A REVIEW ON THE FORMULATION AND CHARACTERIZATION OF CLOVE OIL MICROEMULSIONS

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ABSTRACT

Microemulsions are thermodynamically stable, isotropic systems composed of oil, water, surfactants, and co-surfactants that have gained significant attention for the delivery of natural bioactive compounds. This review highlights the development, properties, and applications of microemulsions, focusing on their role in enhancing the functionality of natural clove oil. Clove oil, rich in eugenol, exhibits potent antimicrobial, antioxidant, anti-inflammatory, and analgesic properties. However, its low aqueous solubility, volatility, and susceptibility to degradation limit its practical applications. Incorporating clove oil into microemulsion systems improves its solubility, chemical stability, and bioavailability, thereby extending its use in pharmaceutical, cosmetic, and food formulations. The review also explores the mechanisms of microemulsion formation, selection of appropriate surfactants and co-surfactants, and their impact on the delivery and release profile of clove oil. The optimized clove oil microemulsion formulation was subjected to an evaluation of various parameters, such as organoleptic properties, %transmittance, pH, viscosity, stability, particle size, zeta potential, and polydispersity index (IP). Moreover, recent studies show that microemulsions can enhance skin penetration and sustain the release of clove oil, making them ideal for topical and transdermal delivery. Thus, microemulsions offer a promising platform for the efficient utilization of clove oil in various therapeutic and commercial applications.

Keywords:
Microemulsion, Clove Oil, Eugenol, Drug Delivery, Natural Oils, Solubility Enhancement, Skin Penetration, Antimicrobial Activity, Topical Formulation, Nanoemulsion.

1. INTRODUCTION

An emulsion is commonly defined as a biphasic system composed of two immiscible liquids, where one liquid (the dispersed phase) is uniformly distributed in the form of small globules within the other liquid (the continuous phase). These systems are thermodynamically unstable, which necessitates the inclusion of a third component—an emulsifier—to maintain stability. Microemulsions (MEs), in contrast, are thermodynamically stable and isotropic systems characterized by a high concentration of surfactants. They are highly effective carriers for oil- based drugs due to their small droplet size, enhanced stability, large interfacial area, and low interfacial tension, forming spontaneously without the need for external energy. A defining feature of microemulsions is their nanoscale droplet size. Unlike nanoemulsions, which require mechanical force for formation, microemulsions self-assemble naturally.[1,2] While emulsions and microemulsions share similarities, MEs maintain a consistent droplet size due to their thermodynamic stability, unlike emulsions where droplets

can grow over time. Another distinguishing factor is the optical clarity of MEs, as they are typically transparent, whereas emulsions appear turbid. The internal droplet size in MEs is usually around 10 nanometers. Additionally, the internal structure of microemulsions varies from that of emulsions. Emulsions typically consist of spherical droplets dispersed in a continuous phase, while MEs can exhibit a range of structural organizations, from micelles to bicontinuous phases. Natural products, particularly essential oils, have gained significant attention in the fields of pharmaceuticals, cosmetics, and food industries due to their bioactive properties and minimal side effects. Among these, clove oil, derived from the flower buds of *Syzygium aromaticum*, stands out for its potent antimicrobial, anti-inflammatory, analgesic, antifungal, and antioxidant activities.[3,4]

Fig 1: Clove

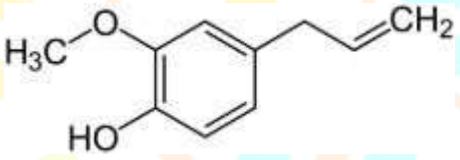
The major active constituent of clove oil, eugenol, is primarily responsible for these therapeutic effects and has been widely studied for its application in traditional and modern medicine. Despite its high potential, clove oil presents certain formulation challenges that limit its widespread use. Its poor water solubility, high volatility, low chemical stability, and strong odor restrict its direct incorporation into aqueous-based formulations or controlled drug delivery systems. These limitations, researchers have explored various advanced delivery systems, among which microemulsions have emerged as a particularly effective strategy.[5] A microemulsion is a thermodynamically stable, transparent, and isotropic system consisting of oil, water, surfactant, and often a co-surfactant. Unlike conventional emulsions, microemulsions have droplet sizes in the nanometer range (typically 10–100 nm), resulting in higher surface area and enhanced solubilization capacity. These unique physicochemical properties allow microemulsions to significantly improve the solubility, bioavailability, stability, and controlled release of hydrophobic bioactive compounds such as those found in clove oil. In the context of clove oil, microemulsions serve not only as solubilizing agents but also as carriers that protect the active components from environmental degradation, enhance penetration through biological membranes, and reduce irritation caused by concentrated oil application. The use of microemulsion systems enables the uniform distribution of clove oil in aqueous environments, which is particularly advantageous for topical formulations, oral administration, and food-grade applications. Moreover, due to their transparent and fluid nature, microemulsions offer better aesthetic appeal and ease of application compared to traditional emulsions or ointments. Several studies have demonstrated the potential of clove oil-loaded microemulsions in treating skin infections, dental conditions, and microbial resistance. For example, the incorporation of clove oil into oil-in-water (O/W) microemulsions has shown improved antimicrobial efficacy against a variety of Gram-positive and Gram- negative bacteria.[6,7] The enhanced permeation capability of microemulsions across the skin barrier has also been reported to increase the therapeutic efficacy of clove oil in transdermal drug delivery systems. In addition, the stability provided by the microemulsion matrix helps in maintaining the integrity and potency of sensitive components such as eugenol, which is otherwise susceptible to oxidation and volatilization. From a formulation perspective, the selection of suitable surfactants and co-surfactants is critical in developing an efficient clove oil microemulsion. Non-ionic surfactants are often preferred due to their low toxicity and skin compatibility. The surfactant-to-co-surfactant ratio (S/C ratio), oil phase concentration, and water content

