

Exploration of traditional oils in a modern balm formulation for topical pain management

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Abstract

Pain remains a significant clinical and public health concern, often managed with synthetic analgesics that may produce systemic side effects with prolonged use. The current study focuses on formulating and evaluating a topical herbal balm using *Vitex negundo* (Nirgundi) oil and *Mentha piperita* (Peppermint) oil- both traditionally acclaimed for their analgesic and anti-inflammatory potential. Four formulations (F1–F4) were prepared by varying the concentration of base excipients while keeping the herbal oil content constant at 13% w/w each. The phytochemical profiles of the oils were confirmed through qualitative and quantitative analyses, indicating the presence of key bioactive constituents such as alkaloids, flavonoids, tannins, and terpenoids. Quantitative assays revealed high phenolic and flavonoid content, further supporting the therapeutic potential. The formulations were evaluated for physicochemical parameters including pH, spread ability, consistency, washability, and stability under accelerated conditions. Among all, formulation F3 exhibited optimal spread ability, acceptable pH, ease of application, and superior stability over 30 days. The findings suggest that the herbal balm developed with *Vitex negundo* and *Mentha piperita* oils is a promising candidate for natural pain relief with improved patient compliance and minimal side effects.

Keywords: Nirgundi Oil, Peppermint Oil; Herbal Balm; Pain Management; Topical Analgesic; Phytochemical Screening; Herbal Formulation Evaluation

1. Introduction

Pain, defined by the International Association for the Study of Pain (IASP) as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage,” serves as a vital protective mechanism [1,2]. Topical delivery of analgesics offers localized relief, reduced systemic side effects, and improved patient compliance [3]. Herbal balms, derived from traditional Ayurvedic formulations, utilize essential oils and natural waxes for pain alleviation via counter-irritation stimulating sensory nerves and distracting from deeper pain sensations [4].

Vitex negundo (Nirgundi) is widely documented for its anti-inflammatory and analgesic properties [6], while *Mentha piperita* (Peppermint) provides a cooling effect due to menthol, which activates TRPM8 receptors for sensory modulation [7]. Despite longstanding traditional use, recent phytopharmacological studies continue to validate these herbs' efficacy [5-7]. This study aimed to formulate and evaluate a natural herbal balm using Nirgundi and Peppermint oil for effective pain relief.

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2. Materials and Methods

2.1. Materials

Nir gundi oil and peppermint oil were procured from Padmavati Ayurvedic Store, Krishnanagar, Ahmedabad, India. The excipients utilized in the formulation included sodium benzoate, soft beeswax, petroleum jelly, and cocoa butter. Soft beeswax was obtained from Research Lab Fine Chem Industries, Mumbai. Petroleum jelly and sodium benzoate were procured from Astron International Pvt. Ltd., Ahmedabad, while cocoa butter was supplied by Oxford Industries Ltd., Mumbai. All the materials used were of analytical or pharmaceutical grade and were utilized without further purification.

2.2. Phytochemical Screening

The phytochemical analysis of *Vitex negundo* (Nirgundi) oil and *Mentha piperita* (Peppermint) oil was conducted to identify the presence of various bioactive constituents. Standard qualitative procedures were employed as per established protocols to determine the chemical composition of the oils [8,9].

2.2.1. Test for alkaloids

Wagner's test: Few ml of extract alcoholic solution + Few drops of Wagner's reagent (Iodine potassium iodide solution) was added by the sides of the test tube. A reddish-brown precipitate confirms the test as positive.

Dragendorff's test: Few ml of filtrate + 1 or 2 ml of Dragendorff's reagent (Potassium bismuth iodide solution) were added. A reddish brown indicates the test positive.

Mayer's test: Few ml of filtrate + 1 or 2 ml of Mayer's reagent (Iodine potassium iodide solution) was added by the sides of the test tube. A white or creamy precipitate indicates the test as positive.

Hager's test: Few ml of filter ate + 1 or 2 ml of Hager's reagent (Saturated solutions of picric acid). A prominent yellow precipitate indicates the test as positive.

2.2.2. Test for tannins and Phenolic compounds

Ferric chloride test: Few ml of extract alcoholic solution + Few drops of neutral 5% ferric chloride solution were added. A dark green color indicates the presence of phenolic compounds.

Gelatin test: The extract (50 mg) was dissolved in 5 ml of distilled water and 2 ml of 1% solution of gelatin containing 10% sodium chloride was added to it. White precipitate indicates the presence of phenolic compounds.

