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PHYTO-THERAPEUTIC BALM FORMULATION OF OCIMUM SANCTUM, CALOTROPIS GIGANTEA, AND CINNAMOMUM ZEYLANICUM: EVALUATION AND HPLC PROFILING OF BIOACTIVE EXTRACTS

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ABSTRACT

Inflammation represents a pathological condition that can potentially contribute to various chronic diseases. This study assesses the anti-inflammatory properties of Calotropis gigantea flowers, Ocimum sanctum (Tulsi) leaves, and Cinnamomum verum (Cinnamon) bark. The phytochemical profile of these plant materials was examined, and they were subsequently incorporated into a formulation for a herbal balm. The increasing demand for herbal medicine can be attributed to its high efficacy, affordability, ease of use, compatibility with the human body, and reduced side effects. Rheumatoid arthritis (RA) and gout are common forms of inflammatory arthritis, characterized by chronic pain, joint inflammation, and substantial detriment to quality of life. Although conventional treatments, such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, may provide symptomatic relief, they frequently entail adverse effects. This investigation explores a novel herbal formulation to mitigate inflammation and pain associated with RA and gout, employing a synergistic combination of Calotropis gigantea flowers, Ocimum sanctum leaves, and Cinnamomum verum bark. The plant materials underwent methanolic extraction utilizing a Soxhlet apparatus, and qualitative phytochemical analyses confirmed the presence of flavonoids. High-Performance Liquid Chromatography (HPLC) was employed to identify bioactive compounds, including Quercetin, Kaempferol, and Apigenin, which are recognized for their significant anti-inflammatory properties. Further in vitro studies are essential to validate the therapeutic efficacy of this novel herbal preparation.

KEYWORD:- Rheumatoid Arthritis, Gout, Calotropis gigantea, Ocimum sanctum, Cinnamomum verum, Anti-inflammatory, HPLC Analysis, Balm.

1. INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease that causes chronic inflammation, potentially damaging joints and various organs, including the heart, kidneys, and lungs. It is characterized by symptoms such as morning stiffness lasting over 30 minutes, fatigue, fever, weight loss, and swollen, tender joints. RA typically begins between the ages of 35 and 60, but can also affect children (juvenile RA). Its prevalence is about 1–2% in the West. In contrast, gout is an inflammatory joint disease caused by elevated uric acid levels, leading to urate crystal deposits in the joints. It is more common in men over 50, affecting approximately 0.12-0.19% of the Indian population, and is less prevalent in premenopausal women due to estrogen aiding urate clearance. Hyperuricemia is a key predictor of gout.[1]

Patients with RA often experience significant joint damage, resulting in deformities and bone erosion, which can be extremely painful. Common symptoms include prolonged morning stiffness lasting more than 30 minutes, fatigue, fever, weight loss, and joints that appear tender, swollen, and warm. Some individuals may also develop rheumatoid nodules beneath the skin. Typically, RA presents between the ages of 35 and 60, although it can also affect younger individuals, sometimes referred to as juvenile RA (JRA), which occurs in those under 16 and generally lacks the presence of rheumatoid factor.[1]

In terms of prevalence, RA affects approximately 1-2% of the population in Western countries, with a global prevalence of around 1%. In contrast, gout, characterized by elevated uric acid levels, leads to painful joint inflammation and can result in kidney complications due to the deposition of urate crystals. In India, gout affects about 0.12-0.19% of the population, with a higher incidence noted in men over 50 years of age. Notably, estrogen in premenopausal women plays a role in urate clearance, contributing to lower prevalence rates in this demographic. Ultimately, hyperuricemia remains a vital risk factor for developing gout.[2]

Arthritis is an umbrella term for over 100 disorders characterized by joint inflammation, commonly affecting fingers, hips, wrists, and knees. Its causes include aging, genetics, gender, joint injuries, and obesity. Rare forms can also affect organs and connective tissues. Management typically involves medications such as acetaminophen, NSAIDs.[3]

Table 1: Scientific classification.

