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Research Article

GC-MS CHARACTERIZATION AND PHYSICOCHEMICAL PROFILING OF SAPLINT PLUS: A POTENT ANTI-INFLAMMATORY LINIMENT

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ABSTRACT

Aches and pains significantly impact an individual's quality of life, often arising from natural aging, chronic conditions like degenerative arthritis, or fibromyalgia. This study aims to characterize the physicochemical properties and chemical composition of Saplint Plus, a potential anti-inflammatory liniment, using advanced analytical techniques. Physicochemical evaluations, including, viscosity, density, solubility, and thermal stability, were conducted to assess its suitability for topical application. Gas chromatography-mass spectrometry (GC-MS) were employed to identify and profile the bioactive phytochemical constituents. The results revealed that Saplint Plus exhibits optimal physicochemical properties, such as ideal pH, viscosity, and stability, for topical use. GC-MS analysis identified 21 bioactive compounds, with cyclohexanol, thymol, and eucalyptol as major constituents, which are known for their anti-inflammatory properties. These findings highlight the robust chemical composition of Saplint Plus, underscoring its potential as a therapeutic liniment. The study concludes that Saplint Plus possesses promising physicochemical characteristics, making it a viable candidate for anti-inflammatory applications. Further research is recommended to explore its therapeutic efficacy and optimize its formulation.

INTRODUCTION

Inflammation is a physiological response to injury or infection, characterized by redness, swelling, and pain. While it is a protective mechanism, chronic inflammation can lead to various health conditions, including arthritis, cardiovascular diseases, and autoimmune disorders. The demand for safe and effective anti-inflammatory therapies has spurred interest in natural and plant-based remedies, particularly those used in traditional medicine. Topical formulations such as liniments are especially popular due to their localized application and reduced systemic side effects [1,2].

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Conventional treatments, including nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, provide symptomatic relief but are often associated with adverse effects such as gastrointestinal irritation, hepatotoxicity, and dependency issues [3]. As a result, there is a growing interest in herbal-based formulations with anti-inflammatory properties, offering safer and more sustainable therapeutic alternatives.

Saplint Plus is a proprietary liniment formulation used for topical application manufactured by Sitaram Ayurveda, composed of plant-based oils and extracts, traditionally employed for its potential anti-inflammatory effects. While its therapeutic applications have been anecdotally reported, a comprehensive scientific evaluation of its physicochemical properties and chemical profile is essential to validate its efficacy and stability [4,5]. The effectiveness of a liniment depends on its physicochemical properties, including pH, viscosity, solubility, and thermal stability, which influence its absorption and therapeutic potential[6]. The physicochemical evaluation of Saplint Plus was conducted to ensure its stability, efficacy, and suitability as a topical anti-inflammatory liniment. Additionally, understanding its chemical composition is essential for validating its pharmacological efficacy. Advanced analytical techniques such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) allow for the identification of bioactive compounds that contribute to anti-inflammatory activity.

This study aims to evaluate the physicochemical properties and chemical composition of Saplint Plus to establish its suitability as an anti-inflammatory liniment. By employing GC-MS, we provide a detailed characterization of its bioactive constituents and assess its formulation stability. The findings from this research could contribute to the development of improved herbal liniments for managing pain and inflammation.

MATERIALS AND METHODS

Materials

The study utilized Saplint Plus, a commercially available Ayurvedic liniment manufactured by Sitaram Ayurveda Pvt. Ltd. This liniment is a proprietary polyherbal formulation comprising a blend of classical Ayurvedic oil preparations and essential oils known for their anti-inflammatory, analgesic, and muscle-relaxant properties. The key ingredients include *Kottamchukkadi Thailam*, extracts of *Mentha viridis*, *Trachyspermum ammi*, and *Cinnamomum camphora*, along with other essential oils. These botanicals were carefully selected based on their traditional use and reported pharmacological activities to synergistically support pain relief and inflammation management.

Physicochemical Characterization.