significantly influence the microemulsion's characteristics, including droplet size, viscosity, and drug release profile. Pseudo-ternary phase diagrams are commonly used to determine the appropriate composition range for microemulsion formation. The potential of clove oil microemulsions extends beyond pharmaceuticals into cosmetics, personal care products, and food preservation, where antimicrobial and antioxidant properties are in high demand. The integration of clove oil into microemulsion-based systems not only enhances its performance but also enables the development of eco-friendly, natural, and effective formulations, aligning with current consumer and regulatory preferences. The application of microemulsion technology in the delivery of clove oil represents a promising and innovative approach to harness its full therapeutic potential.[8,9]

1.1 CHEMICAL STRUCTURE OF CLOVE OIL COMPONENETS

1. Eugenol

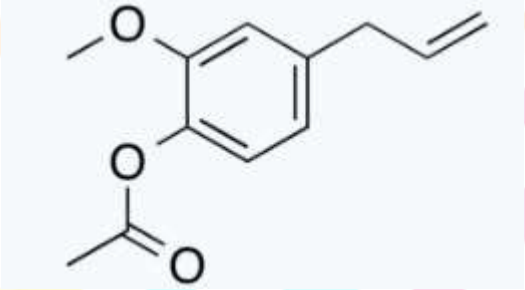
- **IUPAC Name:** 4-allyl-2-methoxyphenol
- **Molecular Formula:** $C_{10}H_{12}O_2$
- **Structure:**



- **Properties:** Main active compound (up to 70–90%), responsible for analgesic, anti- inflammatory, and antimicrobial properties.

2. Eugenyl Acetate

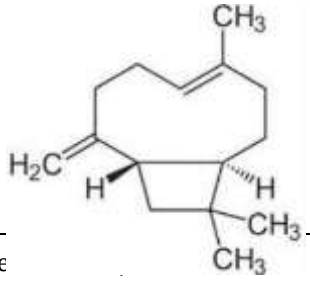
- **IUPAC Name:** 4-allyl-2-methoxyphenyl acetate
- **Molecular Formula:** $C_{12}H_{14}O_3$
- **Structure:**



- **Properties:** Contributes to aroma and some biological activity.

3. β-Caryophyllene

- **IUPAC Name:** (1R,4E,9S)-4,11,11-trimethyl-8-methylene-bicyclo -4-ene
- **Molecular Formula:** $C_{15}H_{24}$
- **Structure:**



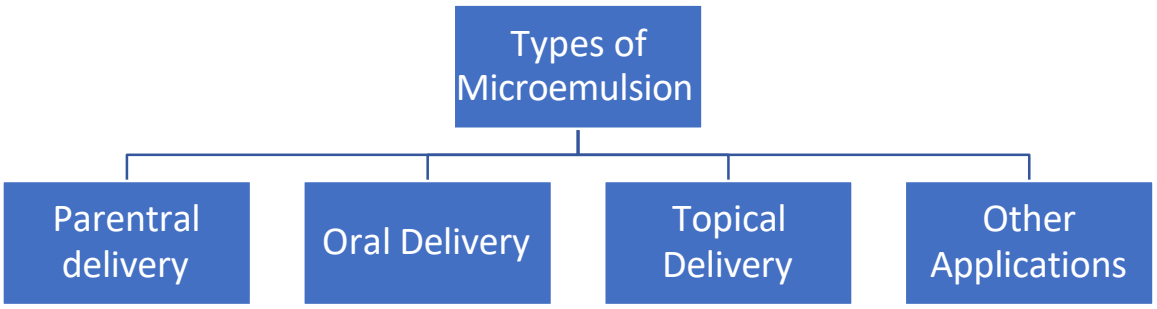
- **Properties:** Anti-inflammatory, antioxidant, binds to CB2 receptors.[10]

