes on top of the two slides, 1 kg weight was added to force air out and create a homogenous gel film between them. Excess gel was scraped off the edges. After that, the top plate was pulled by 50 g weight, and the time needed to cover a distance of 5cm by slide was recorded.

Figure 4: Brookfield Viscometer

Figure 5: Spreadability Apparatus

Formula for Spreadability calculation: -

S= ml/t

Where, S = spreadability (gm.cm/s),

m = weight on pulley (gm),

l = distance travelled by glass slide (cm),

t = time taken for the movement of the slide in seconds.

Drug Content **[6], [7], [8]**:

By dissolving 1g of the gel formulation with 100 ml of distilled water, the drug concentration was estimated. To make dilutions of a different concentration, 1 ml of the prior solution was taken, and it was then diluted further to 10 ml with phosphate buffer 7.4. The absorbance of *Vitex negundo* was estimated at 254 nm using a UV Visible Spectrophotometer. Drug Content was determined from the calibration curve equation **(Equation 1)**.

Physicochemical Parameters:

Table 2: Physical Parameters

Parameters	Observation
Formulations	Topical Gel
Appearance	Smooth
Colour	Yellowish Brown
Odour	Characteristic
Homogeneity	Homogeneous

Evaluation Parameters:

Table 3: Evaluation Parameters

Parameters	Observation
pH	6.2
Viscosity	32561.1 centipoise
Extrudability	Good
Spreadability	22.32 gm.cm/s
Drug Content	96.15%

In Vitro Diffusion Profile [6], [7]:

The Franz Diffusion Cell Apparatus was used to conduct the in vitro diffusion studies and a semi-permeable cellophane membrane (grade 12) was used as a Dialysis membrane. Phosphate buffer 7.4 was used as medium to soak the cellophane membrane overnight for saturation. A phosphate buffer of PH 7.4 was used as a diffusion media. The cellophane membrane was placed between the donor and receiver compartment. Then 1g sample was weighed accurately and deposited on a semipermeable cellophane membrane to occupy the entire surface of the cellophane membrane. The temperature was maintained at 37±1ºC and the speed was maintained at 70 rpm using a magnetic stirrer. 1ml samples were withdrawn at one-hour intervals up to 6 hours. To maintain a constant volume, the equal volume of phosphate buffer 7.4 was added in place of the sample that was withdrawn. The Whattman filter paper was used to filter the sample which was diluted and absorbance was measured using UV-Vis Spectrophotometer at 254 nm.

Figure 6 Franz Diffusion Apparatus

With help of an equation derived from a calibration curve, the cumulative percentage of drug release was calculated. The equation is as follows;

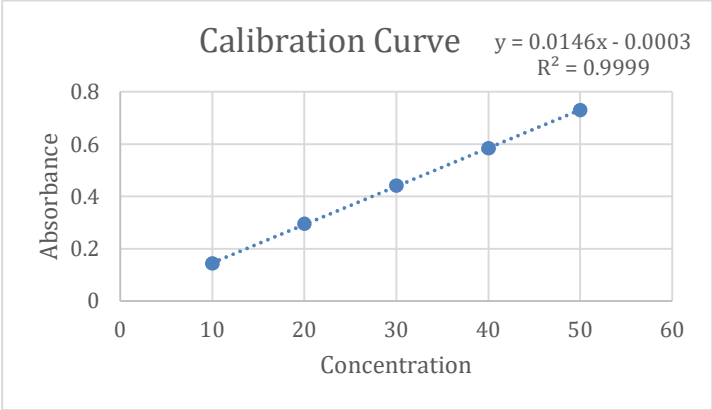

Figure 7 Calibration Curve

The percent drug release obtained is given in **Table 5**. The graph of cumulative drug release percentage over time was plotted and shown in **Figure 4**.

Table 4: Diffusion Study

Sr no.	Time in hrs	% cumulative release
1	1	15.48
2	2	31.40
3	3	48.89
4	4	67.19
5	5	83.73
6	6	92.84

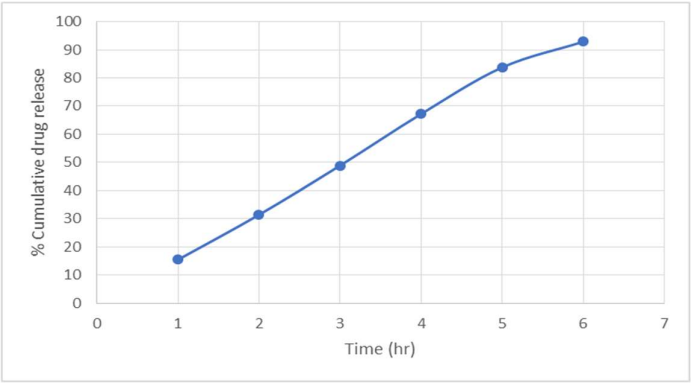

Figure 8 Graph of diffusion study

3. RESULTS AND DISCUSSION:

Four batches of gel were prepared and the optimized batch is reported. All the parameters that are evaluated are mentioned in Table No. 6. From the evaluation the formulation of topical gel was smooth, Yellowish-brown in colour, and homogeneous. It was found that the gel's pH was 6.2, which is identical to the skin's pH. A Brookfield viscometer was used to measure the gel's viscosity, which was found to be 32561.1 centipoise. The extrudability is good as it is more than 80%. The gel's spreadability was assessed to be 22.32 gm/cm/s. The gel was evaluated for content uniformity, the drug content was determined to be 96.15%. The percent cumulative drug release of gel was evaluated through a diffusion study of 6 hours, the drug release at the 6th hour was 92.84%. All the desired parameters of the gel are within specification.

Table 6: Result

Evaluation Parameter	Observations	Inference
Visual Inspection	Homogeneous and smooth.	Absence of particulate matter and no phase separation.
PH measurement	6.2	Matches with pH of skin.
Viscosity	32561.1 centipoise	Sufficiently viscous.
Spreadability	22.32 gm.cm/s	Easily spreadable.
Extrudability	>80%	Good
In Vitro Diffusion Profile	92.15%	Should be between 85 to 115%

4. CONCLUSION:

This research is carried out to develop herbal gel with anti-inflammatory action using the leaves of *Vitex negundo.* The gel was prepared by aqueous extraction of leaves. The formulated herbal gel showed good drug release. The evaluation of herbal gel was done for pH, viscosity, diffusion, spreadability, extrudability, and drug content. The drug release from the gel was good. The gel was homogenous and translucent. The gel's pH was within the limit, and it matched with the pH of the skin. The gel can be easily spread and can be removed easily. The gel was sufficiently viscous, thick, and extrudable from the container. All evaluation parameters were carried out and were within limits. Due to the lack of studies that performed an aqueous extraction of Vitex negundo leaves and the increasing demand in the market for herbal remedies, *Vitex negundo* gel was formulated and evaluated.

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