Plant name	Kingdom	Division	Class	Order	Family	Genus	Species
Calotropis Gigantae	Plantae	Tracheophyta	Magnoliopsida	Gentianales	Apocynaceae	Calotropis	Calotropis gigantea (L.)
Ocimum santum	Plantae	Magnoliophyta	Magnoliopsida	Lamiales	Lamiaceae	Ocimum	O. sanctum
Cinnamomum verum	Plantae	Magnoliophyta	Magnoliopsida	Laurales	Lauraceae	Cinnamomum	Zeylanicum

Corticosteroids, and anti-rheumatic agents, all of which can have side effects. Natural therapies for conditions like osteoarthritis, gout, and rheumatoid arthritis are also being explored. Gout results from the accumulation of monosodium urate crystals, leading to intense inflammation in joints, usually starting in the legs. This condition can recur, potentially leading to the development of tophi after years of episodes. For individuals with serum urate levels above 9.0 mg per deciliter, the annual incidence of acute gout is about 5%. Hyperuricemia may stem from genetic or acquired factors, along with associated health conditions. The global prevalence of gout varies widely, from less than 1% to 8%, with an incidence rate of 0.58 to 2.89 cases per 1,000 individuals annually.[4]

Arthritis is a term that encompasses over 100 disorders characterized by joint inflammation, primarily impacting the fingers, hips, wrists, and knees. Commonly influenced by factors such as aging, genetics, gender, joint injuries, and obesity, arthritis can also manifest in rare forms that affect organs and connective tissues. Management strategies typically involve medications like acetaminophen, NSAIDs, corticosteroids, and anti-rheumatic agents, all of which may carry side effects; therefore, natural therapies for conditions such as osteoarthritis, gout, and rheumatoid arthritis are also under investigation. Gout, a specific type of arthritis, arises from the buildup of monosodium urate crystals in joints, often leading to acute inflammation, particularly in the legs. This condition can recur and may lead to the formation of tophi after prolonged episodes. In individuals with serum urate levels exceeding 9.0 mg per deciliter, the annual incidence of acute gout is around 5%. Hyperuricemia can result from a variety of genetic and acquired factors, alongside associated health conditions. The global burden of gout continues to rise, with its prevalence varying from under 1% to 8%, while the annual incidence is reported to be between 0.58 and 2.89 cases per 1,000 individuals.[4]

This research seeks to create a new pharmaceutical formulation that effectively manages inflammation and reduces adverse effects of traditional treatments. Using in vitro methods, the study will evaluate the anti-rheumatoid and gout activities of specific extracts, potentially leading to safer and more effective treatment options.

1.1 MATERIALS AND METHODS

1.2 Plant description

Fig. 1: Calotropis gigantea linn.

1.2.1 Calotropis gigantea linn

Calotropis gigantea Linn, a member of the Asclepiadaceae family, is indigenous to India, China, and Malaysia, yet its distribution extends globally. Commonly referred to as Madar, this notable medicinal herb has been utilized for centuries within the Unani, Ayurveda, and Siddha systems of medicine. The phytochemical profile of Calotropis gigantea includes constituents such as giganteol, α- and β-calotropeol, β-amyrin, and isogiganteol, among others. Key flavonoids present in the plant include Calotropin, Quercetin, Kaempferol, Rutin, Eugenol, Apigenin, and Luteolin, which can be found in various parts, including the leaves, roots, flowers, and latex. This species typically grows as a robust shrub, reaching heights of 1 to 5 meters (approximately 3 to 16 feet). It thrives in arid environments, along coastal regions, roadsides, and other disturbed habitats. The leaves are simple, arranged oppositely in a decussate manner, and are characterized as sub-sessile and devoid of stipules, with a blade that is oblong, obovate, or broadly obovate, measuring 5–30 cm by 2.5–15.5 cm. The flowers are complete and bisexual, featuring bracts, acting as actinomorphic structures that are pentamerous, hypogynous, and pedunculate. The calyx consists of five sepals that are briefly fused at the base, while the corolla is gamopetalous. The fruit is a

simple, fleshy, inflated structure that is subglobose to obliquely ovoid in shape. Its seeds, measuring approximately 6-5 mm, are flat and compressed, adorned with silky white pappus. Research has demonstrated the anti-inflammatory properties of Calotropis gigantea using the albumin denaturation technique. The percentage inhibition of denaturation induced by this herbal remedy was found to be comparable to that of Ibuprofen (85.71%), thereby indicating a significant anti-inflammatory activity.[5],[6]

Fig. 2: Ocimum Santum.

