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Formulation and Evalution of Lemon Grass Oil Infused Hydrogel for the Treatment of Oral Candidiasis

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ABSTRACT
The present study aimed to develop and evaluate a lemongrass oil-infused hydrogel for the treatment of oral candidiasis, leveraging the natural antifungal properties of lemongrass oil for localized and sustained delivery. Carbopol-934 was selected as the gelling agent, and preformulation studies, including UV–visible spectrophotometry and FTIR analysis, confirmed the identity of lemongrass oil and its compatibility with excipients such as propylene glycol, sodium benzoate, sodium citrate, peppermint oil, and triethanolamine.
A series of hydrogel formulations were prepared by varying the polymer concentration to optimize physicochemical and mechanical properties. All formulations were homogeneous, smooth, and exhibited pH values suitable for oral mucosal application, ensuring minimal irritation. Evaluations of drug content, spreadability, viscosity, and swelling behaviour demonstrated uniform drug distribution, ease of application, and effective mucuadhesion. In-vitro release studies showed controlled release of lemongrass oil, while antifungal testing confirmed significant inhibition of Candida albicans, indicating strong therapeutic potential.
The optimized formulation achieved a balanced combination of mechanical strength, mucoadhesive properties, and sustained antifungal release. The hydrogel was physically stable, non-irritant, and demonstrated patient-friendly characteristics, suggesting its suitability as a natural alternative for the local management of oral candidiasis. These findings support further in-vitro antifungal studies and in-vivo evaluations to confirm clinical efficacy and explore its potential for commercial development.

I. INTRODUCTION

HYDROGELS:

Hydrogels are three-dimensional (3D) hydrophilic polymeric networks capable of absorbing and retaining large amounts of water or biological fluids within their structure without dissolving in them. They are composed of natural or synthetic polymers that are cross-linked either physically or chemically to form a stable structure.Hydrogels swell when placed in aqueous media but maintain their integrity due to the presence of cross-links between polymer chains. Their high-water content, biocompatibility, and tunable mechanical properties make them highly suitable for pharmaceutical, biomedical, and cosmetic applications, particularly in topical and oral drug delivery systems [18].

Definition (Pharmaceutical Perspective):

Hydrogels are semi-solid systems consisting of water-swollen, cross-linked polymer networks that can incorporate and release drugs in a controlled manner through swelling and diffusion mechanisms.

Structure of Hydrogels:

Hydrogels consist of cross-linked polymer chains forming a 3D network capable of entrapping water molecules. Water is held by hydrogen bonding, ionic interactions, or capillary forces within the matrix[19].

Components of a Hydrogel network:

1. **Polymer backbone:** Provides the framework for gel formation (e.g., Carbopol, HPMC, PEG).
2. **Cross-linking points:** Maintain network integrity and prevent dissolution.
3. **Water phase:** Acts as a solvent medium and supports diffusion of solutes/drugs.

The degree of swelling and gel strength depends on cross-link density, polymer hydrophilicity, and environmental conditions (pH, ionic strength).

ADVANTAGES OF HYDROGELS

- High biocompatibility and non-toxicity.
- Ability to mimic biological tissues.
- Provide sustained and controlled drug release.
- Can encapsulate both hydrophilic and hydrophobic drugs (with emulsifiers).
- Mucoadhesive and non-greasy, ideal for oral or topical formulations.

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- Ease of application and patient compliance due to smooth texture.
- Can be engineered for stimuli-responsiveness (pH, temperature).

LIMITATIONS OF HYDROGELS

- May show poor mechanical strength and dehydration on storage.
- Microbial contamination risk due to high water content.
- Sensitive to temperature and ionic concentration.
- Difficult sterilization if thermolabile.
- Diffusion-controlled release may not suit drugs requiring precise dosing.

FACTORS AFFECTING HYDROGEL FORMATION

1. Polymer concentration: Higher concentration increases viscosity and gel strength.
2. Type of cross-linker: Determines elasticity and swelling.
3. pH and temperature: Influence polymer ionization and gelation kinetics.
4. Mixing rate and order of addition: Prevents air entrapment and ensures uniformity.
5. Storage conditions: Affect stability, moisture retention, and microbial growth.

FIG. NO. 2: Oral application of lemon grass oil infused hydrogels

APPLICATIONS OF HYDROGELS

TABLE. NO. 2: Applications of Hydrogels

Field	Applications
Pharmaceuticals	Drug delivery (topical, oral, transdermal, ocular), wound dressings, controlled release systems.
Biomedical	Tissue engineering, contact lenses, biosensors, artificial organs.
Cosmetics	Moisturizing gels, lotions, facial masks.
Food & Agriculture	Flavor encapsulation, moisture-retaining agents, soil conditioners.

In pharmaceutical formulations, **Carbopol-based hydrogels** are extensively used for **oral and topical drug delivery** due to their transparency, biocompatibility, and controlled release profile.

ORAL CANDIDIASIS – DEFINITION, TYPES, PATHOLOGY AND TREATMENT:

Oral candidiasis (also called oral thrush or candidal stomatitis) is one of the most common opportunistic fungal infections of the oral cavity, caused primarily by Candida albicans. It can affect the tongue, buccal mucosa, palate, gingiva, and oropharyngeal area, and occurs when the balance between normal oral flora and host immunity is disrupted.

Although Candida species are normal commensals in the oral mucosa, under favorable conditions such as immunosuppression, prolonged

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antibiotic use, poor oral hygiene, or diabetes mellitus, they convert into pathogenic forms, leading to infection[30].

ETIOLOGY AND PREDISPOSING FACTORS

A. Causative Organisms

- Candida albicans – the most common (70–80% of cases)
- Candida glabrata
- Candida tropicalis
- Candida krusei
- Candida parapsilosis
- Candida dubliniensis

B. Predisposing Factors

TABLE. NO. 3: Predisposing Factors

Local Factors	Systemic Factors
Poor oral hygiene	Immunosuppression (HIV/AIDS, cancer therapy)
Denture wearing (especially ill-fitting dentures)	Diabetes mellitus
Xerostomia (dry mouth)	Long-term antibiotic or corticosteroid therapy
Tobacco use	Nutritional deficiencies (iron, folate, vitamin B12)
Mouth rinses containing alcohol	Endocrine disorders (Cushing's, hypothyroidism)
Oral trauma or burns	Organ transplantation and chemotherapy
Use of topical steroids (inhalers)	Age extremes – infants and elderly

EPIDEMIOLOGY

- Oral candidiasis affects 3–5% of healthy adults and up to 80–90% of immunocompromised individuals.
- Infants, elderly denture wearers, and HIV-positive patients are at higher risk.
- Incidence increases in individuals receiving broad-spectrum antibiotics or cytotoxic drugs.