2.2.3. Test for Saponins

Foam test: The extract powder was dissolved in water and shake vigorously. Persistent foam was observed.

2.2.4. Test for carbohydrates

Molisch's test: 2ml of filtrate + 2 drops of alcoholic solution of alpha-naphthol, the mixture was shaken well and add 1 ml of conc. sulphuric acid slowly along the side wall of test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

Fehling's test: 1 ml of filtrate was boiled on water bath with 1 ml of each of Fehling solutions A and B. A red precipitate indicates the presence of carbohydrates.

2.2.5. Test for Flavonoids

Shinoda Test (Magnesium-HCl Reduction Test): A small quantity of the extract is dissolved in methanol, to which a few magnesium turnings are added, followed by the slow addition of concentrated hydrochloric acid.

2.2.6. Test for Proteins

Biuret test: To 3 ml of extract was added 4% NaOH and few drops of 1% CuSO₄ solution. Violet or pink color indicates the presence of protein.

2.2.7. Test for Glycosides

Keller-Kiliani test: 1 ml of glacial acetic acid containing traces of FeCl_3 and 1 ml of concentrated H_2SO_4 were added to the extracts carefully. A reddish brown colour is formed at the junction of two layers and the upper layers turns bluish green indicates the presence of glycosides.

2.2.8. Test for Terpenoids

Salkowski test: To 2ml of extract 5 ml of chloroform and few drops of concentrated H_2SO_4 were added to form a layer. A reddish-brown coloration formed in the interface indicates the presence of terpenoids.

2.2.9. Detection of Fixed Oils and Fats

Spot Test: A drop of the oil was placed on filter paper. A permanent translucent stain indicated the presence of fixed oils and fats.

Saponification Test: The extract was treated with ethanol and potassium hydroxide and heated. The formation of soap or soapy foam confirmed the presence of fats and oils.

2.3. Quantitative Phytochemical Estimation

Quantitative estimation of key phytoconstituents was carried out using standard spectrophotometric and gravimetric methods. All analyses were performed in triplicate to ensure accuracy and reproducibility of the results.

2.3.1. Total Phenolic Content

The total phenolic content of the oil samples was determined using the Folin- ciocalteu colorimetric method. Briefly, the oil sample was mixed with Folin- ciocalteu reagent and sodium carbonate solution. After incubation, the absorbance of the reaction mixture was measured at 765 nm using a UV-Visible spectrophotometer. Gallic acid was used as the calibration standard, and the results were expressed as milligrams of gallic acid equivalents per gram of oil (mg GAE/g oil) [10].

2.3.2. Total Flavonoid Content

Flavonoid content was quantified by the aluminum chloride colorimetric assay. The oil sample was mixed with methanol, 10% aluminum chloride, potassium acetate, and distilled water. After incubation at room temperature, absorbance was recorded at 415 nm. Quercetin served as the reference standard, and results were expressed in milligrams of quercetin equivalents per gram of oil (mg QE/g oil) [11].

2.3.3. Total Alkaloid Content

Alkaloids were estimated using a gravimetric method. The oil sample was acidified with dilute hydrochloric acid and subjected to liquid-liquid extraction using chloroform. The aqueous phase was basified with ammonium hydroxide, and the resulting precipitate was filtered, dried, and weighed. The total alkaloid content was calculated as the percentage of crude alkaloids present in the sample [12].

2.3.4. Total Tannin Content

Tannin concentration was assessed using the Folin Denis colorimetric method. The sample was mixed with Folin-Denis's reagent and sodium carbonate, and the absorbance was measured at 760 nm. Tannic acid was used to construct the calibration curve, and results were reported as milligrams of tannic acid equivalents per gram of oil (mg TAE/g oil) [13].

2.4. Formulation of Herbal Balm

A total of four formulations (designated as F1 to F4) were developed using varying concentrations of base excipients to optimize the consistency and stability of the herbal balm. Both *Vitex negundo* oil and *Mentha piperita* oil were incorporated at a fixed concentration of 13% w/w in each formulation. The solid excipients like beeswax, petroleum jelly, and cocoa butter were accurately weighed and melted together in a water bath maintained at 70–75°C with continuous stirring to ensure uniform mixing. Once a homogenous melt was obtained, the mixture was allowed to cool to approximately 45°C, at which point the essential oils were added to prevent loss of volatile constituents. The final formulations were poured into pre-sterilized, wide-mouthed glass containers and allowed to solidify at ambient room temperature [14-16].