1.2.2 Ocimum Santum (tulsi)

Tulsi, often referred to as the "Queen of Herbs," is a revered and medicinal plant mentioned in ancient literature. Belonging to the Lamiaceae family, it is characterized by its square stem and distinctive aroma. The botanical designation for Tulsi is Ocimum sanctum (Linn), and it is widely distributed across regions such as Malaysia, Australia, West Africa, and various Arab countries. Within this genus, Ocimum sanctum is the most prominent species. The leaves of this plant hold significant religious importance and are frequently used in Hindu spiritual rituals, such as Tirtha or Prasada. There are two main varieties of Ocimum sanctum: black (Krishna Tulsi) and green (Rama Tulsi), which share similar chemical constituents and therapeutic properties. This erect, branched plant typically reaches a height of 30 to 60 cm upon maturity. Its aromatic leaves are simple, opposite, and can be elliptic, oblong, obtuse, or acute in shape, with margins that are entire or subtly serrated, growing up to 5 cm in length. The extraction of fresh leaves and stems of Ocimum sanctum yields various phenolic compounds with antioxidant properties, including cirsilineol, circimaritin, isothymusin, apigenin, and rosmarinic acid, along with significant quantities of eugenol. The leaves contain approximately 0.7% volatile oil, which comprises around 71% eugenol and 20% methyl eugenol, as well as carvacrol and the sesquiterpene hydrocarbon caryophyllene. Additionally, two flavonoids, orientin and avisin, have been isolated from the aqueous extract of Ocimum sanctum leaves.[4],[7],[8],[9],[10]

Fig. 3: Cinnamomum verum (Dalchini).

1.2.3 Cinnamomum verum (Dalchini)

Cinnamomum verum, or true cinnamon, is part of the Lauraceae family and is primarily cultivated in Sri Lanka and Southern India. This aromatic herb has a rich history in traditional medicine across Korea, China, and Russia. Cinnamomum zeylanicum, known as Dalchini, is an evergreen shrub that can grow 6 to 8 meters tall, characterized by its thick, reddish-brown bark. Cinnamon contains an array of resinous compounds, including cinnamaldehyde, cinnamate, and cinnamic acid, along with essential oils such as trans-cinnamaldehyde, eugenol, and L-borneol. Its phytochemical profile includes aldehydes, alcohols, esters, phenols, and flavonoids. Notably, methanolic and ethanolic extracts of C. zeylanicum have shown the ability to inhibit lipoxygenase (LOX) enzyme activity in mice, indicating potential anti-inflammatory properties.[2],[2],[3],[11]

1.3 Plant material

The collection of Calotropis gigantea flowers was conducted from the roadside in Yashwant Nagar, Khopoli. Additionally, the leaves of Ocimum sanctum were sourced from the herbal garden in Shedung, while the bark powder of cinnamon was obtained from a herbal store in Khopoli.

Reagents and Chemicals

The chemicals and solvents utilized in this study encompassed distilled water, dilute sodium hydroxide (NaOH), lead acetate, ferric chloride (FeCl3), methanol, and reagents such as vanillin hydrochloride. These materials were sourced from the Chhatrapati Shivaji Maharaj University, School of Pharmacy, located in Panvel, Maharashtra, India.

1.3.1 Extraction procedure (Soxhlet extraction)

To prepare the powder sample for analysis, Calotropis gigantea flowers, Ocimum sanctum, and cinnamon were carefully selected and weighed to a total of 60 grams. The measured components were then mixed thoroughly to achieve a homogeneous mixture. The extraction of this powdered mixture was carried out using methanol as the solvent in a Soxhlet apparatus for 5 hours, with the

extraction temperature maintained between 40-60°C to ensure optimal efficiency. Upon completion of the extraction process, the mixture was filtered to eliminate any impurities. The filtered extract was subsequently dried in a hot air oven at a temperature of approximately 70-80°C. This concentrated extract was then ready for further analysis and is expected to contain a variety of bioactive compounds from Calotropis gigantea, Ocimum sanctum, and cinnamon, which could be utilized for various applications.[12],[13],[13],[14]

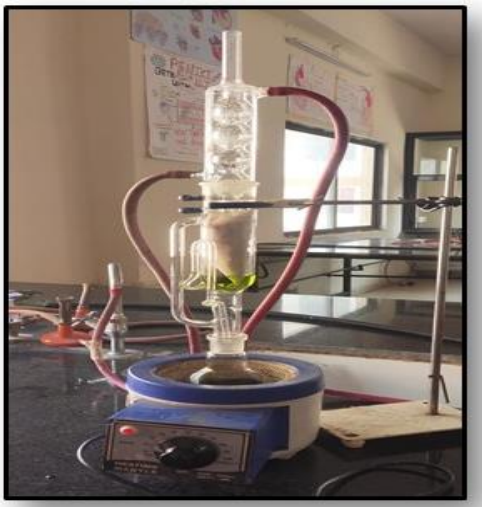


Table 2: Evaluation Tests of the extract for flavonoids.