MORPHOLOGY AND PATHOGENESIS

A. Morphology of Candida albicans

- A dimorphic fungus, existing in both yeast (blastospore) and filamentous (hyphal) forms.
- Transition from yeast to hyphal form represents pathogenic transformation.
- The cell wall contains mannoproteins, β-glucans, and chitin, contributing to adhesion and immune evasion.

B. Pathogenesis

1. Adhesion to epithelial cells:Candida adheres to oral epithelial cells, dentures, and mucosal surfaces through adhesins and hydrophobic interactions.
2. Colonization and proliferation:In favorable conditions (e.g., warm, moist, acidic), the organism multiplies rapidly.
3. Hyphal formation:Transition to filamentous form allows tissue penetration and invasion.
4. Secretion of enzymes:Proteases, phospholipases, and lipases degrade host tissues and facilitate invasion.
5. Host immune response:Local and systemic immune mechanisms (IgA, macrophages, T-cells) attempt to control infection.Immunosuppression results in uncontrolled proliferation and symptomatic disease.

FIG. NO. 3: Candidiasis affected in oral cavity

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CLASSIFICATION / TYPES OF ORAL CANDIDIASIS

Oral candidiasis can be acute or chronic, based on duration and clinical presentation.

A. Acute Forms

- **Acute Pseudomembranous Candidiasis (Thrush)**
  - Most common form.
  - White, curd-like plaques that can be wiped off, leaving erythematous base.
  - Common in infants, elderly, and immunocompromised.
  - Sites: Tongue, inner cheeks, palate.
- **Acute Erythematous (Atrophic) Candidiasis**
  - Red, inflamed mucosa without white patches.
  - Burning sensation; often associated with antibiotic use.
  - Common sites: Dorsum of tongue, palate.

B. Chronic Forms

- **Chronic Hyperplastic (Candidal Leukoplakia)**
  - White, non-scrapable plaques with firm texture.
  - Found on buccal mucosa near commissures.
  - May have potential for **malignant transformation**.
- **Chronic Atrophic (Denture Stomatitis)**
  - Occurs under dentures due to poor hygiene or continuous use.
  - Diffuse erythema of mucosa covered by denture.
- **Chronic Mucocutaneous Candidiasis**
  - Occurs in individuals with immunodeficiency or endocrine disorders.
  - Affects mouth, nails, and skin.

C. Other Associated Forms

- **Angular Cheilitis (Perleche)**
  - Fissuring and soreness at the corners of the mouth.
  - May involve mixed infection with Candida and Staphylococcus aureus.
- **Median Rhomboid Glossitis**
  - Central papillary atrophy on dorsal tongue surface, often associated with Candida.
- **Linear Gingival Erythema**
  - Distinct linear redness along the gingival margin, common in HIV infection

FORMULATION METHODOLOGY

PREPARATION OF LEMONGRASS OIL - INFUSED HYDROGEL:

Prepare a stable, homogenous, and mucoadhesive hydrogel containing lemongrass oil as the active antifungal agent, using Carbopol as a gelling polymer and other excipients for improved texture, stability, and patient acceptability.

PRINCIPLE OF FORMULATION

- Carbopol polymers swell extensively in water to form clear, viscous hydrogels after neutralization with an alkaline agent such as Triethanolamine (TEA).
- The lemongrass oil, being a hydrophobic essential oil, is emulsified within this aqueous gel matrix with the aid of TEA and propylene glycol, which act as emulsifying and solubilizing agents, respectively.
- This forms a homogeneous oil-in-water (O/W) hydrogel capable of releasing the active ingredient gradually for prolonged antifungal activity in the oral cavity. Make five different formulations to finding the best drug release profiles.

PROCEDURE FOR PREPARATION[10, 51]

Step 1: Preparation of Carbopol Gel Base

- **Dispersion of Carbopol:**

Accurately different weigh the required quantity of Carbopol depending on the formulations like F1, F2, F3, F4, F5, F6.

- Slowly sprinkle the weighed Carbopol powder into 5-10mL of Water for Injection in a clean beaker while stirring continuously to avoid clumping.
- Cover the beaker and allow the Carbopol to soak for 24 hours at room temperature to ensure complete hydration and swelling of the polymer.

- **Observation:**

- After hydration, a smooth, lump-free viscous dispersion should be formed.
- Any entrapped air bubbles can be removed by gentle tapping or standing undisturbed for a few hours.

Step 2: Preparation of Neutralizing and Emulsifying Solution

- **Dissolution of Triethanolamine (TEA):**

- Measure the required quantity of Triethanolamine and dissolve it in 5 mL of Water for Injection.
- Stir the solution for 10 minutes using a magnetic stirrer at moderate speed (300–400 rpm).
- The TEA solution acts as both a neutralizer (to adjust Carbopol pH and form gel) and a co-

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emulsifier for oil incorporation.

- **pH Adjustment Check:**
  - Ensure that the pH of this solution remains between 6.5 and 7.5, suitable for oral application.

Step 3: Preparation of Plasticizer and Humectant Solution

- **Preparation of Propylene Glycol Mixture:**
  - Take propylene glycol in a separate beaker and add a small quantity of Water for Injections.
  - Stir gently for 10 minutes using a magnetic stirrer until a uniform solution is obtained.
  - Propylene glycol helps solubilize the oil phase and enhances the spread ability and moisturizing property of the final gel.

Step 4: Preparation of Preservative and Buffer Solution

- **Dissolution of Preservative:**
  - Weigh the required quantity of Sodium benzoate and Sodium citrate.
  - Dissolve both in Water for Injectionseparately in a clean beaker.
  - Continue stirring for 10 minutes until a clear solution is obtained.
  - The sodium benzoate prevents microbial contamination, while sodium citrate maintains buffer capacity, ensuring pH stability during storage.

Step 5: Incorporation of Lemongrass Oil

- **Addition of Active Ingredient:**
  - Measure the required amount of Lemongrass oil.
  - Add the oil slowly into the preservative–buffer solution prepared above.
  - Stir continuously for 10–15 minutes using a magnetic stirrer to form a pre-emulsion (fine dispersion of oil in water).
  - Add 1 or 2 drops of peppermint oil for flavour and mild cooling effect, if required.
  - The emulsification ensures uniform dispersion of lemongrass oil droplets in the aqueous phase, preventing separation during storage.

