

FORMULATION AND EVALUATION OF A EUCALYPTUS (*EUCALYPTUS GLOBULUS*)-BASED HERBAL MASSAGE OIL: PHYSICOCHEMICAL, ANTIBACTERIAL, AND ANTIOXIDANT STUDIES

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ABSTRACT

Herbal topical formulations are widely utilized for the management of musculoskeletal disorders due to their safety, efficacy, and minimal side effects. The present study aimed to formulate and evaluate an herbal massage oil containing *Eucalyptus globulus* leaves using a simple and rapid heat infusion method. The formulation incorporated eucalyptus leaves as the active ingredient, coconut oil or sesame oil as the base, along with camphor, vitamin E, parabens, and propylene glycol to enhance therapeutic efficacy, stability, and preservation.

The prepared oil was evaluated for organoleptic properties, spreadability, homogeneity, clarity, stability, and skin irritation. The formulation exhibited a characteristic aroma, good consistency, excellent spreadability, and stability without any signs of phase separation or degradation. Skin irritation studies conducted on human volunteers confirmed its safety for topical application.

The antibacterial activity was assessed using the agar well diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*, demonstrating significant concentration-dependent inhibition, with higher susceptibility observed in Gram-positive bacteria. Antioxidant activity evaluated by the DPPH assay revealed notable free radical scavenging potential, increasing with concentration.

The results indicate that the formulated eucalyptus herbal massage oil possesses significant analgesic, anti-inflammatory, antibacterial, and antioxidant properties, primarily attributed to bioactive constituents such as eucalyptol. The study concludes that this formulation is a safe, effective, economical, and easily preparable herbal product suitable for topical application in pain relief and inflammation management, with potential applications in pharmaceutical, cosmetic, and traditional medicine fields.

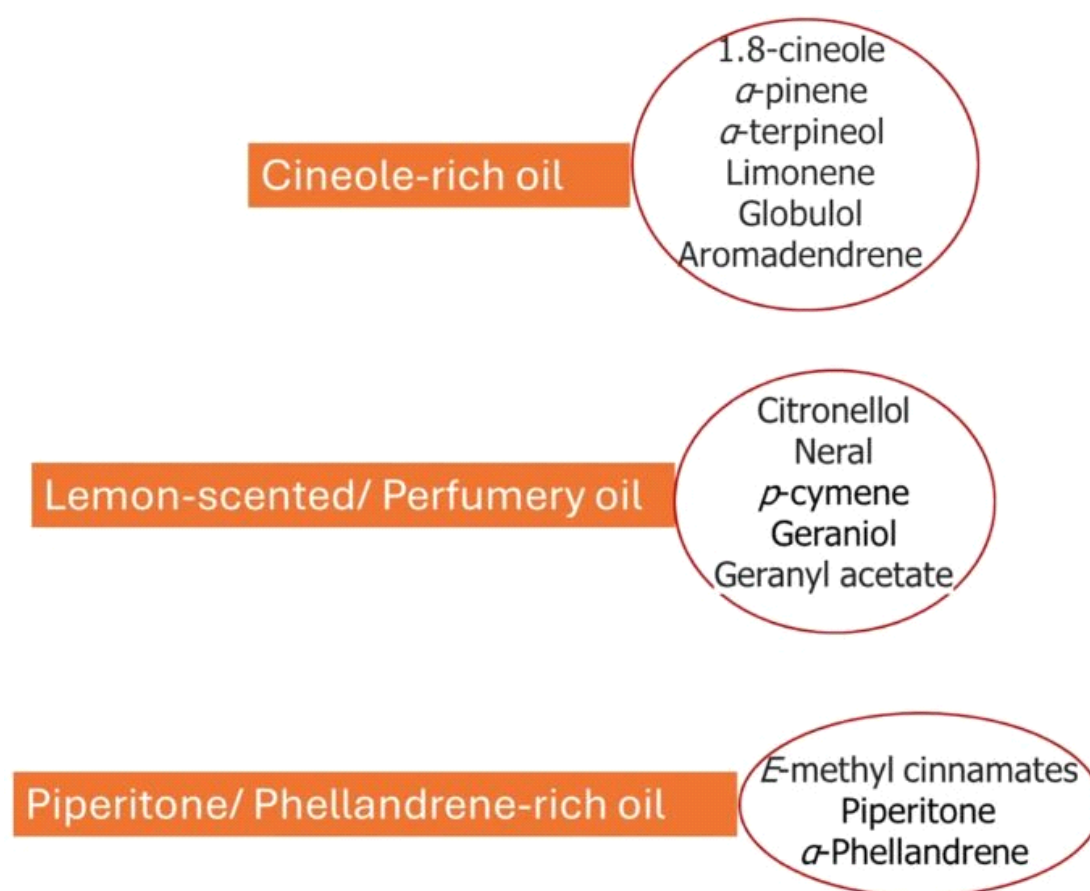
Keywords: Herbal massage oil, Eucalyptus leaves, Heat infusion method, Topical formulation, Evaluation