2. MICROEMULSION IN TOPICAL TARGETED DRUG DELIVERY

Ophthalmic, rectal, vaginal, and cutaneous topical routes are all examples of localized drug delivery methods used in topical medication administration. Many treatments, medicines, and delivery systems have been developed as a result of efforts to treat diseases. Microemulsion (MEs) has attracted much interest for several years in terms of delivery and target potential. MEs containing natural oils have been of increasing interest to researchers and have shown great potential in industrial applications. MEs' utility lies in their ability to incorporate a large amount of active natural oil products in the continuous or dispersed phase, which is otherwise difficult to formulate. Due to the therapeutic advantages and the complex composition of the natural oil, various formulation approaches including carrier technology such as MEs offer an intelligent approach for the delivery of bioactive compounds from natural oil products. Therefore, this work aimed to review the various advantages of natural oils-loaded ME systems to be used as delivery systems for these bioactive compounds.[11,12] The present review is divided into two sections. Firstly, the state of the art of parameters involved in the ME formation, including the basic concepts of the physicochemical formulation of the ME systems, and the main aspects of production and the energy responsible for their formation were reported. The second section describes the use of ME systems and reviews the recent applications of natural oil loaded in the ME systems as the bioactive compound in the formulation. Natural oil products have promising potential in maintaining and promoting health, as well as preventing and potentially treating some diseases. Several studies have been conducted over the last few decades concerning the formulation of new ME systems containing bioactive compounds from natural oil products. Such studies reveal that these systems are promising and are also an innovative approach with potential applications in medicinal and health research, which can result in decreasing the dose, enhancing the absorption and the bioavailability as well as reducing the systemic side effects and the patient variability. Therefore, bioactive oil-loaded ME represents a potential strategy to increase the therapeutic properties of natural oils. Microemulsions are simply smaller versions of emulsions, which are a subclass of colloidal systems. All three forms of matter—gas, liquid, and solid—are capable of supporting colloidal systems. The emulsions are created by emulsification, a key property of surface-active agents that makes them adaptable for a variety of real-world applications, including milk and cream, espresso, cutting fluid for metalworking, and photo-sensitizer.

Specific Drug Delivery: It has proven possible to overcome hepatic first-pass metabolism by increasing the pharmacokinetic characteristics through the promotion of lymphatic transport. The utilization of lipid-based delivery methods, complexation, and pH manipulation are some of these tactics. The pharmacokinetic properties of hydrophobic drugs are improved by self- micro-emulsifying formulations (droplet size 100 nm), which are lipid-based. This is primarily because of their effectiveness in promoting solubilization and in presenting the hydrophobic drug in a solubilized form where the dissolution process can be avoided.[13,14] The chemists Hoar and Schulman introduced the microemulsion concept in the 1940s. Schulman and Stoeckenius subsequently coined the term microemulsion. The best definition, as emphasized by Lawrence and Ress, was the one provided by Danielsson and Lindman in 1981. Microemulsion. Microemulsions also have a controlled drug release rate, slow degradation, and most important of all, it has target specificity.[15]

2.1 There are various types of microemulsion:



1. Parentral delivery
2. Oral delivery
3. Topical delivery
4. Other applications
a). Nasal
b). Drug Targeting
c). Cell Targeting
d). Brain Targeting

1. Parenteral Delivery

Microemulsions are suitable for intravenous, intramuscular, or subcutaneous administration due to their small droplet size and stability.

- Enhance solubility of poorly water-soluble drugs.
- Enable controlled and sustained release of drugs into the bloodstream.
- Reduce irritation at the injection site compared to traditional emulsions.
- Improve bioavailability and therapeutic efficacy.[16]

Example: Delivery of anticancer agents or anaesthetics intravenously.

2. Oral Delivery

Microemulsions improve the oral bioavailability of drugs with poor water solubility or poor gastrointestinal absorption.

- Increase drug dissolution and absorption in the gastrointestinal tract.
- Protect sensitive drugs from enzymatic degradation.
- Bypass first-pass metabolism in some cases.
- Enhance patient compliance due to ease of administration.[17]

Example: Oral formulations of lipophilic drugs like cyclosporine or curcumin.

3. Topical Delivery

Microemulsions provide an effective medium for dermal and transdermal delivery of drugs.

- Enhance drug permeation through the skin by disrupting lipid layers.

- Provide a non-greasy, smooth texture, making them cosmetically appealing.
- Useful for local and systemic effects depending on the formulation.
- Can be used for anti-inflammatory, antifungal, or cosmetic purposes.[18,19]

Example: Microemulsion-based gels or creams for psoriasis, acne, or pain relief.

4. Other Applications

a) Nasal Delivery

- Used for systemic drug delivery through nasal mucosa.
- Allows rapid onset of action.

Example: Nasal insulin or flu vaccines.

b) Drug Targeting

- Microemulsions can be surface-modified for active or passive targeting.
- Useful in oncology and infectious disease treatments.

Example: Targeted delivery of chemotherapeutics to tumor cells.

c) Cell Targeting

- Engineered microemulsions can bind specific cell surface receptors.
- Enable precise intracellular drug delivery.
- Aid in gene therapy and cell-specific diagnostics.[20]

Example: Delivery of siRNA or DNA to immune cells or cancer cells.

d) Brain Targeting

- Overcomes the blood-brain barrier (BBB), a major challenge in CNS drug delivery.
- Enables treatment of neurological disorders like Alzheimer's, Parkinson's, or brain tumors.

