Research Article

Int Jou Hea Bio Sci, 6(2), 2025, 1-6

International Journal of Health care and Biological Sciences

Content available at www.saap.org.in Online ISSN: 2582-7499

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DEVELOPMENT AND EVALUATION OF NATURAL PAIN RELIEF SPRAY USING POLYHERBAL EXTRACTS

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Article History

Received on: 11-03-2025
Revised on: 19-04-2025
Accepted on: 05-05-2025

Abstract

Pain management is a critical aspect of healthcare, with growing interest in natural and herbal-based alternatives to synthetic analgesics. This study focuses on the development of a natural pain relief spray formulated using bioactive extracts from Mentha piperita (peppermint), Aloe vera, Arnica montana, and Calendula officinalis. The methodology involves the careful selection and authentication of raw materials, followed by optimized extraction techniques such as steam distillation, maceration, and freeze-drying to obtain key bioactive compounds. The extracted compounds were blended into a emulsion-based spray formulation using a combination of aqueous and ethanolic phases, ensuring uniform dispersion and stability. Natural stabilizers and preservatives were incorporated to enhance consistency and shelf-life, while pH adjustments were made to ensure skin compatibility. Standardization and quality control were conducted through UV-Vis spectroscopy, microbial load testing, viscosity measurement, and stability assessment under various environmental conditions. Furthermore, in-vitro anti-inflammatory and antioxidant assays were performed to evaluate the efficacy of the formulation. The developed pain relief spray demonstrated significant anti-inflammatory potential by inhibiting protein denaturation and stabilizing cell membranes, along with strong antioxidant activity. The final product was packaged in UV protected amber glass bottles to prevent photodegradation and ensure prolonged stability. This study presents a scientifically validated, herbal-based pain relief spray as a safe and effective alternative for topical pain management.

Keywords: Pain, pain relief spray, Mentha piperita, Aloe vera, Arnica montana and Calendula officinalis.

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DOI: https://doi.org/10.46795/ijhcbs.v6i2.680

Introduction

Pain is a common and debilitating condition that affects individuals worldwide, arising from various causes such as inflammation, injury, muscle strain, arthritis, and neuropathic disorders. Peppermint (Mentha piperita) contains menthol, which provides a cooling effect, desensitizing pain receptors and reducing discomfort. Aloe vera is rich in polysaccharides and bioactive compounds that reduce inflammation, promote healing, and soothe irritated skin. Arnica montana is widely recognized for its ability to reduce pain, swelling, and muscle soreness, making it a popular remedy in sports and musculoskeletal injuries. Calendula officinalis possesses flavonoids and triterpenoids that help in skin repair, wound healing, and inflammation reduction [1,2].

Despite the potential of herbal-based pain relief formulations, their standardization, bioavailability, and stability remain major challenges. This study aims to develop a scientifically validated, natural pain relief spray by integrating modern extraction, formulation, and analytical techniques to ensure efficacy, stability, and safety. The objective of the study as to develop a scientifically validated, herbal-based pain relief spray formulated using bioactive plant extracts (Mentha piperita, Aloe vera, Arnica montana, and Calendula officinalis), ensuring optimal efficacy, safety, and stability for topical application [3].

Materials & Methodology

Procurement of Plants

Plant materials were procured from certified suppliers or organic farms to ensure authenticity and safety. Supplier audit was conducted to verify Good Agricultural and Collection Practices (GACP) compliance and to ensure that all plant materials are free from pesticides, heavy metals, and microbial contamination. Raw materials were stored in controlled environments (temperature: 15–25°C, humidity: 30–50%) to maintain potency.

Preparation

Wash all raw materials thoroughly under running water to remove dirt and microbial contaminants. Dry materials at controlled conditions (temperature: <40°C) to prevent degradation of heat-sensitive compounds. Use a mechanical grinder to obtain a fine powder for efficient extraction. Store processed powders in airtight containers under nitrogen flushing to prevent oxidation [4].