1. Introduction

Herbal topical formulations play an important role in the management of musculoskeletal disorders and inflammatory conditions. Massage oils prepared from medicinal plants are widely used due to their safety, ease of application, and therapeutic benefits. Herbal massage oils provide localized relief from pain, stiffness, and inflammation while improving blood circulation.

Eucalyptus (*Eucalyptus globulus*) leaves are a rich source of essential oils containing eucalyptol (1,8-cineole), which possesses analgesic, anti-inflammatory, antimicrobial, and soothing properties. Traditionally, eucalyptus oil is used for muscle pain, joint disorders, headache, and respiratory conditions. Coconut oil or sesame oil is commonly used as a carrier oil because of its emollient properties and ability to enhance skin penetration. Camphor provides a cooling and analgesic effect, while vitamin E acts as an antioxidant and skin conditioner.

The present work focuses on the formulation and evaluation of a herbal massage oil using eucalyptus leaves by a simple and less time-consuming heat infusion method suitable for laboratory and academic purposes.



The diverse range of Eucalyptus species not only provides essential oils with therapeutic properties but also plays a crucial role in biodiversity and ecosystem health. Their adaptability to different climates has made them valuable for reforestation and landscaping efforts around the world.

The versatility of Eucalyptus species continues to make them an essential resource in various industries, contributing to both economic development and ecological sustainability. As research progresses, new applications and benefits of these trees are likely to emerge, further enhancing their significance in global agriculture and health.

These properties have led to increased research into the potential therapeutic benefits of these essential oils, highlighting their roles in natural remedies and wellness products. Furthermore, their antimicrobial and anti-inflammatory effects make them appealing for use in both traditional and modern medicinal practices.

These therapeutic benefits underscore the potential of EEOs as complementary treatments in respiratory care. As research continues to explore their mechanisms of action, the relevance of these essential oils in modern medicine is likely to grow.

Such evaluations could lead to enhanced understanding of their mechanisms and potential synergies with existing therapies. Additionally, further research may uncover new applications for EEOs in the treatment of various respiratory conditions.

2. Aim and Objectives

Aim

To formulate and evaluate an herbal massage oil using eucalyptus leaves by a simple and rapid heating method.

Objectives

- To prepare herbal massage oil using eucalyptus leaves
- To evaluate the formulation for physical and safety parameters
- To assess its suitability for topical application
- To prepare oil by the heat infusion method
- To evaluate the effectiveness in pain relief

3. Materials and Methods

3.1 Materials

- Fresh eucalyptus leaves
- Coconut oil / Sesame oil
- Camphor
- Vitamin E capsule
- Beaker / steel bowl
- Water bath or low flame heater
- Muslin cloth / filter paper
- Glass rod
- Clean, dry bottle