Example: Microemulsion-based formulation of anti-Parkinsonian drugs.

3. APPLICATIONS OF MICROEMULSION

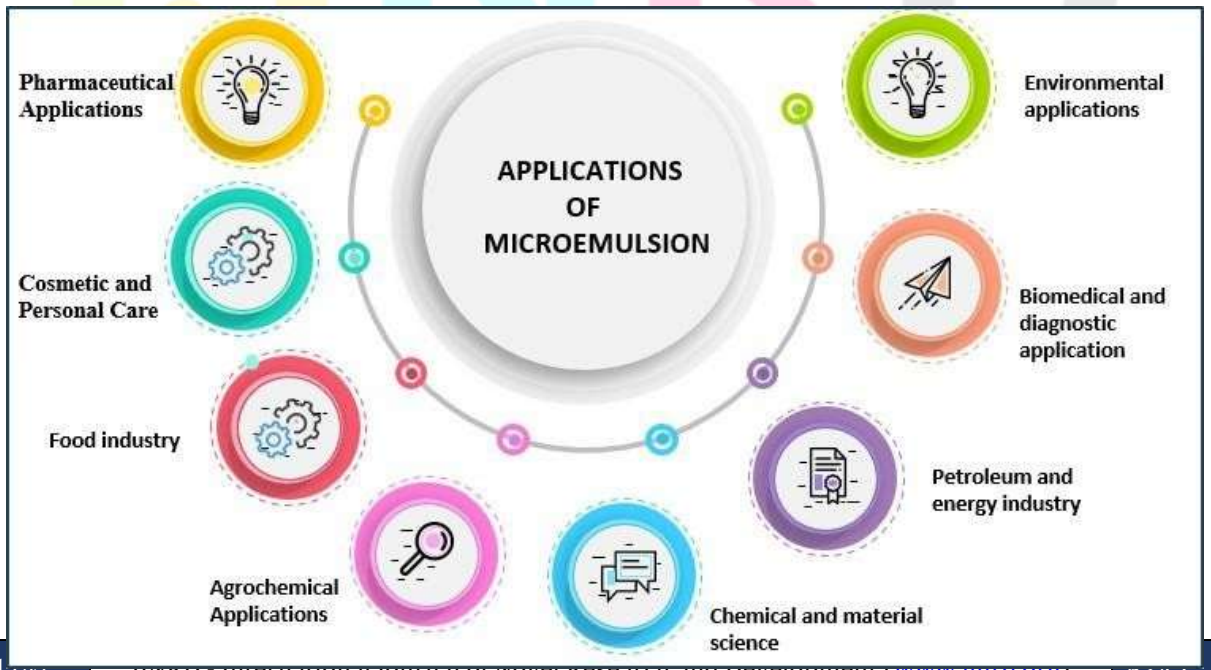


Fig 2: Applications of Microemulsion

VARIOUS ACTIVITIES OF CLOVE OIL

Clove oil, derived from the dried flower buds of the clove tree (*Syzygium aromaticum*), is rich in eugenol, its primary active component. It has been used in traditional medicine, dentistry, and modern pharmaceuticals due to its broad spectrum of biological activities.[21,22]