Tests	Procedure	Result
Ferric Chloride	Method: Add the test solution to a solution of ferric chloride, and the development of colour (Usually blue or green) indicates the presence of flavonoids.	Pass
Lead Acetate	Mix the test solution with lead acetate, and the formation of a yellow precipitate indicates the presence of flavonoids.	Pass
Alkaline Reagent Test/ Sodium Hydroxide Test	Mix the plant extract sample with a few drops of dilute NaOH solution. Observe an intense yellow colour.	Pass
Vanillin-HCL	Mix the test solution with Vanillin-HCL reagent, and the appearance of colour (Usually pink to red) indicates the presence of flavonoids.	Pass

1.3.2 HPLC result

1. **Materials:** - Calotropis gigantae flower, Tulsi leaves, cinnamon bark
2. **Extraction procedure:** - Combined (60gm) of Calotropis gigantae flower, Tulsi leaves, cinnamon bark powder was extracted with 100 mL of methanol using a Soxhlet apparatus for 6 hours at 60-70^{o}c temp.

HPLC analysis

To identify and quantify the presence of

1. Twelve active ingredients: Quercetin, Kaempferol, Rutin, Apigenin, Luteolin, cirsilineol, circimaritin, isothymusin, orientin, andvicenin, Catechin
2. Bio active compounds: Calotropin, Giantolide, Rosmarinic acid, Caryophellene and Cinnamic acid. These compounds are known for their potential Anti-rheumatoid and anti-gout activity.

HPLC Conditions

- **Materials**

1. Plant Material: (Combined 60gm)

- Calotropis gigantae flower (25 g)
- Tulsi leaves (30 g)
- Cinnamon bark (05 g)

2. Extraction Solvent: Methanol
3. Instrument: Agilent 1100 Series HPLC system

- **HPLC Instrumentation**

1. HPLC system with UV detector at 254 nm
2. C18 reverse-phase column (Luna C18, 5 µm particle size, 250 mm × 4.6 mm)
3. Mobile Phase: Gradient elution using water (solvent A) and acetonitrile (solvent B) with 0.1% formic acid added to both solvents (specific gradient program optimized for separation)
4. Gradient Program:

- 0-5 min: 5% B
- 5-10 min: 5-15% B
- 10-20 min: 15-40% B
- 20-25 min: 40-95% B
- 25-30 min: 95% B
- 30-35 min: 95-5% B

5. Flow Rate: 1.0 mL/min
6. Detection: UV absorbance at 254 nm
7. Injection Volume: 20 µL

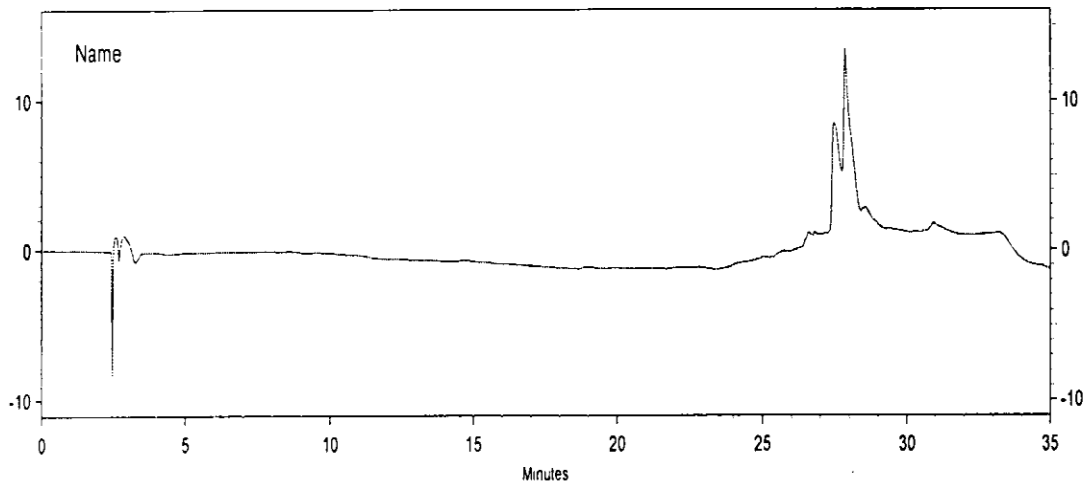
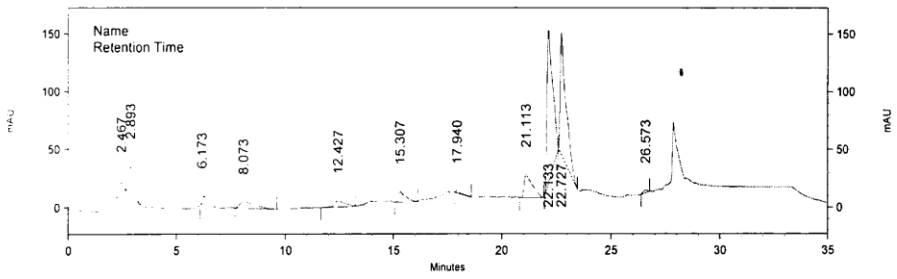