3.2 Master Formula

S. No.	Ingredient	Quantity	Role
1	Fresh eucalyptus leaves	q.s.	Analgesic, anti-inflammatory
2	Coconut oil / Sesame oil	100 ml	Base oil, emollient
3	Camphor	2–3 g	Analgesic, cooling agent
4	Vitamin E	1 capsule	Antioxidant
5	methyl paraben	0.0075gm	Antimicrobial
6	propyl paraben	0.0015gm	Antimicrobial
7	propylene glycol	5ml	Humectant, solvent

Eucalyptus Leaves

Eucalyptus leaves, obtained from species of the genus *Eucalyptus* (family Myrtaceae), are known for their high content of essential oil, particularly eucalyptol (1,8-cineole). These leaves possess significant analgesic, anti-inflammatory, and antimicrobial activities. In topical formulations, eucalyptus extract improves blood circulation and provides relief from pain and inflammation, making it a valuable component in herbal massage oil preparations.

Coconut Oil

Coconut oil, obtained from the dried kernel of *Cocos nucifera* (family Arecaceae), is commonly used as a base in topical formulations due to its excellent emollient properties. It contains medium-chain fatty acids, primarily lauric acid, which exhibit antimicrobial and anti-inflammatory activities. The oil enhances skin hydration and facilitates the penetration of active phytoconstituents, making it a suitable carrier in herbal massage oil formulations. Its stability and non-irritant nature further support its use in topical therapeutic applications.

Sesame Oil

Sesame oil, derived from the seeds of *Sesamum indicum* (family Pedaliaceae), is widely utilized in pharmaceutical and traditional medicine systems. It is rich in unsaturated fatty acids such as oleic and linoleic acids, along with natural antioxidants like sesamol and sesamin. These constituents contribute to its anti-inflammatory and antioxidant properties. Due to its excellent skin penetration ability and warming effect, sesame oil serves as an effective base for enhancing the therapeutic efficacy of herbal massage oils.

Camphor

Camphor is a natural terpenoid obtained from the wood of *Cinnamomum camphora* and is widely used in topical pharmaceutical preparations due to its counterirritant and analgesic properties. It produces a cooling sensation on the skin by stimulating sensory nerve endings and improving local blood circulation. Camphor also exhibits mild antimicrobial and anti-inflammatory activities, making it useful in formulations intended for the relief of muscle pain and inflammation. Its rapid skin absorption enhances the overall therapeutic efficacy of massage oils.

Vitamin E

Vitamin E, commonly in the form of tocopherol, is a fat-soluble antioxidant widely incorporated in topical formulations to prevent oxidative degradation of oils and enhance skin health. It protects the formulation from rancidity by inhibiting lipid peroxidation and also provides beneficial effects on the skin, including moisturizing and anti-inflammatory actions. The inclusion of vitamin E improves the stability and shelf life of herbal massage oils.

Methylparaben

Methylparaben is a widely used antimicrobial preservative in pharmaceutical and cosmetic formulations. It is effective against a broad spectrum of microorganisms, particularly bacteria and fungi, and is commonly used to prevent microbial contamination in topical preparations. Due to its stability and compatibility with other excipients, methylparaben is frequently included in low concentrations to enhance the shelf life and safety of herbal formulations.

Propyl paraben

Propylparaben is another ester of p-hydroxybenzoic acid used as a preservative in topical formulations. It is particularly effective against molds and yeasts and is often used in combination with methylparaben to achieve a synergistic antimicrobial effect. This combination enhances preservation efficiency and ensures long-term stability of the formulation.

Propylene Glycol

Propylene glycol is a widely used pharmaceutical excipient that functions as a solvent, humectant, and penetration enhancer in topical formulations. It improves the solubility of active ingredients and facilitates their absorption through the skin. Additionally, propylene glycol helps maintain moisture content and enhances the overall consistency and stability of the formulation.