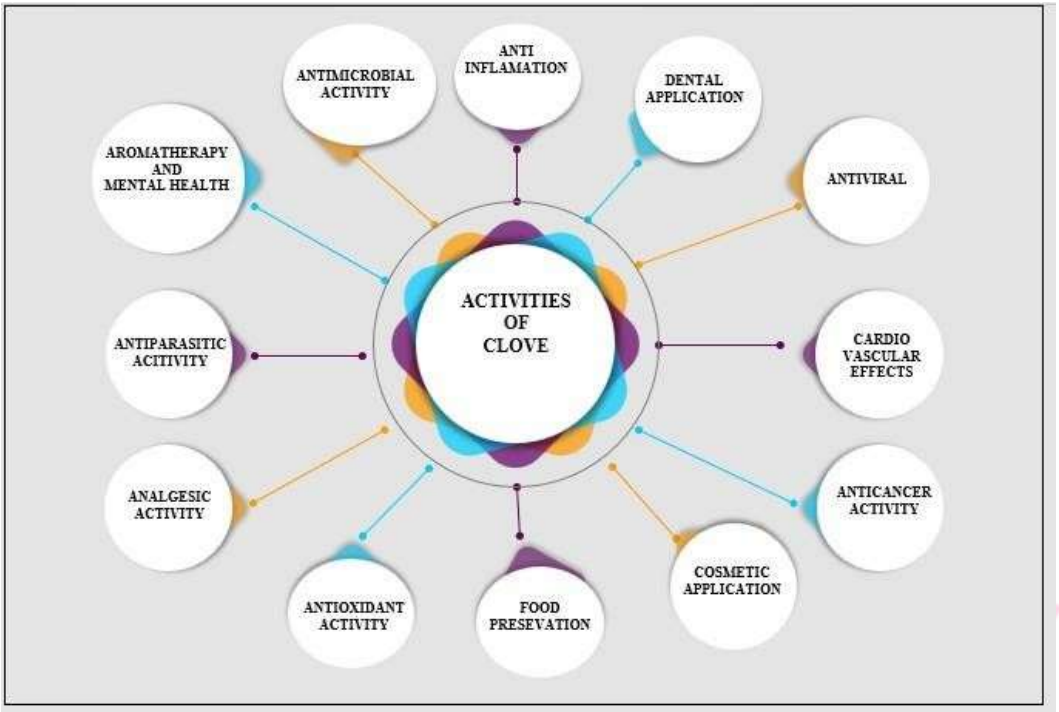


Fig 3: Activities of Clove

1. Antimicrobial Activity

Clove oil exhibits strong antimicrobial effects against a wide range of bacteria, fungi, and viruses.

- **Antibacterial**: Effective against *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, etc.
- **Antifungal**: Inhibits growth of *Candida albicans*, *Aspergillus niger*, and other fungal strains.
- **Antiviral**: Eugenol has shown activity against herpes simplex virus and hepatitis C in lab studies.

Mechanism: Disrupts microbial cell walls, inhibits enzyme activity, and alters membrane permeability.

2. Antioxidant Activity

Clove oil is known for its potent antioxidant potential, which helps in neutralizing free radicals and reducing oxidative stress.

- Scavenges reactive oxygen species (ROS).
- Protects DNA, proteins, and lipids from oxidative damage.
- Supports anti-aging and anti-inflammatory functions in cells.

Applications: Used in cosmetics, anti-aging creams, and nutraceuticals.

3. Anti-inflammatory Activity

Eugenol in clove oil can reduce inflammation by inhibiting pro-inflammatory cytokines and enzymes like COX-2.

- Reduces swelling, redness, and pain.

- Effective in arthritis, dermatitis, and inflammatory bowel disease models.
- Promotes wound healing and reduces tissue irritation.

4. Analgesic (Pain-Relieving) Activity

- Clove oil is widely used for its natural analgesic properties.
- Topically applied for toothache relief, muscle pain, and minor cuts.
- Acts on TRPV1 receptors and local nerves to numb the affected area.
- Often used in dentistry as an anesthetic and anti-irritant. Form: Dental pastes, gels, and oral sprays.

5. Anticancer Activity

Preliminary studies show that clove oil exhibits anticancer and chemopreventive potential.

- Induces apoptosis (programmed cell death) in cancer cells.
- Inhibits proliferation in cell lines of breast, colon, liver, and prostate cancers.
- Modulates key pathways such as p53, NF-κB, and MAPK.

6. Antidiabetic Activity

Clove oil may help in the management of diabetes by improving glucose metabolism.

- Increases insulin sensitivity.
- Lowers blood glucose and HbA1c levels in diabetic models.
- Protects pancreatic β-cells from oxidative damage.[23,24]

7. Insecticidal and Repellent Activity

Clove oil serves as a natural insecticide and repellent.

- Effective against mosquitoes, ants, houseflies, and termites.
- Often used in natural insect repellents and sprays.
- Safe and eco-friendly alternative to synthetic pesticides.

8. **Antispasmodic and Muscle Relaxant**

Clove oil can reduce muscle spasms and cramps.

- Relaxes smooth muscles.
- Used in aromatherapy or topical application to reduce muscle fatigue.
- Helpful in menstrual cramps, gastrointestinal colic, and general muscle tension.

9. Hepatoprotective Activity

- Clove oil helps in protecting the liver from toxins and oxidative stress.
- Reduces lipid peroxidation and enhances liver enzyme activity.
- Prevents damage from drugs, alcohol, and heavy metals.

10. Dental Applications

- Clove oil has a long-standing use in oral health care.
- Used in toothache relief, mouthwashes, and gum infection treatments.

- Reduces plaque and gingivitis.
- Component of dental cements and filling materials due to its antimicrobial action.[25,26]

11. Anthelmintic Activity

Shows activity against intestinal parasites like worms.

- Disrupts parasite metabolism and neuromuscular function.
- Used in traditional medicine for deworming.

12. Antioxidant Activity

- Antioxidants are compounds that neutralize free radicals—unstable molecules that can damage cells, proteins, and DNA through oxidative stress.
- If not controlled, oxidative stress contributes to aging, inflammation, and chronic diseases like cancer, diabetes, and cardiovascular disorders.[27]