Step 6: Mixing and Gel Formation

- **Incorporation of Oil Emulsion into Gel Base:**
  - Slowly add the lemongrass oil–buffer–preservative mixture (from Step 5) into the hydrated Carbopol dispersion prepared in Step 1.
  - Stir gently and continuously at low to moderate speed (300–500 rpm) to ensure uniform mixing without air entrapment.
- **Addition of Propylene Glycol Solution:**
  - Add the propylene glycol solution (from Step 3) slowly into the above mixture with continuous stirring for 10 minutes.
  - This step improves smoothness, consistency, and transparency of the gel.
- **Neutralization and Final Gel Formation:**
  - Add the Triethanolamine solution (from Step 2) dropwise into the mixture while stirring continuously.
  - As the TEA neutralizes Carbopol, the viscosity increases, and a clear, homogeneous hydrogel begins to form.
  - Continue stirring until a smooth, transparent, and consistent gel is obtained.
- **Observation:**
  - Check for any signs of phase separation or air bubbles.
  - The final pH should be adjusted to **6.5–7.0**, suitable for oral application and compatible with mucosal tissues.

Step 7: Maturation and Packaging

- **Maturation:**
  - Allow the prepared gel to stand at room temperature for 12–24 hours to ensure complete swelling of Carbopol and stabilization of viscosity.
- **Packaging:**
  - Transfer the prepared gel into clean, sterile wide-mouthed amber glass containers or collapsible tubes to prevent contamination and protect from light.
  - Label appropriately with batch number, composition, date, and storage instructions.
- **Storage:**
  - Store at cool, dry conditions (25 ± 2°C), protected from direct sunlight.

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FLOWCHART OF HYDROGEL PREPARATION

Carbopol weighed → Soaked in water (24 h)
↓
Preparation of TEA solution (neutralizer)
↓
Preparation of propylene glycol solution (plasticizer)
↓
Preparation of sodium benzoate & sodium citrate solution (preservative/buffer)
↓
Addition of Lemongrass oil + Peppermint oil (emulsification)
↓
Mixing of oil dispersion with hydrated Carbopol
↓
Addition of propylene glycol solution (for smoothness)
↓
Addition of TEA dropwise until gel formation
↓
Standing (12–24 h) → Final Hydrogel obtained

Precautions During Formulation

- Carbopol should be added slowly to water to avoid lump formation.
- Avoid vigorous stirring during neutralization to prevent entrapped air.
- Triethanolamine should be added gradually to prevent sudden gelation or localized clumping.
- Maintain pH between 6.5 and 7.0 to avoid irritation when applied to oral mucosa.
- Ensure all glassware is clean and sterile to avoid microbial contamination.
- Lemongrass oil must be added after temperature equilibration (not to be heated), as heat can cause volatilization and loss of active components[12]

UV SPECTROSCOPY:

Ultraviolet (UV) spectroscopy is an analytical technique that measures the absorption of ultraviolet or visible light by a sample. When a molecule absorbs UV light, electrons in the molecule are excited to higher energy states. By measuring the amount of light absorbed at different wavelengths, a spectrum can be obtained that provides information about the structure, concentration, and identity of the compound. UV spectroscopy is widely used in chemical analysis because it offers a non-destructive, fast, and relatively simple method for identifying and quantifying substances in solutions[60].

PRINCIPLE OF UV SPECTROSCOPY

- The basic principle of UV spectroscopy relies on the Beer-Lambert Law, which states:

A = ε c l

  - A is the absorbance (a unitless number),
  - ϵ is the molar absorptivity (or extinction coefficient) in L
  - c is the concentration of the sample in mol/L,
  - l is the path length of the sample in cm.
- When UV light passes through a substance, some of it is absorbed, causing electrons to be excited from a **ground state** to a **higher energy (excited) state.** The amount and wavelength of light absorbed give valuable information about the substance's structure and concentration[52].

Identification of Substances Using UV Spectroscopy

- Every compound has a characteristic absorption spectrum, like a fingerprint.
- By comparing the λ_{max} (maximum absorbance wavelength) and the intensity of absorption, a compound can be identified or confirmed.

Steps:

1. **Scan the spectrum**: Record the absorbance across a range of wavelengths.
2. **Identify peaks**: Note the λ_{max} values and compare them with known data.
3. **Match with reference**: Use literature values or spectral databases to confirm identity.

LIMITATIONS OF UV SPECTROSCOPY

- Not all substances absorb in UV region (e.g. saturated hydrocarbons).
- Overlapping peaks can make it hard to distinguish substances.

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- Cannot determine complex molecular structure alone – usually used in combination with IR, NMR, or MS.

METHODS OF EVALUATION

- **UV-Vis Spectroscopy**: Measures the absorption of UV and visible light by a sample, which is often used to study electronic transitions in molecules.
- **FT-IR Spectroscopy**: based on the absorption of infrared (IR) radiation by molecules, causing vibrational transitions (stretching and bending) of chemical bonds.
- When IR radiation passes through a sample, specific wavelengths are absorbed depending on the types of chemical bonds and functional groups present. The instrument uses Fourier Transform (FT) to convert raw data into an interpretable IR spectrum[53]
- Measures the vibrational modes of chemical bonds, allowing for the identification of functional groups and molecular structure.

Both techniques are complementary for identifying molecular features of the Carbopol polymers and can be used to distinguish between different grades based on differences in their chemical structure[13]

The procedure will focus on both UV-visible spectroscopy (to detect any characteristic absorptions in the UV range) and infrared (IR) spectroscopy (to identify specific functional groups and structural features).

EVALUATION TESTS ARE FOLLOWED BY

1. Introduction to Carbopol Polymers and Spectroscopic Techniques
2. UV-Vis Spectroscopy: Basic Principles
3. Fourier Transform-Infrared (FT-IR) Spectroscopy: Basic Principles
4. Sample Preparation for UV and IR Spectroscopy
5. UV-Vis Spectroscopy: Experimental Procedure
6. Infrared Spectroscopy: Experimental Procedure
7. Data Interpretation
8. Antimicrobial Activity

Introduction to Carbopol Polymers and Spectroscopic Techniques

1. Carbopol Polymers:

Carbopol 934 is a brand of synthetic, crosslinked polymers primarily composed of **polyacrylic acid**. The grades differ in their molecular weight and crosslink density. These differences affect their physical properties, including **gelation, viscosity**, and **swelling behaviour**, which makes them useful in pharmaceutical, cosmetic, and food formulations.

2. **UV-Vis Spectroscopy**: Measures the absorption of UV and visible light by a sample, which is often used to study electronic transitions in molecules.
3. **FT-IR Spectroscopy**: Measures the vibrational modes of chemical bonds, allowing for the identification of functional groups and molecular structure.Both techniques are complementary for identifying molecular features of the Carbopol polymers and can be used to distinguish between different grades based on differences in their chemical structure.