Table 1 Composition of Herbal Balm Formulations (F1-F4)

Ingredients	F1 (% w/w)	F2 (% w/w)	F3 (% w/w)	F4 (% w/w)
<i>Vitex negundo</i> Oil	13	13	13	13
<i>Mentha piperita</i> Oil	13	13	13	13
Beeswax	10	12	14	16
Petroleum Jelly	30	28	26	24
Cocoa Butter	30	28	26	24
Sodium Benzoate	0.5	0.5	0.5	0.5
Total	100	100	100	100

2.5. Evaluation of Herbal Balm Formulations

All four formulations (F1 to F4) were subjected to a series of physicochemical evaluations to determine their suitability for topical application. The parameters assessed included physical appearance, pH, spreadability, consistency, washability, and stability [17-21].

2.5.1. Physical Appearance

Each formulation was visually examined for color, homogeneity, texture, and the presence of any phase separation. The final balm was expected to be smooth, semi-solid, and free from grittiness or lumps.

2.5.2. pH Determination

The pH of each formulation was measured by dispersing 1 g of balm in 10 mL of distilled water and analyzing the mixture using a calibrated digital pH meter. An ideal topical preparation should exhibit a skin-compatible pH (typically between 4.5 and 6.5).

2.5.3. Spreadability

Spreadability was assessed using the slip and drag method. A fixed amount (1 g) of balm was sandwiched between two glass slides, and the time (in seconds) required to separate the slides under a specific weight (500 g) was recorded. Spread ability was calculated using the formula:

$$S = M \times L / T,$$

Where S is spreadability, M is the applied weight (g), L is the length moved by the upper slide (cm), and T is the time taken (s).

2.5.4. Consistency (Penetration Test)

A penetrometer was used to determine the consistency by measuring the depth (mm) a standard needle penetrated into the formulation under a fixed load and time.

2.5.5. Washability

Washability was evaluated by applying a small amount of balm on the skin and washing under running tap water. The ease of removal was noted qualitatively (Easy / Moderate / Difficult).

2.5.6. Stability Study

Preliminary stability tests were conducted under accelerated conditions ($40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH) for 30 days. The formulations were evaluated for changes in appearance, pH, and phase separation at 0, 15, and 30 days.

3. Results and Discussion

3.1. Phytochemical Screening

Qualitative tests indicated the presence of multiple bioactive phytochemicals in both *Vitex negundo* and *Mentha piperita* oils.

Table 2 Phytochemical Screening of *Vitex negundo* and *Mentha piperita* Oils

Phytoconstituent	Test Performed	<i>Vitex negundo</i> Oil	<i>Mentha piperita</i> Oil
Alkaloids	Wagner's, Dragendorff's, Mayer's, Hager's test	+	+
Tannins& Phenolics	Ferric Chloride, Gelatine Test	+	+
Saponins	Foam Test	+	–
Carbohydrates	Molisch's, Fehling's Test	+	+
Flavonoids	Shinoda Test	+	+
Proteins	Biuret Test	–	–
Glycosides	Keller–Killian Test	+	+
Terpenoids	Salkowski Test	+	+
Fixed Oils and Fats	Spot, Saponification	+	+
Note: (+) Present; (–) Absent			

All major phytochemical groups like alkaloids, flavonoids, tannins, sterols, terpenoids, glycosides, proteins, and fixed oils were detected. The results validate the traditional claims regarding their therapeutic efficacy.

The presence of alkaloids and flavonoids in *Vitex negundo* suggests potential anti-inflammatory and analgesic action through inhibition of prostaglandin synthesis and antioxidant activity. Menthol, the key constituent of *Mentha piperita*, activates TRPM8 receptors, producing a cooling sensation that aids in counter-irritation- a well-established mechanism in topical analgesics.

3.2. Quantitative Phytochemical Estimation

Quantitative analysis confirmed the richness of these oils in pharmacologically active constituents.