4. MATERIAL AND METHODS

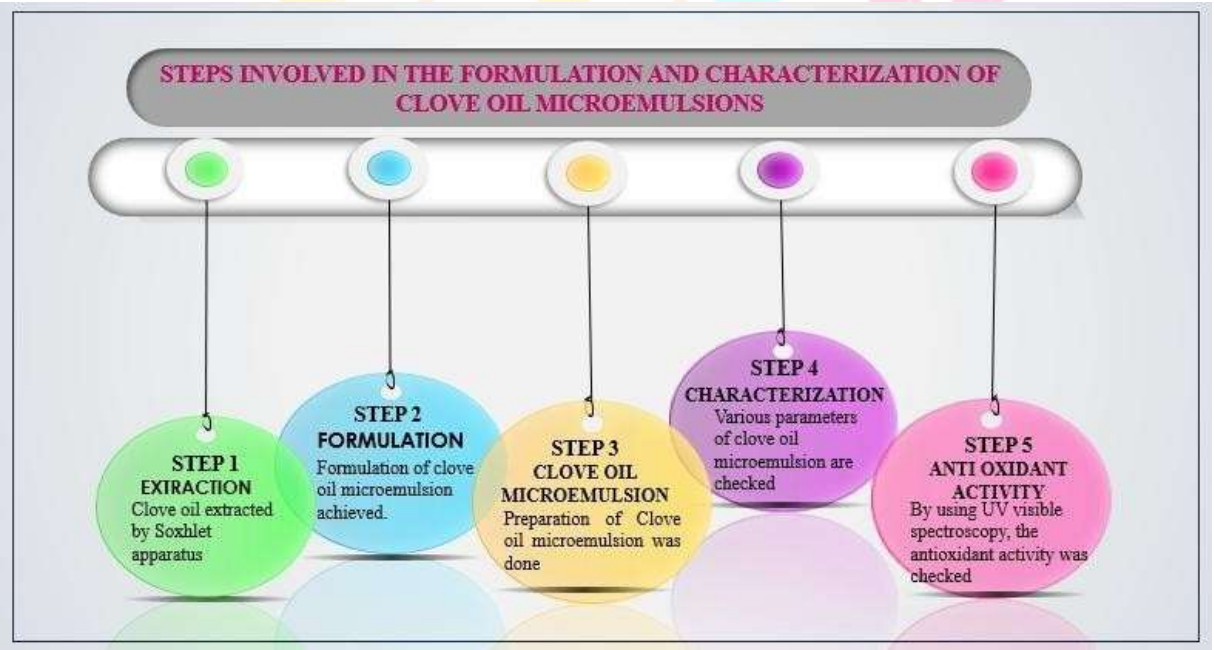


Fig 4: Steps involved in the formulation and characterization of clove oil microemulsion

Materials

- **Clove Oil (Eugenia caryophyllata) –** Natural essential oil, analytical grade
- **Surfactants –** Tween 80, Span 80 (non-ionic surfactants)
- **Co-surfactants –** Ethanol, Propylene glycol
- **Aqueous phase –** Distilled water
- **Other reagents –** For analysis: methanol, chloroform, HPLC-grade solvents
- **Analytical standards –** Eugenol standard (for chemical assay)
- **Equipment** – Magnetic stirrer, centrifuge, pH meter, viscometer, UV-Vis spectrophotometer.

4.1 METHODS

Materials Required for extraction of clove oil

- Clove buds (dried)
- Round bottom flask (RBF)
- Distillation setup
- Heating mantle
- Water
- Clamps and stands
- Separating funnel
- Collection flask
- Anhydrous sodium sulphate (for drying oil)

Extraction Procedure:

1. Preparation of the Sample

- Grind dried clove buds into coarse powder.

2. Assembly of Distillation Setup

- Set up the steam distillation apparatus:
  - Round bottom flask (containing cloves and water)
  - Heating source
  - Condenser
  - Collection receiver

3. Steam Distillation

- Add sufficient water to the RBF containing powdered cloves.
- Heat gently; steam carries volatile clove oil vapours.
- Vapours pass through the condenser, where they cool and condense into liquid.

4. Collection

- Collect the distillate (a mixture of oil and water) in a separating funnel.[28]

5. Separation

- Let the mixture settle; clove oil separates out as a distinct layer (usually floats due to lower density).
- Carefully separate the clove oil layer.

6. Drying

- Dry the oil using anhydrous sodium sulphate to remove any remaining moisture.

7. Storage

- Store the pure clove oil in a sealed amber bottle away from sunlight. [29,30]

4.2 Method for microemulsion formation of clove oil

The most stable microemulsion base was achieved. The manufacture of clove oil microemulsions was carried out by weighing all ingredients using a 10% clove oil concentration. The surfactant and co-surfactants were mixed in a beaker glass, to produce a surfactant mixture by stirring at 500 rpm using magnetic stirrer for 5 minutes until homogeneity was achieved. Oil was added by continuously stirring with the same stirring speed using a magnetic stirrer for 5 minutes until homogeneity was achieved. Weighed clove oil was added to the mixture by continuously stirring with the same stirring speed using a magnetic stirrer for 5 minutes until homogeneity was achieved. Distilled water was also added little by little to the mixture until all the distilled water was added. Stirring was carried out continuously using a magnetic stirrer for 15 minutes until homogeneity was achieved. [31,32]