Instruments Required

S.NO	Instruments required	Glassware Required
1	Magnetic stirrer	Beakers
2	Water bath	Measuring cylinder
3	Hot plate	Conical flask
4	Analytical balance	Funnel
5	Thermometer	Storage bottle

3.3 Method of Preparation

Heat infusion method

3.4 Procedure

1. Fresh eucalyptus leaves were washed thoroughly and dried. The leaves were lightly crushed to release active constituents. The base oil was taken in a beaker and heated gently on a water bath. Crushed eucalyptus leaves were added and the mixture was heated for 10–15

minutes on low flame. The mixture was allowed to cool slightly and filtered using muslin cloth. Camphor and vitamin E were added to the filtered oil and mixed well. The prepared herbal massage oil was transferred into a clean, dry bottle and properly labeled

2. Fresh eucalyptus leaves are washed, shade-dried, and coarsely powdered. Take propylene glycol (5 ml) in a beaker and add the crushed leaves. Stir the mixture using a magnetic stirrer for 20–30 minutes to extract active constituents. Slightly warm the mixture on a water bath (not exceeding 50°C) to enhance extraction. Filter the mixture using muslin cloth to obtain the extract. In a separate beaker, take coconut oil or sesame oil (100 ml). Add the filtered extract slowly into the oil with continuous stirring. Dissolve methyl paraben and propyl paraben in a small quantity of warm propylene glycol or oil and add to the mixture. Add camphor and mix until completely dissolved. Finally, add vitamin E and mix thoroughly. Transfer the prepared oil into a clean, dry, airtight container and label.

4. Evaluation of Herbal Massage Oil

4.1 Organoleptic Evaluation

The formulation was evaluated for color, odor, and appearance.

- **Color:** Light green
- **Odour:** Characteristic eucalyptus aroma
- **Appearance:** Clear and free from suspended particles

4.2 Spreadability

A small quantity of oil was applied on the skin surface and gently rubbed.

Observation:

The oil spread easily with smooth application, indicating good spreadability.

4.3 Skin Irritation Test

The oil was applied on a small area of healthy skin and observed for 24 hours.

Observation:

No redness, itching, or irritation was observed.

4.4 Stability Test

The oil was stored at room temperature and observed for changes in color and odor.

Observation:

No significant change was observed, indicating good stability.

5. Results and Discussion

The herbal massage oil containing eucalyptus leaves was successfully prepared using a simple heat infusion method. The formulation exhibited good physical appearance, pleasant odor, easy spreadability, and stability. The absence of skin irritation confirms its safety for topical use. The therapeutic properties of eucalyptus leaves, combined with the emollient nature of the base oil, contribute to the effectiveness of the formulation in relieving muscle pain and inflammation.

1. Color: A white background was used to assess the color of the formulation. No, Particles of any color are shown.
2. Odour: The odour of the formulated oil was evaluated by organoleptic method and was found to possess a characteristic, pleasant, and camphoraceous smell, indicating the presence of eucalyptus oil.
3. Consistency: oil was applied to the skin to assess the consistency. Easily and smoothly applied onto the skin.
4. Grittiness: Under 40x magnification, the formulation was assessed under a microscope. There are no aggregates or particulate matter present.
5. Homogeneity: Visual inspection with the unaided eye was used to test homogeneity after allowing them to be placed in a container while their appearance and the presence of aggregates.
6. Clarity: Visual inspection was used to assess the clarity of different formulations under There is nothing special on the white background.
7. Skin irritancy test: The skin irritation study was carried out on 10 healthy human volunteers of either gender after obtaining their informed consent. Approximately 0.5 g of the formulated eucalyptus oil was applied to a marked area of about 6 cm² on the dorsal surface of the hand and covered with a sterile gauze patch. The patch was kept in contact with the skin for a duration of 1 hour. After removal, any remaining oil was gently wiped off without disturbing the skin surface. The treated area was carefully observed for signs of irritation such as redness, itching, or inflammation at intervals of 1, 3, 6, 12, 24, 48, and 72 hours. No visible signs of skin irritation or adverse reactions were observed during the study period, indicating that the formulation is safe for topical application.
8. Antibacterial test: The agar well diffusion method was used to assess the antibacterial activity of eucalyptus oil against both Gram-positive and Gram-negative bacterial strains, such as *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. The zone of inhibition (measured in millimeters) was recorded following a 24-hour incubation period at 37°C.