Table 3 Quantitative Phytochemical Estimation of Oils

Phytoconstituent	<i>Vitex negundo</i> (Mean $\pm$ SD) (mg GAE/g oil)	<i>Mentha piperita</i> (Mean $\pm$ SD) (mg GAE/g oil)
Total Phenolic Content	86.4 $\pm$ 2.3	78.1 $\pm$ 1.8
Total Flavonoid Content	69.5 $\pm$ 2.1	63.2 $\pm$ 2.5
Total Tannin Content	42.7 $\pm$ 1.9	38.9 $\pm$ 1.6
Total Alkaloid Content	3.2 $\pm$ 0.2	2.5 $\pm$ 0.3

These values corroborate the oils' analgesic efficacy and justify their inclusion in a topical delivery system.

3.3. Formulation Development and Evaluation

The four formulations (F1-F4) showed differences in physical characteristics due to the variation in the base excipients. Key observations are as follows:

Table 4 Evaluation of Balm Formulations (F1-F4)

Parameters	F1	F2	F3	F4
Physical Appearance	Smooth, semi-solid	Smooth, semi-solid	Smooth, semi-solid	Slightly firmer
pH	5.1 ± 0.1	5.5 ± 0.2	5.9 ± 0.1	6.2 ± 0.2
Spreadability (gm./s)	11.5 ± 0.4	12.9 ± 0.5	14.6 ± 0.3	10.8 ± 0.6
Consistency (mm)	6.2 ± 0.3	5.7 ± 0.2	5.3 ± 0.1	4.9 ± 0.2
Washability	Easy	Easy	Moderate	Moderate
Stability (30 Days)	Slight oil separation	Stable	Stable	Slight texture change

All four formulations were designed by keeping the concentration of herbal oils constant and adjusting the ratios of base excipients like beeswax, petroleum jelly, and cocoa butter to achieve optimal consistency and therapeutic retention. All formulations exhibited a smooth, semi-solid appearance, which is desirable for topical applications, with F4 appearing slightly firmer due to its higher beeswax content (14%). Beeswax acts as a stiffening agent and plays a significant role in determining the structural integrity of topical formulations. This increased firmness in F4 was supported by its lower penetration value (4.9 mm), indicating greater consistency and hardness compared to F1 (6.2 mm), which had the lowest wax content.

The pH values of all four formulations ranged between 5.1 and 6.2, which falls within the skin-friendly range and is therefore unlikely to cause irritation upon topical application. This range also ensures compatibility with the skin's natural acidic mantle, which is essential for maintaining microbial balance and skin barrier function.

Spreadability, a critical factor influencing patient compliance and ease of application, was highest in F3 (14.6 g·cm/s), followed by F2 and F1. The increase in spreadability may be linked to the optimal ratio of softening and lipid components like petroleum jelly and cocoa/shear butter, which reduce drag during application. F4 showed the lowest spreadability (10.8 g·cm/s), possibly due to its higher wax concentration, making it thicker and less pliable during application.

In terms of washability, F1 and F2 were rated as easy to wash off, which can be advantageous for short-term or repeated applications. However, F3 and F4, with higher wax and lipid content, showed moderate washability, suggesting longer retention time on the skin, which might be beneficial for sustained therapeutic action but may require more effort to clean.

The stability study conducted over 30 days at room temperature indicated that F2 and F3 remained stable, showing no phase separation, color change, or odor alteration. In contrast, F1 exhibited slight oil separation, possibly due to a lower emulsifier and wax ratio, while F4 showed minor texture changes, which may be due to its dense structure and high wax content leading to recrystallization or phase compression.

Overall, F3 emerged as the most balanced formulation, demonstrating optimal spreadability, good stability, adequate firmness, and acceptable washability.

4. Conclusion

The present study successfully formulated a topical herbal balm incorporating *Vitex negundo* and *Mentha piperita* oils, harnessing their documented analgesic and anti-inflammatory properties. Phytochemical screening confirmed the presence of key bioactive constituents, while quantitative assays validated their pharmacological richness. The evaluation of four formulations revealed that F3 offered the most favorable characteristics, including optimal pH, superior spread ability, ideal consistency, ease of washability, and excellent stability under accelerated conditions. These findings support the therapeutic potential of herbal balms as a safe and effective alternative to conventional

topical analgesics. Further in vivo and clinical studies are warranted to substantiate the balm's efficacy and to explore its commercial viability in the natural pain relief segment.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author Contributions

RP, GJ, DM, SP, and PR. were involved in conceptualization, formulation development, experimental work, data collection, literature review, and drafting of the manuscript. KP supervised the study, provided resources and guidance, contributed to manuscript revision, and approved the final version for submission. All authors have read and agreed to the final manuscript.

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