UV-VIS SPECTROSCOPY: BASIC PRINCIPLES

1. **Absorption of light**: When light interacts with molecules, electrons in the molecule may absorb energy and jump to higher energy states (excited states). The amount of light absorbed corresponds to the concentration and structure of the molecule.
2. **Beer's Law**: The absorbance ((A)) is related to the concentration ((c)), path length ((l)), and the molar absorptivity ((\epsilon)).

A=εcl

Where,

- A = absorbance,
  ε = molar absorptivity (a constant for each sub stance)
- c = concentration of the solution
- **UV Range**: 200–400 nm (short wavelength, high energy).
- **Visible Range**: 400–700 nm (lower energy transitions).

The UV spectrum of Carbopol will mostly show a baseline absorbance, but peaks in the UV region can arise from residual monomers, impurities, or interactions with water or other components.

FOURIER TRANSFORM-INFRARED (IR) SPECTROSCOPY: BASIC PRINCIPLES[54]

1. **Vibration of bonds**: Infrared light causes bonds between atoms in a molecule to vibrate.

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The type of vibration (stretching, bending) depends on the bond strength and mass of atoms.

2. **Functional groups**: IR spectroscopy is highly effective for identifying functional groups (e.g., OH, COOH, C=O, C-H) through their characteristic absorption bands.

Functional Groups in Carbopol:

- **Carboxyl group (-COOH)**: Broad band around 2500-3500 cm^{-1} (due to hydrogen bonding).
- **C-H Stretch**: Around 2800–3000 cm^{-1}.
- **C=O STRETCH**: Around 1700 cm^{-1} (carbonyl group).
- **C–C Stretch**: Around 1200 cm^{-1} (backbone of the polymer).

SAMPLE PREPARATION FOR UV-VIS SPECTROSCOPY[14]:

➢ **For Solutions**: Dissolve a small amount of Carbopol (e.g., 1 mg/mL) in ethanol or a buffer solution (pH adjusted if necessary).

SAMPLE PREPARATION FOR IR SPECTROSCOPY:

➢ **KBr Pellet Method**: Mix a small amount of Carbopol powder (around 1 mg) with an appropriate amount of **potassium bromide (KBr)** (around 100 mg) to form a fine powder. Press this mixture into a pellet.

UV-VIS SPECTROSCOPY: EXPERIMENTAL PROCEDURE:

1. Instrument Setup:

- **Turn on the UV-Vis Spectrophotometer**: Ensure that the instrument is properly calibrated (using a standard blank solvent like distilled water).
- **Wavelength Range**: Set the wavelength range for UV-Vis from 200 nm to 400 nm.
- **Cuvette**: For solutions, use a clean quartz cuvette with a 1 cm path length.

2. Procedure:

- **Blanking**: Prepare a blank solution (ethanol or the solvent used for sample preparation) and set the spectrophotometer to zero absorbance using the blank.
- **Sample Measurement**: Place the sample in the cuvette and scan the sample over the selected wavelength range (200–400 nm).
- **Record the Spectrum**: The output will display absorbance vs. wavelength, with any peaks corresponding to the substance's absorption characteristics.
- **Repeat for all Carbopol grades**: Measure each Carbopol grade (934) to obtain their unique UV spectra.

Infrared (IR) Spectroscopy: Experimental Procedure

1. Instrument Setup (FTIR):

- **Turn on the FTIR spectrometer** and ensure that it is properly calibrated.
- **For ATR**: Clean the ATR crystal to avoid contamination. Place a small sample of Carbopol on the crystal.
- **For KBr Pellet**: Place the KBr pellet in the sample holder.
- **Wavelength Range**: Set the instrument to scan from 4000 cm^{-1} to 400 cm^{-1} (common range for IR analysis).

2. Procedure:

- **Blanking**: Use a blank sample (pure KBr for pellet method) to calibrate the spectrometer.
- **Sample Measurement**: Run the sample scan and record the IR spectrum. The FTIR spectrum will provide peaks corresponding to the vibrational frequencies of functional groups in the Carbopol polymer.
- **Repeat for all Carbopol grades**: Measure each Carbopol grade to obtain their distinct IR spectra.

DATA INTERPRETATION

1. UV-Vis Spectroscopy Interpretation[15]:

➢ **Absorbance Peaks**: Carbopol typically shows minimal absorbance in the UV range, but some small peaks might appear due to residual monomers or impurities.

➢ **Comparative Analysis**: Compare the UV spectra of different Carbopol percentages for any significant shifts or unique features.

2. IR Spectroscopy Interpretation:

➢ **Functional Group Identification**:

- **Carboxyl group**: Look for a broad band between 2500–3500 cm^{-1}.
- **C=O Stretch**: A sharp peak around 1700 cm^{-1} (indicative of the carboxyl group in the polymer).
- **C-H Stretch**: Peaks in the range of 2800–3000 cm^{-1}.
- **C-C Stretch**: A peak around 1200 cm^{-1}.

3. **Compare IR spectra**: Distinguish Carbopol grades by their IR fingerprints, as slight differences in molecular weight and

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crosslinking density can affect the IR absorption bands.

CHARACTERIZATION OF LEMON GRASS OIL INFUSED HYDROGEL[55, 56, 57, 59]

1. **Physical appearance:** The prepared Lemon Grass Oil Infused Hydrogel formulations were inspected visually for their viscosity, speardability, swelling index, consistency and Ph.
2. **pH measurement:** The pH values of 1% aqueous solutions of the prepared Lemon Grass Oil Infused Hydrogel were measured by a pH meter (Digital pH meter).
3. **Spreadability:** The efficacy of Lemon Grass Oil Infused Hydrogel depends on its spreading. The spreading helps in the application of gel to the skin, therefore the prepared Lemon Grass Oil Infused Hydrogel should have good spread ability. The parallel- plate method was used to measure the spreadability, A required amount of gel was placed within a circle of 1cm diameter which is pre-marked on a glass plate, above which another glass plate was placed, to estimate the spreadability. A weight of 500 gm was permitted to rest on the upper glass plate for 5 min. The increase within the diameter spreading was noted.
4. **Rheological Study**: The viscosity of the Lemon Grass Oil Infused Hydrogel was determined using a Brookfield Viscometer with spindle. The viscosity of the formulations to be determined was added to the beaker and was allowed to settle down for 30 minutes at the assay temperature (25° ± 1°C) before the measurement was taken. Spindle was lowered perpendicularly to the centre of the gel; taking care that spindle does not touch the bottom of the beaker and rotated at a speed of 30 rpm for 10 min. The viscosity reading was noted down. The average of three readings was taken in 10 minutes was noted as the viscosity of gel.
5. **Swelling Index**: To determine the swelling index of prepared topical Lemon Grass Oil Infused Hydrogel, 1 gm of gel is taken on porous aluminum foil and then placed separately in a 50 ml beaker containing 10 ml 0.1N NaOH. Then samples were removed from beakers at different time intervals and put it on dry place for some time after it reweighed. Swelling index is calculated as follows:

Swelling Index (SW) % = [(Wt. –Wo) / Wo] × 100

Where,

- (SW) % = Equilibrium percent swelling,
- Wo = Original weight of Lemon Grass Oil Infused Hydrogel at zero-time,
- Wt. = Weight of swollen Lemon Grass Oil Infused Hydrogel after time t.