The physicochemical evaluation of Saplint Plus Liniment was carried out as per standard protocols [7,8,9] and relevant in-house specifications. The following parameters were assessed:

Chemicals and Reagents: Analytical-grade solvents and chemicals were obtained from NICE chemicals.

Viscosity: Viscosity was determined using a Brookfield viscometer at 25°C with varying spindle speeds.

Specific gravity: The density was measured using a pycnometer at room temperature, and the values were recorded in g/cm^3.

Acid value, Iodine value, refractive index and saponification value were determined according to the procedure provided in the Ayurvedic Pharmacopeia of India (API), and the values were recorded.

Solubility Test: The solubility of the formulation was evaluated in polar and nonpolar solvents, including water, ethanol, and hexane.

GCMS Analysis

Sample Preparation: 200 µL Sample was made up to 5 mL with Hexane and filtered through a syringe filter (Nylon 13 mm 0.2um) into a vial and injected to GCMS

Instrument details and analysis

Instrument Make – Agilent Technologies

Instrument Model -7890 A GC with 5975C with triple axis detector

Column - DB 5MS 30 m x 0.250mm Diameter x 0.25 Micro Meter Thickness

Analysis was performed by injecting 2 μL of the sample in a split mode with a split ratio of 100:1. Helium gas (99.9995%) was used as the carrier gas at a flow rate of 0.6 mL/min. The analysis was performed in the EI (electron impact) mode with 70 eV of ionization energy. The injector temperature was maintained at 280°C (constant). The column oven temperature program is

Oven	Rate °C/min	Value °C/ min	Hold Time
Initial	-	40	5
Ramp 1	5	100	10
Ramp 2	20	280	5

The compounds were identified after comparing the spectral configurations obtained with that of available mass spectral database (NIST -08 SPECTRAL DATA)

RESULTS AND DISCUSSION

Physicochemical Properties

Physicochemical evaluation is essential to determine the quality, stability, and suitability of Saplint Plus as an anti-inflammatory liniment base. Results for the physicochemical parameters of Saplint Oil, evaluated as per standard methodologies [10], and it was represented in the Table -1.

Table 1: Physicochemical properties of Saplint Plus

S.No.	Parameter	Result	Methodology	Significance
1.	Colour	Yellowish Green	Visual inspection	Indicates the oil's purity and processing quality.
2.	Odour	Characteristic	Sensory evaluation	Confirms the presence of essential oils and actives.
3.	Viscosity	22.6 cP at 60 RPM using spindle L3 at 27.5 ± 0.5°C.	Brookfield Viscometer	Ensures optimal Spreadability during topical application.
4.	Acid value	5.6 ± 0.15	Titration method (API)	Indicating low levels of free fatty acids, ensuring stability and quality.
5.	Iodine value	108.93 ± 1.25	Wijs method (API)	Indicating the degree of unsaturation in the oil.
6.	Refractive index	1.478 ± 0.002	Abbe Refractometer	Indicates the oil's purity and consistency.
7.	Saponification value	98.76 ± 0.85	Titration method (API)	Assesses fatty acid content for emulsifying properties.
8.	Specific gravity	0.928 ± 0.005	Pycnometer method	Assesses oil density for formulation stability.

Solubility Test

The oil was soluble in non-polar solvents like hexane and toluene but insoluble in polar solvents like water, confirming its non-polar nature. The result is represented in the Table -2

Table 2: Solubility test result of Saplint Plus

S.No	Solvent	Polarity	Observation	Result
1.	Water	Polar	Layered separation	In soluble
2.	Ethanol	Polar	Turbid and becomes clear at 60^{0}C	Partially soluble becomes completely soluble at 60^{0}C
3.	Methanol	Polar	Clear Solution	Soluble
4.	Acetone	Polar	Clear Solution	Soluble
5.	Isopropyl Alcohol	Polar	Clear Solution	Soluble
6.	Hexane	Non-Polar	Clear Solution	Soluble
7.	Chloroform	Non-Polar	Clear Solution	Soluble
8.	Toluene	Non-Polar	Clear Solution	Soluble
9.	Diethyl ether	Non-Polar	Clear Solution	Soluble
10.	Ethyl Acetate	Semi-Polar	Clear Solution	Soluble
11.	Dimethyl Sulfoxide	Semi-Polar	Turbid	Partially Soluble