Determination of Antioxidant Activity of Clove oil

The method for determining the antioxidant content of clove essential oil samples was by using the DPPH method (1,1-diphenyl-2-picrylhydrazil). DPPH method was used to test the ability of a component as a free radical scavenger in an ingredient or extract. The advantage of the DPPH method was quick and simple sensitive and only required small sample. The parameter used for the DPPH radical capture test was IC 50, which was the concentration of the extract or test fraction needed to capture the DPPH radical by 50% The antioxidant analysis procedure of the DPPH (IC50) method was as follows: extract of the sample (distillate) was weighed as much as 25 mg then put in a 25 ml volumetric flask, then added with ethanol solvent up to a concentration of 1000 ppm. Then a dilution series was carried out to obtain a solution of 10, 30, 50, 70 and 90 ppm. The solution was then taken as much as 0.2 ml and added with 3.8 ml of DPPH 50µM solution. The mixture was homogenized and left for 30 minutes in a dark place. Then the absorption was measured at a wavelength of 517nm with spectrophotometer. Tests were also carried out on DPPH solutions. The absorbance value obtained was used to determine the% inhibition.[33]

5. Characterization of Microemulsion of clove oil

Microemulsions are thermodynamically stable, optically transparent, and isotropic mixtures composed of oil, water, surfactant, and often a co-surfactant. When incorporating natural oils like clove oil into microemulsions, characterization becomes essential to understand their physicochemical properties, stability, and drug delivery potential.[35,34]

Characterization involves both physical and chemical assessments using advanced techniques to ensure the microemulsion system is suitable for topical, oral, or injectable use.

Steps involved in characterization of microemulsion are:

- **Visual and Physical Appearance:** The visual and physical appearance is the first and simplest step in characterizing a microemulsion system containing natural oils like clove oil. It gives a preliminary indication of whether the formulation has been successfully developed. In visual and physical appearance, the main objective is to evaluate clarity, homogeneity, and phase behaviour.

Parameters which are observed under this are:

- **Clarity**: A true microemulsion appears clear, transparent, or slightly bluish due to nanometer-sized droplets (10–100 nm). Lack of turbidity or cloudiness suggests proper dispersion of oil in the aqueous phase.
- **Homogeneity**: The formulation should appear uniform with no signs of phase separation or layering. Indicates proper mixing and stable microstructure.
- **Fluidity**: Observed by tilting or pouring the sample. Most microemulsions are low- viscosity, water-like fluids, although viscosity can vary based on components.

- **Zeta Potential**

Zeta potential is a key parameter in the characterization of microemulsions, including those loaded with clove oil, as it provides insight into the electrostatic stability of the formulation. The main objective is to determine the electrical charge on droplet surfaces. Helps in predicting the shelf life and physical stability of the clove oil microemulsion. Provides insight into interfacial interactions between oil, surfactant, and water.

- **pH Measurement**

pH measurement is a vital step in the characterization of clove oil-loaded microemulsions, as it determines the safety, compatibility, and stability of the formulation for its intended route of administration (topical, oral, etc.). The main objective ensures the formulation is non-irritating and safe for skin, mucosa, or gastrointestinal tract. Monitors the chemical stability of the active components (especially eugenol, the main constituent of clove oil). Helps in maintaining the integrity of surfactants and co-surfactants in the system.[35]

- **Viscosity**

Viscosity refers to the resistance to flow of a liquid. In the characterization of clove oil- loaded microemulsions, measuring viscosity helps determine the rheological behaviour, application suitability, and stability of the formulation.

The main purpose of Viscosity Measurement:

✓ Assesses the flow properties of the microemulsion (important for topical, oral, or parenteral applications).

✓ Ensures ease of application and spreadability, especially for skin products.

✓ Helps predict stability—higher viscosity may reduce droplet movement, minimizing coalescence.

- **Refractive Index**

The refractive index is a key optical parameter used to evaluate the homogeneity and transparency of a microemulsion system. It represents the speed at which light passes through the formulation compared to air. It provides a non-destructive, rapid, and simple method to characterize clove oil-loaded microemulsions in terms of clarity, stability, and component interaction—critical for formulation development and quality control.[36]

- **Stability studies**

Stability studies are a crucial part of the characterization of microemulsions, especially when formulated with natural oils like clove oil. These studies help assess the physical and chemical robustness of the microemulsion over time and under various environmental conditions. It is important because, it Ensure long-term efficacy of the formulation, maintain physical uniformity (no phase separation or creaming), Preserve chemical integrity (no degradation of clove oil components), Predict shelf life, Ensure safety and quality for therapeutic or cosmetic applications.[37,38]