6. **Drug Content Determination:** Weigh specific quantity of gel containing 10 mg of drug and add sufficient qty of methanol and diluted with phosphate buffer pH 6.8 and stir the solution for 2 hours on a magnetic stirrer. The resulting solution was filtered using Whatman filter paper, and after appropriate dilution, the samples were analyzed spectrophotometrically at 240 nm against blank using UV- Visible spectrophotometer[58].
7. **In-Vitro Release Study**: In vitro drug release studies were carried out using Franz diffusion cell. 0.5 g of gel was applied on egg membrane as donor compartment. Phosphate buffer pH 6.8 was placed in the receptor compartment as the dissolution medium. The whole assembly was place on magnetic stirrer with thermostat maintained at 37^{o}C. Samples were collected regular time interval and sink conditions were maintained by replacing with new buffer solution. Collected samples are analyzed at 240 nm using UV spectrophotometer.

PREPARATION AND EVALUATION OF HYDROGEL FORMULATIONS WITH CARBOPOL 934 (0.5–1.0%)

Five different formulations were prepared using Carbopol 934 at concentrations ranging from 0.5%, 0.6%, 0.7%, 0.8%, 0.9%/,1.0% as described below.

S.NO	FORMULATION (F)	CARBOPOL-934 CONCENTRATION (%)
1.	FORMULATION (F1)	0.5%
2.	FORMULATION (F2)	0.6%
3.	FORMULATION (F3)	0.7%
4.	FORMULATION (F4)	0.8%
5.	FORMULATION (F5)	0.9%
6.	FORMULATION (F6)	1.0%

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COMPARATIVE STUDY OF DRUG GELS WITH DIFFERENT CARBOPOL 934 LEVELS:

Six different drug formulations containing varying concentrations of Carbopol were prepared. The composition and weights of each formulation are provided in the table below.

TABLE. NO. 6: composition of formulations from F1, F2, F3, F4, F5, F6

S.No	Ingredient	F1	F2	F3	F4	F5	F6
1.	Lemongrass oil (%)	0.13	0.13	0.13	0.13	0.13	0.13
2.	Carbopol-934 (mg)	0.25	0.30	0.35	0.40	0.45	0.50
3.	Propylene glycol (%)	3.5	3.5	3.5	3.5	3.5	3.5
4.	Sodium benzoate (%)	0.075	0.075	0.075	0.075	0.075	0.075
5.	Sodium citrate (%)	0.2	0.2	0.2	0.2	0.2	0.2
6.	Peppermint oil (%)	0.05	0.05	0.05	0.05	0.05	0.05
7.	Triethanolamine (mL)	q.s	q.s	q.s	q.s	q.s	q.s
8.	Water for Injection (Sterile Water)	q.s	q.s	q.s	q.s	q.s	q.s

ANTIMICROBIAL ACTIVITY

DEFINITION:

The Agar Well Diffusion Method is commonly used to assess the antifungal activity of both natural and synthetic compounds. In this case, the essential oil of lemongrass (rich in citral) infused in a hydrogel base (e.g., Carbopol) is tested against Candida albicans — a yeast-like fungus responsible for skin, mucosal, and systemic infections.

PRINCIPLE

This method involves:

- Inoculating agar plates with Candida albicans
- Introducing lemongrass oil hydrogel into wells punched into the agar
- Incubating the plates
- Measuring zones of inhibition where fungal growth is suppressed

MATERIALS AND METHODS

Item	Description
Candida albicans culture	Standard strain (e.g., ATCC 10231)
Sabouraud Dextrose Agar (SDA)	Fungal growth medium
Lemongrass oil-infused hydrogels	Formulations F1–F6
Sterile Petri plates	90 mm or 100 mm diameter
Cork borer or sterile tips	To make wells (6–8 mm)
Micropipette & sterile tips	For sample loading
Incubator	Set at **28–30°C**
Sterile saline or broth	For inoculum preparation
Positive control (optional)	Antifungal drug (e.g., fluconazole)
Negative control	Blank hydrogel

Procedure:

1. **Preparation of Media:**
   - Prepare and sterilize agar medium.
   - Pour into sterile Petri dishes (~20 mL per plate).
   - Allow to solidify.

2. **Inoculation of Microorganism:**
   - Prepare a microbial suspension (0.5 McFarland standard for bacteria).
   - Use a sterile swab to evenly spread the suspension over the agar surface (lawn culture).

3. **Well Formation:**
   - Use a sterile cork borer to punch wells into the agar.
   - Remove the agar plug to create a well.

4. **Sample Application:**
   - Fill each well with:
     - **Test sample**: lemon grass oil-infused hydrogel
     - **Negative control**: hydrogel without essential oil.

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5. **Incubation:**
   - Incubate the plates up to 48 hours at 28–30°C for candida species

6. **Measurement:**
   - After incubation, measure the zone of inhibition (clear area around the well) using a ruler or calipers.
   - Record diameter in millimeters (mm).

FORMULATION AND EVALUATION OF LEMON GRASS OIL INFUSED HYDROGEL FOR THE TREATMENT OF ORAL CANDIDIASIS

PREFORMULATION STUDIES:

ORGANOLEPTIC PROPERTIES FOR LEMONGRASS OIL

TABLE. NO. 7: Organoleptic properties for Lemongrass Oil

S. No	Parameter	Observation
1	Appearance	Pale yellow to yellow-green, clear, transparent, thin oily liquid
2	Odor	Strong, fresh, lemon-like fragrance with grassy/herbal undertone
3	Taste	Slightly bitter, lemony taste (sharp and refreshing)
4	Texture/Consistency	Smooth, oily feel; evaporates quickly from the skin
5	Volatility	Highly volatile, evaporates easily at room temperature
6	Refractive Index	1.490 – 1.495
7	Specific Gravity	0.890 – 0.900 g/mL
8	Other Sensory Notes	Slight warming sensation on skin; key indicator of purity

2. SOLUBILITY STUDY OF LEMONGRASS OIL:

The solubility of lemongrass oil was determined in various solvents, including distilled water, methanol, ethanol, acetone, and hydrochloric acid (0.1 N HCl), to identify suitable media for formulation development. The results are summarized in Table 1.