The solubility profile of Saplint Plus provides critical insights into its chemical nature and potential applications. The results indicate that the oil is soluble in non-polar solvents such as hexane, chloroform, toluene, and diethyl ether, confirming its non-polar characteristics. This suggests that Saplint Plus consists predominantly of lipophilic (fat-soluble) compounds, which aligns with its formulation as an oil-based liniment.

In polar solvents, the solubility varied. The Saplint Plus was insoluble in water, as expected for a non-polar substance, leading to layered separation. It showed partial solubility in ethanol, with complete dissolution occurring upon heating to 60°C, indicating the presence of compounds that may require higher energy input to dissolve in polar media. However, the oil exhibited good solubility in methanol, acetone, and isopropyl alcohol, suggesting that certain constituents have moderate polarity and interact favourably with these solvents.

In semi-polar solvents, the solubility behaviour was mixed. Ethyl acetate, which has both polar and non-polar characteristics, allowed complete dissolution, whereas dimethyl sulfoxide (DMSO) resulted in a turbid solution, indicating partial solubility. This could be due to the presence of certain medium-polarity components that do not fully interact with the highly polar environment of DMSO.

These findings reinforce the non-polar nature of Saplint Plus, making it well-suited for oil-based formulations and lipophilic drug delivery systems. Its solubility in organic solvents also suggests compatibility with extraction, formulation, and bioavailability enhancement techniques.

GCMS Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed to elucidate the chemical composition of Saplint Plus, a liniment-based formulation developed for the management of joint pain and inflammation. The analysis revealed the presence of 21 distinct volatile organic compounds, with their retention times (RT), molecular identities, and relative abundance (area %) documented in Table 3. And the chromatogram is represented in the Figure -1. These compounds, predominantly terpenoids, phenolic derivatives, and cyclic alcohols, are known for their pharmacological properties, including anti-inflammatory, analgesic, antioxidant, and antimicrobial effects, which collectively contribute to the therapeutic efficacy of the formulation.

Table 3: GC- MS profile compounds and its significance (identified using NIST Library Data)

S.No	RT	Molecules	Area %	Properties	
				Primary	Secondary
1.	11.03	α-Pinene	2.21	Anti-inflammatory	Analgesic, Antioxidant
2.	11.64	Camphene	0.02	Anti-inflammatory	Antimicrobial
3.	12.72	β-Pinene	0.06	Antimicrobial	Bronchodilator
4.	13.84	α-Phellandrene	0.08	Anti-inflammatory	Antioxidant
5.	14.55	o-Cymene	0.29	Antioxidant	Anti-inflammatory
6.	14.74	Eucalyptol	10.32	Pain relief	Analgesic, Expectorant
7.	15.71	γ-Terpinen	0.14	Anti-inflammatory	Antimicrobial
8.	18.91	Camphor	0.29	Analgesic	Counterirritant, Cooling agent
9.	19.14	β-Citronellal	0.07	Muscle relaxant	Insecticidal
10.	19.88	Isoborneol	3.85	Anti-inflammatory	Antimicrobial, CNS stimulant
11.	20.84	Cyclohexanol	37.50	Anti-inflammatory	Local anesthetic
12.	21.36	Methyl salicylate	6.80	Antibacterial	Pain reliever, Counterirritant
13.	23.94	β-Citral	4.14	Anti-inflammatory	Antioxidant
14.	25.19	Geraniol	0.54	Antimicrobial	Anti-inflammatory, Antioxidant
15.	26.31	α-Citral	5.89	Anti-inflammatory	Antioxidant, Spasmolytic
16.	28.75	Thymol	27.02	Anti-inflammatory	Analgesic, Anti-inflammatory
17.	29.44	Carvacrol	0.10	Anti-inflammatory	Antioxidant, Antibacterial
18.	30.11	Geraniol acetate	0.26	Antibacterial	Antioxidant
19.	30.78	Caryophyllene	0.17	Anti-inflammatory	Pain modulator
20.	31.04	Aromadendrene	0.11	Anti-inflammatory	Anti-inflammatory
21.	32.64	Caryophyllene oxide	0.15	Anti-inflammatory	Analgesic