For every microorganism tested, the eucalyptus oil demonstrated concentration-dependent antibacterial activity. At 100% concentration, the greatest activity was seen, whereas zones of inhibition were comparatively smaller at lower concentrations.

The findings unequivocally show that eucalyptus oil has strong antibacterial activity against both Gram-positive and Gram-negative bacteria. *Staphylococcus aureus* and *Bacillus subtilis* were the most sensitive of the tested organisms, while *Escherichia coli* was relatively less sensitive.

Active ingredients like eucalyptol (1, 8-cineole), which damage bacterial cell membranes and prevent microbial growth, are responsible for the antibacterial effect of eucalyptus oil. A dose-dependent response is confirmed by the observed increase in the zone of inhibition with increasing concentration.

It was discovered that Gram-positive bacteria were more vulnerable than Gram-negative bacteria, perhaps because of variations in the structure of their cell walls. Gram-negative bacteria have an outer membrane that functions as a barrier, preventing hydrophobic substances like essential oils from penetrating.

Overall, the results lend credence to the possibility of using eucalyptus oil as a natural antibacterial agent in herbal formulations for therapeutic purposes, such as massage oils.

Bacteria	Concentration (%)	Zone of inhibition (mm)
Staphylococcus aureus	25%	8 ± 0.5
	50%	12 ± 0.6
	75%	16 ± 0.4
	100%	20 ± 0.5
Bacillus subtilis	25%	7 ± 0.4
	50%	11 ± 0.5
	75%	15 ± 0.6
	100%	18 ± 0.5
Escherichia coli	25%	6 ± 0.5
	50%	10 ± 0.4
	75%	13 ± 0.5
	100%	16 ± 0.6

8. Antioxidant: The DPPH assay (2, 2-diphenyl-1-picrylhydrazyl) method was used to assess the antioxidant activity of eucalyptus oil. The assay is based on the reduction of the DPPH radical, which is detected spectrophotometrically at 517 nm and causes a color shift from deep violet to yellow.

Significant free radical scavenging activity was demonstrated by eucalyptus oil in a concentration-dependent manner. As the oil's concentration rose, the percentage inhibition rose as well, suggesting strong antioxidant potential.

S.NO	Concentration (µg/ml)	Absorbance (517nm)	%Inhibition
1	20	0.682 ± 0.02	18.5 ± 0.8
2	40	0.601 ± 0.03	28.2 ± 0.9
3	60	0.512 ± 0.02	38.7 ± 0.7
4	80	0.421 ± 0.01	49.8 ± 0.6
5	100	0.335 ± 0.02	60.5 ± 0.8

The ability of eucalyptus oil to scavenge DPPH free radicals showed that it has significant antioxidant activity. A dose-dependent antioxidant effect is confirmed by the rise in percentage inhibition with increasing concentration.

The presence of bioactive substances like phenolics and terpenoids, especially eucalyptol, which are known to donate hydrogen atoms or electrons to neutralize free radicals, is responsible for the antioxidant potential of eucalyptus oil.

The steady decline in absorbance values suggests that DPPH radicals have been effectively reduced. Eucalyptus oil may be a natural source of antioxidants because the results are comparable to those of standard antioxidant agents documented in the literature.

6. Conclusion

The present study concludes that herbal massage oil containing eucalyptus leaves can be effectively formulated using a simple, economical, and less time-consuming method. The prepared oil demonstrated satisfactory evaluation parameters and was found to be safe and suitable for topical application. This formulation can be recommended for academic, laboratory, and traditional therapeutic use.

The oil's therapeutic potential is further enhanced by its noteworthy antioxidant and antibacterial properties. Its analgesic, anti-inflammatory, and antimicrobial properties are attributed to the presence of active ingredients like eucalyptol.

All things considered, the developed herbal massage oil can be regarded as a safe, efficient, and reasonably priced formulation for reducing inflammation, easing muscle pain, and encouraging relaxation. It can be used in traditional medical applications, academic research, and laboratory preparation.

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