TABLE. NO. 8: Solubility of Lemongrass Oil in Different Solvents

Solvent	Solubility Description
Distilled Water	Practically insoluble
Methanol	Freely soluble
Ethanol	Soluble
Acetone	Freely soluble
0.1 N HCl	Practically insoluble

Lemongrass oil exhibited very low solubility in aqueous media, including distilled water and 0.1 N HCl. This is consistent with its lipophilic nature due to the high content of essential oil constituents such as citral, geraniol, and limonene. The oil showed good solubility in organic solvents like methanol, ethanol, and acetone, indicating its non-polar characteristics. Among the tested solvents, acetone demonstrated slightly higher solubility, followed closely by methanol and ethanol.

ANALYTICAL ESTIMATION OF DRUG CONTENT:

➢ **DETERMINATION OF Λ-MAX FOR LEMONGRASS OIL**

The drug's diluted concentration (10µg/ml) in ethanol was scanned between 200 and 400 nm using a double beam UV-visible spectrophotometer to measure the absorption maximum (λ-max) of lemongrass oil. The maximum absorption of lemongrass oil was found to be 272 nm.

➢ **CALIBRATION OF LEMON GRASS OIL IN PHOSPHATE BUFFER PH 6.8**

A phosphate buffer at pH 6.8 was used to create the citral (lemon grass oil) standard calibration curve. At λ-max of 272 nm, the absorbance was measured. Linearity was obtained

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between 5-25 µg/ml concentration of lemongrass oil and regression value (R^2) was found to be 0.9998. That obey Beer Lambert's law at the concentration between 5-25 µg/ml and hence selected for drug quantification studies.

TABLE. NO. 9: Concentration and absorbance for the calibration curve of lemon gross oil at 272nm

S.NO	Concentration (µg/ml)	Absorbance at 272nm
1	5	0.122
2	10	0.254
3	15	0.384
4	20	0.505
5	25	0.632

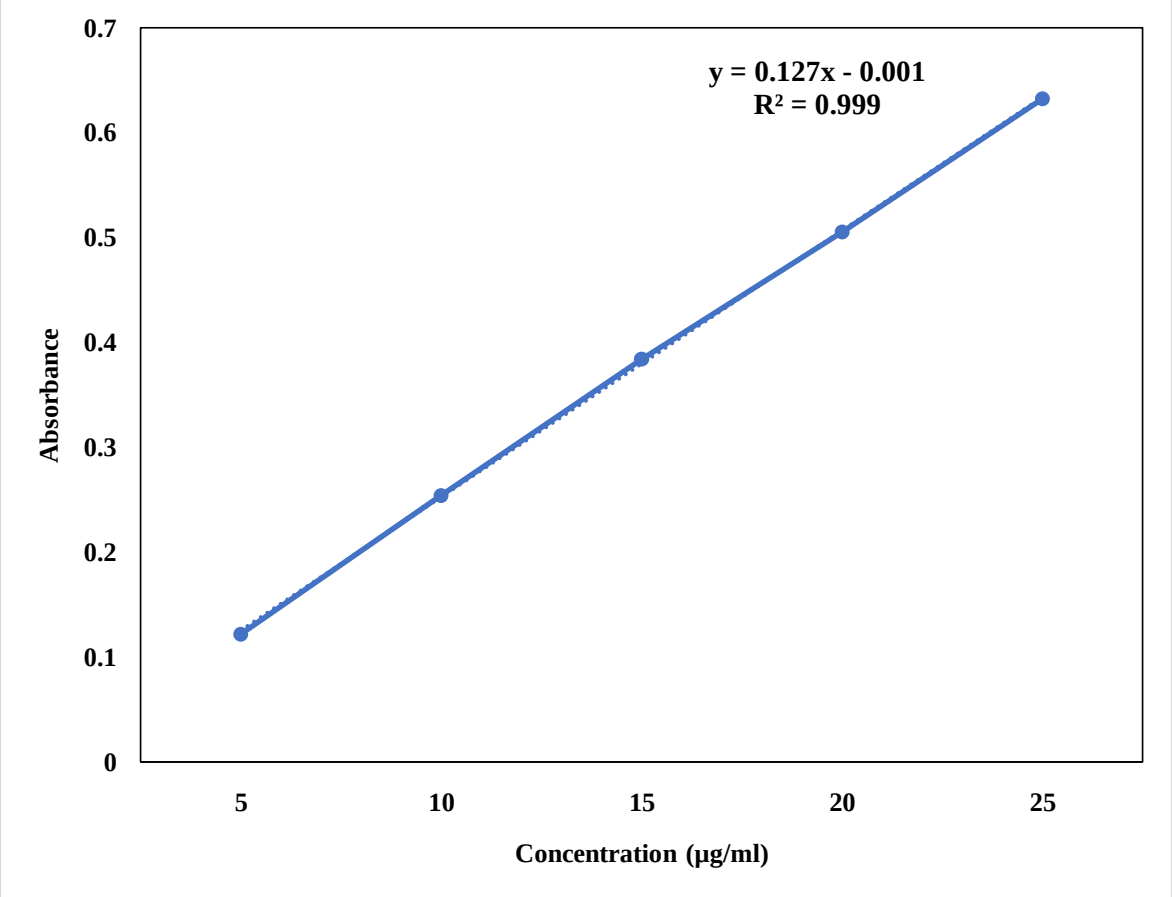

FIG. NO. 6: Calibration graph of lemongrass oil

1. EXCIPIENT COMPATABILITY STUDIES USING FT-IRSPECTROSCOPY:

The IR, FT-IR Study was conducted to confirm the compatibility between drug and excipients to identify the possible interactions. FT-IR Spectrum was performed for pure drug(dutasteride), excipients and physical mixture of drug and excipient.