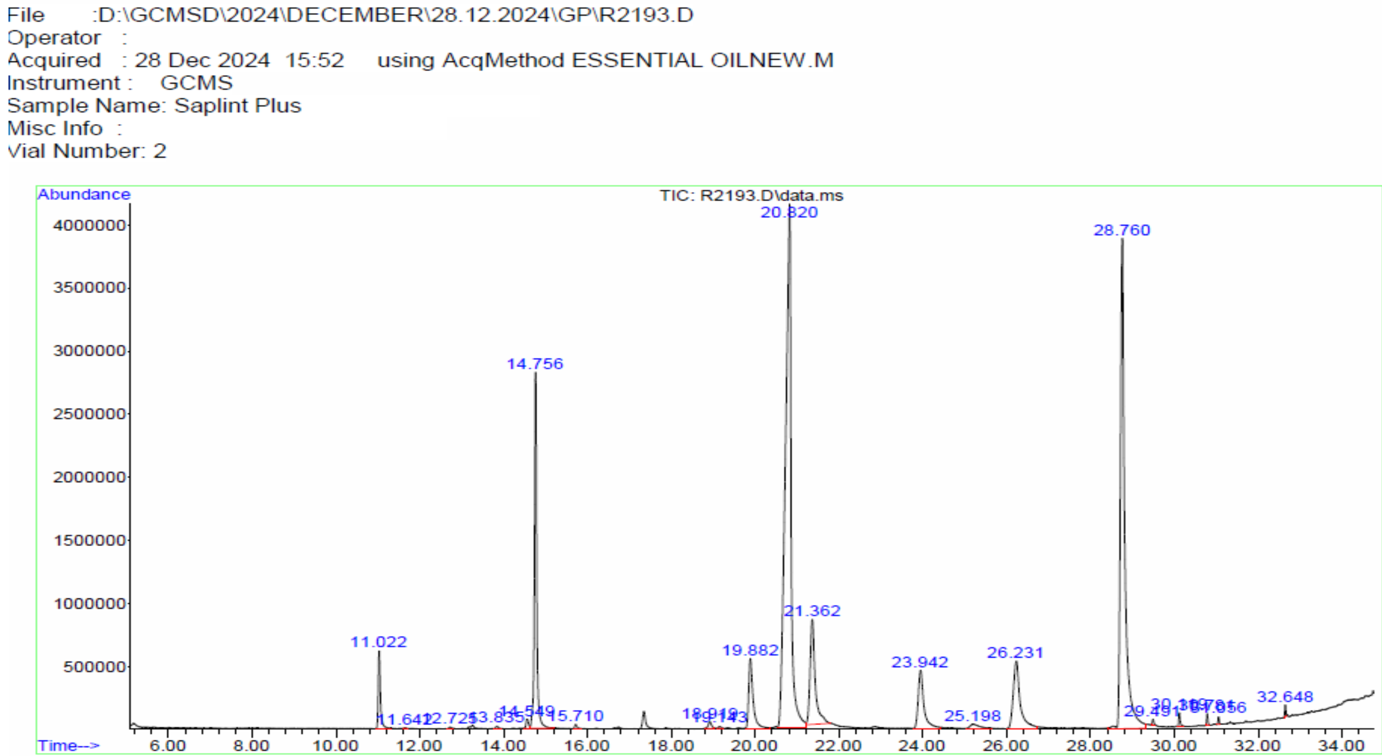

Figure1: GCMS chromatogram of Saplint Plus

The most abundant constituent identified was Cyclohexanol (RT 20.84 min), accounting for 37.50% of the total area, suggesting it plays a significant role in the formulation. Cyclohexanol is recognized for its muscle relaxant and local anesthetic properties, which may enhance the liniment's ability to alleviate joint stiffness and discomfort. Following this, Thymol (RT 28.75 min) was detected at 27.02%, a phenolic monoterpene renowned for its potent analgesic, antimicrobial, and anti-inflammatory activities, making it a key contributor to the product's efficacy in reducing inflammation and pain.

Eucalyptol (RT 14.74 min), constituting 10.32%, is another major component, valued for its analgesic, anti-inflammatory, and expectorant properties, potentially aiding in the relief of musculoskeletal discomfort. Methyl salicylate (RT 21.36 min), present at 6.80%, is a well-documented counterirritant and pain reliever, structurally related to salicylic acid, which underscores its role in topical pain management. The formulation also contains α-Citral (RT 26.31 min, 5.89%) and β-Citral (RT 23.94 min, 4.14%), isomers of citral with anti-inflammatory, antioxidant, and spasmolytic effects, further supporting the liniment's therapeutic profile.

Other notable constituents include Isoborneol (RT 19.88 min, 3.85%), a bicyclic monoterpene alcohol with analgesic, antimicrobial, and central nervous system (CNS) stimulant properties, and α-Pinene (RT 11.03 min, 2.21%), a monoterpene with anti-inflammatory, analgesic, and antioxidant activities. Minor components such as Geraniol (RT 25.19 min, 0.54%), Caryophyllene (RT 30.78 min, 0.17%), and Carvacrol (RT 29.44 min, 0.10%) were also identified, each contributing antibacterial, anti-inflammatory, and antioxidant effects, albeit in smaller proportions. Out of the 21 identified compounds, 16 molecules exhibited anti-inflammatory activity either as a primary or secondary property.