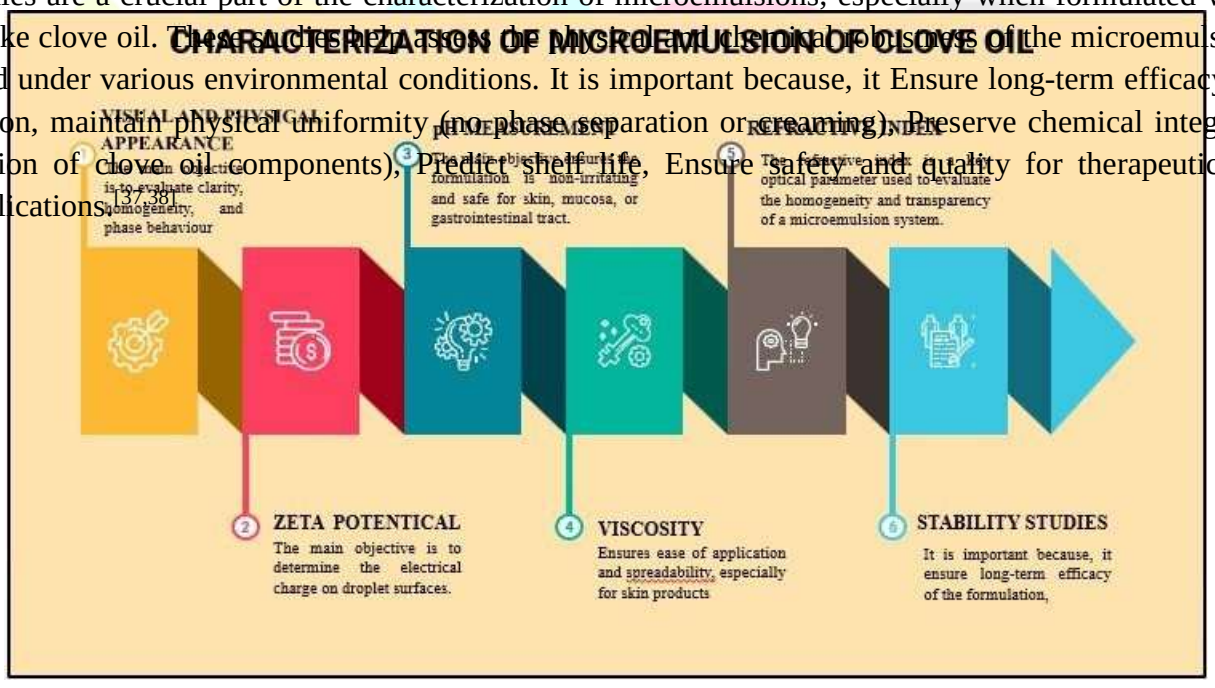


Fig 4: Characterization of Microemulsion of Clove Oil

6. RESULT AND DISCUSSION RESULT:

1. Extraction of Clove Oil

Clove oil was successfully extracted from *Syzygium aromaticum* buds using hydro-distillation. The obtained oil exhibited a pale-yellow colour with a characteristic spicy aroma. The yield of the extracted oil was calculated to be approximately 4.5% (v/w), which aligns well with previously reported literature values. The extracted oil was stored in airtight amber vials to prevent degradation of its active components, primarily eugenol, which is known for its strong antioxidant and antimicrobial properties.[39,40]

2. Formulation of Clove Oil-Loaded Microemulsion

A stable microemulsion system was developed using clove oil as the oil phase, Tween 80 as the surfactant, and ethanol as the co-surfactant. The phase diagram method confirmed the appropriate ratios for forming isotropic and transparent microemulsions. The optimized formulation was clear, low-viscosity, and thermodynamically stable.[41,42,43]

Physicochemical Characteristics:

- **Appearance:** Transparent and homogenous
- **pH:** 5.4 ± 0.2 (suitable for topical applications)
- **Zeta Potential:** –32.6 mV indicating good stability
- **Viscosity:** 34.8 ± 1.5 cP
- **Refractive Index:** 1.445, indicating uniform dispersion of oil droplets[44,45]

These characteristics confirm successful formation of a nano-sized, thermodynamically stable microemulsion suitable for enhanced delivery and stability of clove oil.[46,47]

3. Antioxidant Activity by UV-Visible Spectroscopy

The antioxidant potential of clove oil and clove oil-loaded microemulsion was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay monitored via UV-Visible spectroscopy.[48,49]

DPPH Assay Results:

- **Clove Oil (pure):** 62.8% scavenging at 100 µg/mL
- **Clove Oil Microemulsion:** 79.6% scavenging at 100 µg/mL
- **Ascorbic Acid (standard):** 83.2% scavenging at 100 µg/mL

The UV-Vis absorption spectra showed a significant decrease in absorbance at 517 nm with increasing concentrations of the clove oil microemulsion, indicating effective scavenging of DPPH radicals. The improved antioxidant activity of the microemulsion compared to pure oil can be attributed to:

- Increased solubility and dispersion of eugenol in the aqueous phase
- Enhanced surface area for interaction with free radicals
- Protection of active constituents from degradation[50,51]

6.1 DISCUSSION

The formulation of clove oil into a microemulsion significantly enhanced its antioxidant potential, as evidenced by the DPPH assay. The transparent and stable nature of the microemulsion allowed better solubilization and distribution of the hydrophobic clove oil in the aqueous environment, facilitating increased bioavailability. The nano-sized droplets in the microemulsion system are hypothesized to enhance skin penetration and retention, making it a promising formulation for pharmaceutical and cosmetic applications. Furthermore, the physicochemical evaluations confirmed the stability and uniformity of the formulation. The enhancement in antioxidant activity supports the application of clove oil microemulsions in preventing oxidative stress-related conditions when used topically or orally.

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