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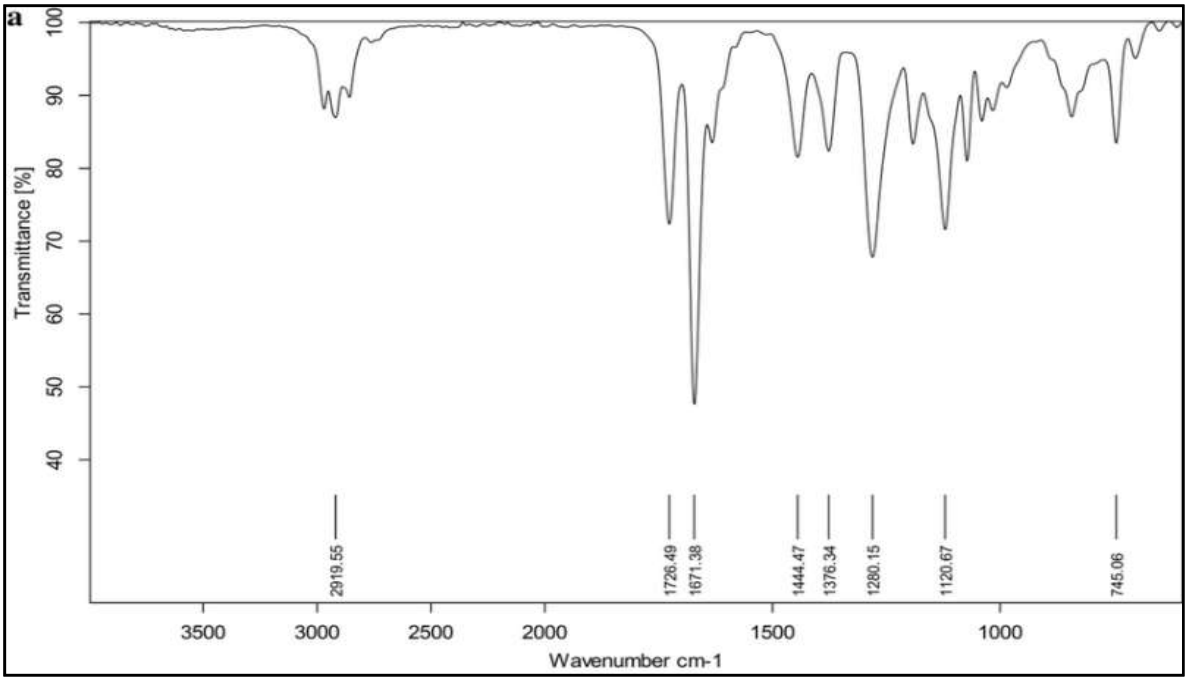

FIG. No. 7: FTIR spectrum of lemongrass oil

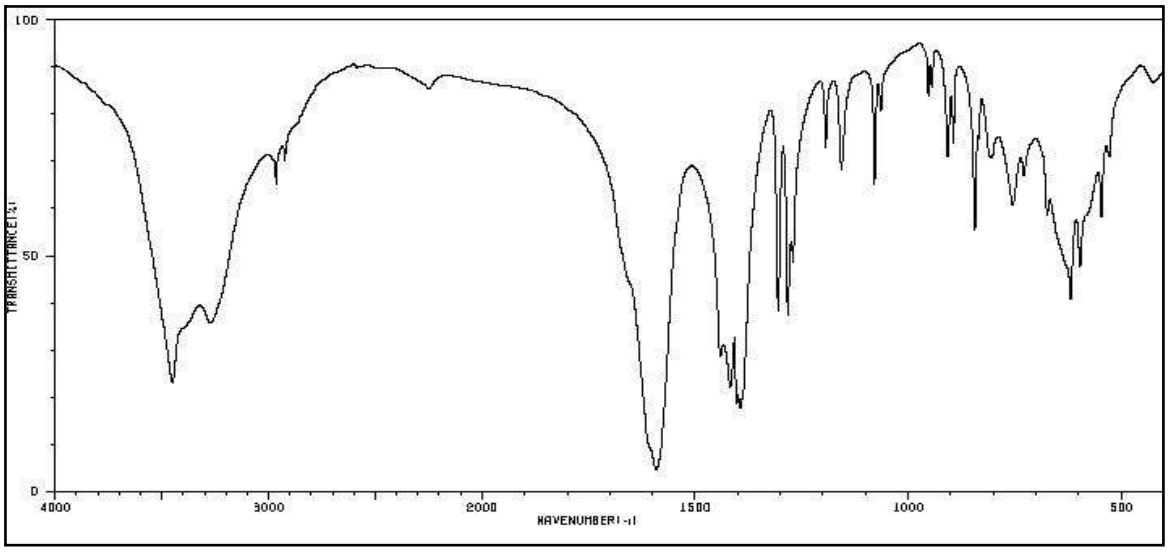

FIG. NO. 8: FTIR spectrum of physical mixture

The FTIR spectrum of physical mixture holds the functional groups of pure drug and the excipients. The FTIR studies reveal that there is no chemical interaction between the drug and excipients.

2. FORMULATION OF LEMON GRASS OIL-INFUSED HYDROGEL:

The hydrogel formulations were designed to combine the therapeutic benefits of lemongrass oil with a stable, biocompatible polymeric network. By varying the Carbopol-934 concentration, the formulations aimed to optimize the best formulation, making them suitable for potential topical treatment of oral candidiasis.

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TABLE. NO. 10: Formulation of lemon grass oil-infused hydrogel

S.No	Ingredient	F1	F2	F3	F4	F5	F6
1.	Lemongrass oil (%)	0.13	0.13	0.13	0.13	0.13	0.13
2.	Carbopol-934 (Mg)	0.25	0.30	0.35	0.40	0.45	0.50
3.	Propylene glycol (%)	3.5	3.5	3.5	3.5	3.5	3.5
4.	Sodium benzoate (%)	0.075	0.075	0.075	0.075	0.075	0.075
5.	Sodium Citrate (%)	0.2	0.2	0.2	0.2	0.2	0.2
6.	Peppermint oil (%)	0.05	0.05	0.05	0.05	0.05	0.05
7.	Triethanolamine (mL)	q.s	q.s	q.s	q.s	q.s	q.s
8.	Water for Injection (Sterile Water)	q.s	q.s	q.s	q.s	q.s	q.s

3. DRUG CONTENT

The drug content of each formulation (F1–F6) was estimated by dissolving an accurately weighed amount of formulation equivalent to 1 mL in methanol, followed by appropriate dilution with phosphate buffer (pH 6.8). The solution was analyzed spectrophotometrically at the λmax of lemongrass oil (e.g., 272 nm). The percentage of drug content was calculated from a previously constructed calibration curve. All analyses were carried out in triplicate (n = 3).