Extraction of Active Compounds

Mentha piperita

Crush fresh or dried leaves to release volatile oils. Perform steam distillation by heating plant material with water, condensing vapor, and separating essential oil. Store the extracted oil in amber glass bottles at 4°C to prevent oxidation [5].

Aloe vera

Mature Aloe vera leaves (>2 years old) were harvested to ensure maximum bioactive content. The leaves were cut lengthwise, and the inner gel was collected. The gel was filtered using muslin cloth to remove fibers and large debris. Freeze-drying (lyophilization) was used to retain polysaccharides such as acemannan. The dried extract was stored in vacuum-sealed containers at low temperatures [6].

Arnica Montana

Dried Arnica flowers were soaked in a 70% ethanol solution to extract helenalin and flavonoids. The mixture was macerated under continuous agitation for 48–72 hours to ensure maximum compound release. It was then filtered and concentrated using a rotary evaporator under reduced pressure. The extracts were stored in nitrogen-flushed vials to maintain stability [7].

Calendula officinalis

Dried Calendula petals were macerated in a hydroalcoholic solution (50% ethanol, 50% water) to extract flavonoids and triterpenoids. Periodic agitation was performed at room temperature for 48 hours. The extract was filtered using vacuum filtration and concentrated under reduced pressure. The final product was stored in dark glass bottles to prevent photodegradation [8].

Formulation of the Spray for natural pain relief

Preparation of Solvent Phases

Aqueous Phase Preparation

Water-soluble extracts (e.g., Aloe vera gel) were dissolved in deionized water under constant stirring.

Ethanolic Phase Preparation

Oil-based and hydroalcoholic extracts (peppermint oil, arnica, calendula) were dissolved in ethanol.

Liposomal Encapsulation (Optional)

Active ingredients were encapsulated in liposomes to enhance bioavailability and skin penetration, where applicable.

Blending of Extracts

The aqueous phase was slowly added to the ethanolic phase under constant stirring to prevent phase separation and maintain uniformity. Stirring was maintained at a speed of 500–1000 rpm for 20–30 minutes to ensure proper mixing.

Homogenization

A high-shear homogenizer was used at 10,000–15,000 rpm for 30 minutes to ensure uniform dispersion of active ingredients.

Incorporation of Stabilizers and Preservatives

Natural stabilizers such as lecithin and xanthan gum were added to improve consistency. Natural preservatives, including Tocopherol (vitamin E) and grapefruit seed extract, were incorporated to extend shelf life and prevent microbial growth. A stable nano emulsion with uniform particle distribution and no visible phase separation [9].

Adjustment of pH

The pH was measured using a calibrated pH meter and adjusted to 4.5–5.5 using citric acid or sodium hydroxide to ensure skin compatibility.

Table 1. Formulation Composition

S.No.	Ingredient	Function	Amount (100 ml)
1	*Aloe vera* Gel	Hydrating & soothing agent	15 mL
2	Deionized Water	Solvent (Aqueous Phase)	10 mL
3	Peppermint Oil	Cooling & antimicrobial	2 mL
4	Arnica Extract	Anti-inflammatory	2 mL
5	Calendula Extract	Skin repair & soothing	2 mL
6	Ethanol (70%)	Solvent (Ethanolic Phase)	28 mL
7	Lecithin	Stabilizer	2 g
8	Xanthan Gum	Thickener	0.5 g

9	Tocopherol (Vit E)	Antioxidant preservative	0.3 g
10	Grapefruit Seed extract	Antimicrobial & preservative	0.2 g
11	Citric Acid	pH Adjuster	0.05 g

Packaging

Sterilized spray bottles made of UV-protected amber glass or BPA-free plastic was used. The bottles were filled using aseptic techniques to prevent contamination. Finished products were stored at controlled temperatures to maintain stability.

Filling Process

Aseptic filling is performed under sterile conditions to prevent microbial contamination. Each batch contains 1L of spray, which is filled into 100 units of 10 mL bottles.

Storage Conditions

Temperature: 15–25°C
Humidity: <50% RH

Standardization:

UV-Vis spectroscopy was used for standardization, with the following detection wavelengths: Menthol (280 nm), Aloin (296 nm), Helenalin (228 nm), and Quercetin (370 nm).