The GC-MS profile indicates a synergistic blend of bioactive molecules, with a predominance of compounds exhibiting anti-inflammatory and analgesic properties, aligning with the intended application of Saplint Plus for joint pain and inflammation relief. The diversity of terpenoids (e.g., α-Pinene, β-Pinene, Camphene, Eucalyptol), phenolic compounds (e.g., Thymol, Carvacrol), and other cyclic structures (e.g., Cyclohexanol, Methyl salicylate) suggests a multi-target mechanism of action, potentially enhancing efficacy through complementary pharmacological effects. Trace constituents such as Camphene (RT 11.64 min, 0.02%) and β-Citronellal (RT 19.14 min, 0.07%) were present in minimal amounts but may still contribute subtly to the overall antioxidant and antimicrobial profile of the formulation.

This compositional analysis highlights the complex phytochemistry of Saplint Plus, providing a scientific basis for its therapeutic claims. Further studies, including *in vitro* and *in vivo* assays, are recommended to validate the bioactivity of these constituents and their synergistic interactions in the context of joint pain and inflammation management.

CONCLUSION

The present study successfully evaluated the physicochemical properties and chemical composition of Saplint Plus, an Ayurvedic liniment developed for the management of joint pain and inflammation. Physicochemical characterization demonstrated the formulation's stability, non-polar nature, and suitability for topical application. GC-MS analysis revealed the presence of 21 bioactive compounds, of which 16 exhibited anti-inflammatory activity either as a primary or secondary property. Key constituents such as Cyclohexanol, Thymol, Eucalyptol, and Methyl salicylate contribute significantly to the formulation’s therapeutic efficacy. The synergistic interplay of terpenoids, phenolic compounds, and cyclic alcohols likely underpins the potent anti-inflammatory and analgesic effects observed with Saplint Plus. These findings provide scientific validation for its traditional use and support its potential as a safe, stable, and effective herbal-based alternative for managing musculoskeletal discomfort and inflammation.

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