Table 5: Standard retention time of the compound.

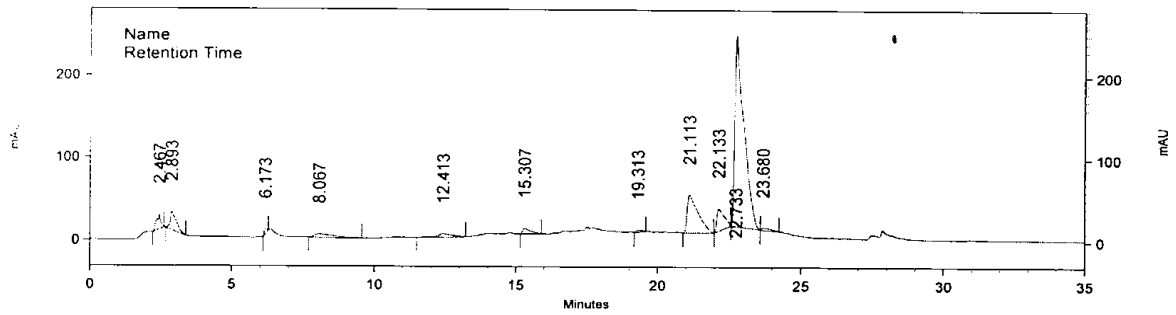
S. N	Compound	Retention time in min.
1	Quercetin	3.6-14.4
2	Kaempferol	3.88-16.3
3	Rutin	2.3-7.0
4	Eugenol	3.0-14
5	Apigenin	3.53-8.30
6	Luteolin	2.9-3.0
7	Calotropin	12.3
8	Giantolide	5-10
9	Rosmarinic acid	15-20
10	Caryophellene	3-35
11	Cinnamic acid.	10-18
12	Isothymusin	12.3
13	Cirsilineol	1.23
14	Circimaritin	13-15



DAD: Signal A, 254 nm/Bw:4 nm

Results

Peak No.	Name	Retention Time	Area	Area Percent
1		2.47	239116	1.913
2		2.89	693410	5.548
3		6.17	83003	0.664
4		8.07	566753	4.534
5		12.43	340149	2.721
6		15.31	404007	3.232
7		17.94	53549	0.428
8		21.11	1075624	8.605
9		22.13	4963591	39.711
10		22.73	4022529	32.182
11		26.57	57658	0.461
Totals			12499389	100.000



DAD: Signal B, 270 nm/Bw:4 nm
Results

Peak No.	Name	Retention Time	Area	Area Percent
1		2.47	367653	2.257
2		2.89	676963	4.155
3		6.17	76540	0.470
4		8.07	346347	2.126
5		12.41	257927	1.583
6		15.31	256196	1.573
7		19.31	56675	0.348
8		21.11	2498153	15.334
9		22.13	979311	6.011
10		22.73	10656535	65.413
11		23.68	118772	0.729
Totals			16291072	100.000

1.4 Formulation of polyherbal balm

1.4.1 Materials

Herbal extract (Calotropis gigantea flower, Tulsi leaves, and cinnamon bark); Coconut oil; Bees Wax; Petroleum jelly; Vitamin C oil; Menthol; Methyl salicylate; Camphor; Benzoic acid.