Physicochemical Testing

Appearance, Viscosity, Stability Testing, Spray Performance, Efficacy and Safety Testing In-vitro Anti-inflammatory Testing, Protein Denaturation Assay were evaluated.

Protein Denaturation Assay

Test sample: The spray formulation was diluted in PBS to prepare five concentrations: 25, 50, 100, 150, and 200 µg/mL.
Standard drug: Diclofenac Sodium was dissolved in PBS to prepare matching concentrations 25, 50, 100, 150, and 200 µg/mL.
Control: PBS solution

Procedure

1% BSA was incubated with different concentrations of the test sample at 37°C for 30 minutes. Heat-induced denaturation was performed at 70°C for 10 minutes. The absorbance was measured at 660 nm. % Inhibition of protein denaturation was calculated.

Antioxidant Activity

Test Sample (Spray Extract) Dilutions: The test formulation was diluted in methanol to obtain five different concentrations: 25, 50, 100, 150, and 200 µg/mL
Standard (Ascorbic Acid) Dilutions: Ascorbic Acid, a well-known antioxidant, was used as a positive control and was also diluted in methanol to prepare: 25, 50, 100, 150, and 200 µg/mL.

DPPH radical scavenging assay

0.1 mM DPPH solution was prepared fresh in methanol and kept in the dark to prevent degradation. 2 mL of DPPH solution was mixed with 2 mL of test sample at different concentrations. 2 mL of DPPH solution was mixed with 2 mL standard sample at different concentrations. A control was prepared using DPPH + methanol without any antioxidant. The reaction mixtures were incubated in the dark at room temperature for 30 minutes to allow complete interaction between DPPH and antioxidants. After incubation, absorbance was recorded at 517 nm using a UV-Vis spectrophotometer. Lower absorbance indicates stronger antioxidant activity [10].

Results and Discussion

The formulation methodology ensures stable nano emulsion formation (particle size ~100–200 nm), Long shelf life (12–18 months) due to antioxidants and preservatives, balanced pH (4.5–5.5) for skin compatibility, effective blend of botanical actives (Aloe vera, Peppermint, Arnica, Calendula) and optimized storage conditions to prevent degradation.

Table 2. Standardization of extracts by UV-Vis spectroscopy

S.No.	Compound	Test Sample Concentration (µg/mL)	Standard Drug Concentration (µg/mL)	Difference (µg/mL)	UV Wavelength (nm)	Conclusion
1	Menthol	4.83	5	0.17	280	Close match, small variation, efficient extraction
2	Aloin	4.94	5	0.06	296	Very close match, highly efficient extraction
3	Helenalin	5.67	5	0.67	228	Slightly higher, extraction likely more efficient
4	Quercetin	6.00	6	0	370	Perfect match, excellent extraction efficiency

Table 3. Absorbance of Protein Denaturation Assay

S.No.	Concentration (µg/mL)	Test Sample Absorbance	Diclofenac (Standard) Absorbance
1	25	0.754	0.706
2	50	0.583	0.515
3	100	0.328	0.264
4	150	0.215	0.150
5	200	0.107	0.088