1.4.2 Procedure

The formulation process begins with the selection of an appropriate container, which should be weighed before adding 4 grams of petroleum jelly. This container is then placed on a hot plate to heat the mixture until the petroleum jelly is completely dissolved. Subsequently, 2 ml of methyl salicylate is measured and brought to a boil in the same setup. After this, 10 grams of beeswax are weighed and added to the dissolved petroleum jelly solution, with continuous stirring maintained during the heating process to ensure full incorporation of the beeswax. Once dissolved, 4 grams of menthol crystals are introduced, and the mixture is heated and stirred until the menthol is entirely dissolved. Following this step, 0.5 grams of herbal extract is weighed and integrated into the solution under thorough heating. The formulation continues with the addition of 1 ml of vitamin C oil, which is stirred into the mixture while boiling, followed by the careful weighing and incorporation of 1 gram of benzoic acid, ensuring all components are fully dissolved. After achieving a uniform liquid state, the solution is carefully removed from the hot plate and allowed to cool, resulting in a semi-solid balm. Finally, the cooled balm is poured into a suitable container,

sealed securely, and stored in a cool, dry environment for further evaluation and testing.

Table 3: Formulation table.

Sr. No.	Ingredients	Quantity	Medicinal uses
1.	Herbal extract	0.5gm	Relieve arthritis pain & prevent inflammation
2.	Coconut oil	3ml	Solvent
3.	Bees Wax	10gm	Base, Thickener
4.	Petroleum jelly	4gm	Relieves dry skin
5.	Vitamin C oil	1ml	Anti-inflammatory properties
6.	Menthol	4gm	Counter irritant
7.	Methyl salicylate	2ml	Analgesic, skin absorbent
8.	Camphor	1gm	Cooling effect
9.	Benzoic acid	1gm	Preservative

1.4.3 Evaluation parameter

1.4.3.1 Organoleptic evaluation

It refers to the evaluation of herbal balm by its colour, odour, appearance, texture etc. The external character of the formulation was examined.

1.4.3.2 Colour: The balm should have a Lime green colour

1.4.3.3 Odour: The balm has a strong fragrance of herbal extract.

1.4.3.4 Consistency: The consistency of the formulation was checked by applying to the skin. It has a smooth, uniform consistency without lumps or graininess.

1.4.3.5 Determination of pH: The pH of the prepared formulation was determined by using a Digital pH meter by preparing 10% solution and dipping the glass electrode completely in the solution system to cover the electrode.

1.4.3.6 pH Level: The pH of the balm is Neutral.

1.4.3.7 Phase separation: The prepared balm was transferred in a suitable wide-mouth container. Set aside for storage. The balm was stable at room temperature. And No Phase separation was observed.

1.4.3.8 Spreadability: Spreadability was determined by placing a sample between two glass slides, which were compressed to uniform thickness by applying a definite time period. The time required to separate two slides was measured as spreadability less the time taken for the separation of two slides, showing better spreadability calculated by formula.

$S=M*L/T$

S = Spreadibility

M = Weight applied to two slides

L = Length of slide

T = Time taken to separate the slide

1.4.3.9 Solubility: Soluble in boiling water, miscible in alcohol and ether

1.4.3.10 Non-irritancy: The Prepared formulation was applied to the skin of a Human being and the effect

1.4.3.11 Stability study: Physical stability of prepared balm was carried out for 3 months at various temperature conditions like 20 °C, 25^0C and 37^0C

1.4.3.12 Washability: Balm was applied to the skin the washability with water was checked.

2. CONCLUSION

This study successfully developed and assessed a phyto-therapeutic balm that incorporates a methanolic extract derived from the leaves of Ocimum sanctum, the flowers of Calotropis gigantea, and the bark of Cinnamomum verum. These medicinal plants are characterized by their high flavonoid content and notable anti-inflammatory properties. High-Performance Liquid Chromatography (HPLC) analysis confirmed the presence of bioactive compounds within the formulation. Following the identification of these constituents, the preparation of the anti-inflammatory balm commenced. In vitro testing demonstrated a significant anti-inflammatory activity of the balm. These findings underscore the potential of the herbal formulation as an effective alternative for the management of inflammation associated with rheumatoid arthritis and gout, thus necessitating further in vitro and clinical investigations to validate its therapeutic efficacy.

Table 4: Evaluation result of herbal balm.

Sr. No.	Parameter	Results
1	Colour	Lime green
2	Odour	Strong Aromatic
3	pH	Neutral
4	Consistency	Fair
5	Spreadibility	Good
6	Phase separation	No phase separation

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