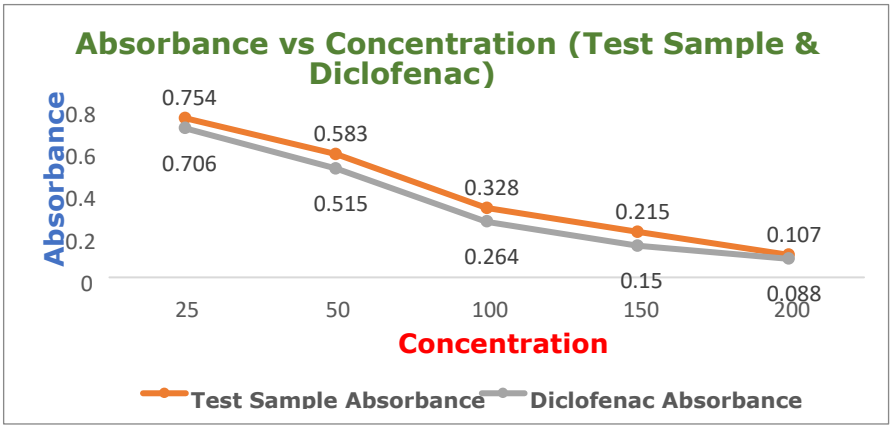

Figure 1. Absorbance for Protein Denaturation Assay

The test formulation demonstrated significant inhibition of protein denaturation in a dose dependent manner. At 200 µg/mL, the formulation exhibited 89.3% inhibition, which is comparable to Diclofenac Sodium (91.2%) indicating strong anti-inflammatory potential.

Table 4. Inhibition Results for Protein Denaturation Assay

S.No.	Concentration (µg/mL)	Test Sample Inhibition (%)	Diclofenac (Standard) Inhibition (%)
1	25	24.6	29.4
2	50	41.7	48.5
3	100	67.2	73.6
4	150	78.5	85.0
5	200	89.3	91.2

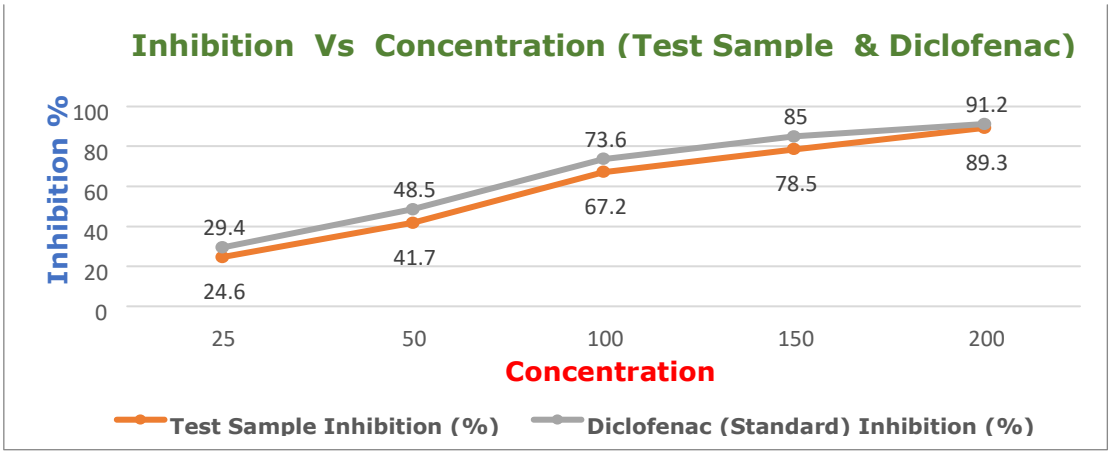

Figure 2. Inhibition Results for Protein Denaturation

Table 5. Absorbance for DPPH free radical scavenging assay

S.No.	Concentration (µg/mL)	Test Sample Absorbance	Ascorbic Acid Absorbance
1	25	0.762	0.726
2	50	0.535	0.479
3	100	0.341	0.288
4	150	0.218	0.170
5	200	0.153	0.119

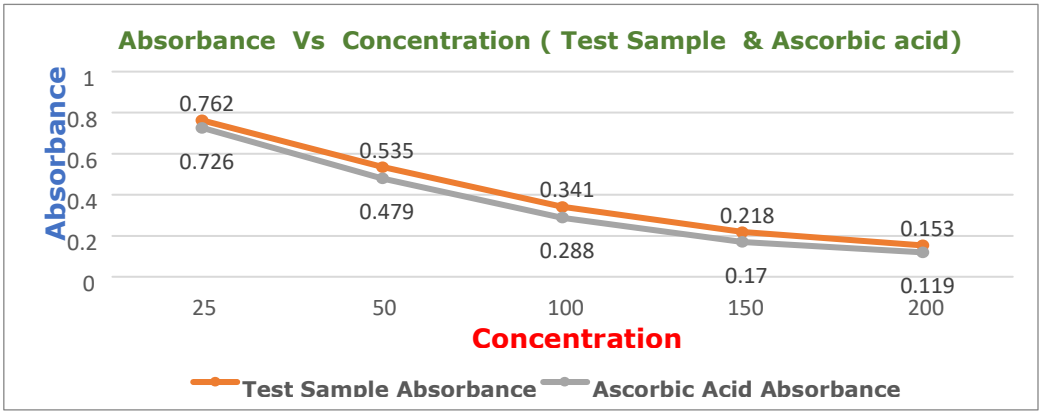

Figure 3. Absorbance for DPPH free radical scavenging assay

The test formulation demonstrated a dose-dependent increase in DPPH radical scavenging activity. At 200 µg/mL, the test sample exhibited 84.7% inhibition, which was comparable to the standard Ascorbic Acid (88.1%).

Table 6. DPPH Free Radical Scavenging assay

S.No.	Concentration (µg/mL)	Test Sample Inhibition (%)	Ascorbic Acid Inhibition (%)
1	25	23.8	27.4
2	50	46.5	52.1
3	100	65.9	71.2
4	150	78.2	83.0
5	200	84.7	88.1

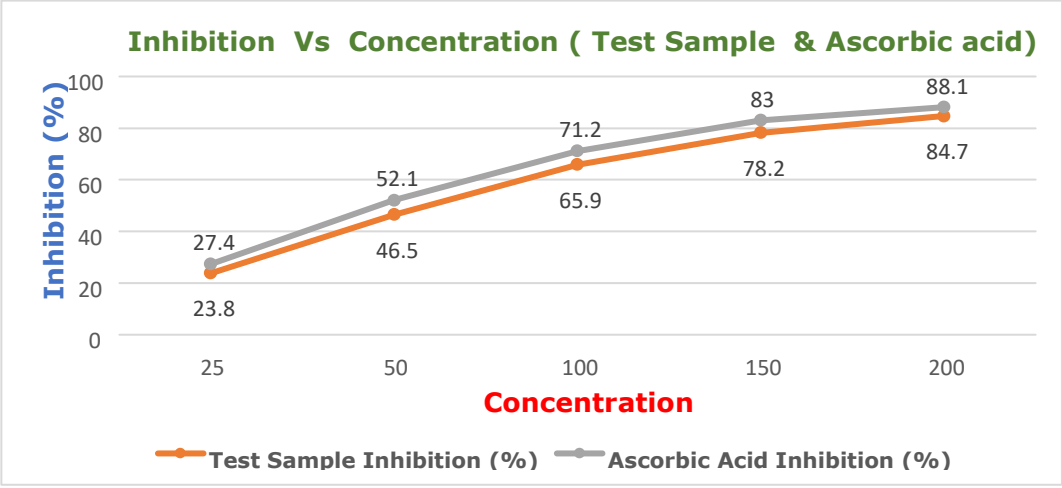

Figure 4. Inhibition for DPPH Free Radical Scavenging Assay

Conclusion

All extraction methods (steam distillation, freeze-drying, maceration, and hydroalcoholic extraction) were effective in isolating bioactive compounds, with concentrations near or matching standard drug values. The combination of peppermint, Aloe vera, arnica, and calendula extracts resulted in a formulation with significant anti-inflammatory and antioxidant activities. The formulation showed good potential for use in topical treatments for skin conditions. The spray formulation demonstrated bioactivity comparable to standard pharmaceuticals, making it a promising candidate for future dermatological and cosmetic applications.

Funding

No funding was received for this study.

Acknowledgement

The authors are thankful to the management of Sri Venkateswara College of Pharmacy, for providing facilities and support to carry out this work.

Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

Concept: T.M. Poorna Chandra, design: T.M. Poorna Chandra, data collection: M. Gowtham Vivek Reddy, K.V. Rekha, D. Chandana, G. Lahari, A. Fazil, analysis: T.M. Poorna Chandra, writing: M. Gowtham Vivek Reddy, K.V. Rekha, D. Chandana, G. Lahari, A. Fazil.

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