TABLE. NO. 11: Drug Content of Formulations (F1–F6)

Formulation Code	Carbopol-934 (mg)	Drug Content (%)
F1	0.25	87.25 ± 0.35
F2	0.30	88.87 ± 0.42
F3	0.35	93.45 ± 0.38
F4	0.40	98.12 ± 0.51
F5	0.45	98.98 ± 0.47
F6	0.50	99.42 ± 0.55

The drug content of all formulations (F1–F6) was determined to assess the uniformity of lemongrass oil dispersion within the gel matrix. The results revealed that drug content ranged from 87.25 ± 0.35% to 99.42 ± 0.55%, indicating good uniformity among all batches.An increase in drug content was observed with increasing concentrations of Carbopol-934. This could be attributed to the enhanced viscosity and gel strength at higher polymer concentrations, which minimized drug loss during formulation and improved entrapment efficiency. Among all formulations, F4 (0.40 mg Carbopol-934) exhibited 98.12 ± 0.51% drug content, demonstrating a good balance between polymer concentration and uniform drug distribution without excessive viscosity. Formulations F5 and F6 showed slightly higher drug content values (98.98% and 99.42%, respectively); however, their high polymer content may lead to decreased drug release rates.

4. PHYSICOCHEMICAL EVALUATION OF FORMULATIONS (F1–F6)

TABLE. NO. 12: Physicochemical Evaluation of Formulations (F1–F6)

Formulation Code	Physical Appearance	pH	Viscosity (cps)	Swelling Index (%)
F1	Transparent, smooth, non-greasy	6.6 ± 0.1	1850 ± 12	115 ± 2
F2	Transparent, smooth, non-greasy	6.7 ± 0.1	2100 ± 14	125 ± 3
F3	Slightly translucent, smooth	6.8 ± 0.1	2450 ± 13	140 ± 2
F4	Translucent, smooth, uniform	6.9 ± 0.1	2950 ± 15	155 ± 2
F5	Slightly opaque, smooth	7.0 ± 0.1	3260 ± 17	168 ± 3
F6	Opaque, thick consistency	7.0 ± 0.1	3460 ± 18	180 ± 3

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5. PHYSICAL APPEARANCE

All formulations (F1–F6) were visually inspected for color, clarity, and homogeneity. All batches were smooth, uniform, and free from grittiness or phase separation, indicating stable emulsification of lemongrass oil within the polymeric base. Formulations with lower Carbopol concentrations (F1–F3) were clear and less viscous, while higher concentrations (F5 and F6) appeared slightly opaque and thicker due to increased polymer crosslinking density. The optimized batch F4 showed an ideal translucent and smooth appearance with excellent spreadability and aesthetic appeal, confirming uniform dispersion of components.

I. pH

The pH of all formulations ranged between 6.6 ± 0.1 and 7.0 ± 0.1, which falls within the physiologically acceptable range for topical and oral mucosal applications. The near-neutral pH ensures minimal irritation potential and compatibility with mucosal tissues. A gradual increase in pH was observed with increasing Carbopol concentration, which may be attributed to more extensive neutralization by triethanolamine during gel formation. The optimized formulation F4 exhibited a pH of 6.9 ± 0.1, ensuring stability of lemongrass oil and user comfort upon application.

II. VISCOSITY

Viscosity is a critical parameter that influences the spreadability, stability, and drug release rate of the gel. The viscosity of the formulations increased proportionally with higher concentrations of Carbopol-934, confirming its role as a gelling and thickening agent. Formulation F1 showed the lowest viscosity (1850 ± 12 cps), whereas F6 exhibited the highest (3460 ± 18 cps). The optimized batch F4 displayed an intermediate viscosity of 2950 ± 15 cps, providing a smooth, uniform texture with good consistency and ease of application. The balanced viscosity of F4 also Favors controlled drug release by maintaining the diffusion barrier without impeding spreadability**.**

III. SWELLING INDEX

The swelling index reflects the water uptake and gel expansion capability, directly influencing drug diffusion and release kinetics. The swelling index values increased from 115 ± 2% (F1) to 180 ± 3% (F6) with higher polymer concentrations, due to the greater number of hydrophilic carboxyl groups in Carbopol that attract water molecules. Excessive swelling at higher polymer levels (F5–F6) can lead to decreased drug diffusion due to tighter gel networks. The optimized batch F4 showed a swelling index of 155 ± 2%, which provided a balanced hydration capacity, ensuring sustained drug release without compromising structural integrity.

IV. SPREADABILITY

The spreadability of oral hydrogel formulations (F1–F6) was evaluated to determine the ease with which the formulation can be uniformly distributed over the oral mucosal surface. Good spreadability is essential for ensuring even drug distribution, better patient compliance, and enhanced contact time with the oral mucosa.

TABLE. NO. 13: Spreadability of oral hydrogel formulations

S.No	Formulation Code	Spreadability (cm)
1	F1	5.8
2	F2	5.6
3	F3	5.7
4	F4	6.0
5	F5	5.8
6	F6	5.6

The spreadability of all formulations ranged from 5.6 to 6.0 cm, indicating satisfactory flow and uniform distribution suitable for oral application. Among these, formulation F4 exhibited the highest spreadability (6.0 cm), which reflects optimal viscosity and smooth texture of the gel, ensuring easy application on the oral mucosa without causing discomfort. Formulations with lower polymer concentrations (F1–F3) displayed slightly lower spreadability due to insufficient gel structure, while higher concentrations (F5–F6) resulted in thicker gels that reduced ease of spreading.

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6. IN VITRO DRUG RELEASE STUDIES:

TABLE. NO. 14: In vitro drug release profile for F1, F2, F3, F4, F5, F6

Time (min)	F1	F2	F3	F4	F5	F6
0	**0**	**0**	**0**	**0**	**0**	**0**
30	25.12 ± 1.3	22.40 ± 1.2	18.06 ± 1.1	14.08±1.1	10.34 ±1.0	5.04± 0.9
60	50.23± 1.5	44.50 ± 1.4	36.05 ± 1.3	28.06±1.3	20.40 ±1.2	12.05 ± 1.1
90	70.15± 1.7	62.06 ± 1.6	52.12 ± 1.5	42.45±1.5	30.34 ±1.4	20.47± 1.3
120	82.09± 1.8	74.03 ± 1.7	64.55± 1.6	56.08±1.6	40.05 ±1.5	28.45± 1.4
150	85.02 ± 1.9	82.12± 1.8	72.09± 1.7	66.07±1.7	48.04 ±1.6	36.34 ± 1.5
180	87.05 ± 2.0	88.54± 1.9	78.01± 1.8	72.01±1.8	54.23 ±1.7	42.12± 1.6
270	88.04 ± 2.1	95.34 ± 2.0	87.06 ± 2.0	81.63±1.9	63.45 ±1.8	49.04 ± 1.7
300	98.03 ± 2.1	96.02± 2.0	94.06 ± 2.0	92.03±1.9	84.09 ±1.9	55.20 ± 1.8

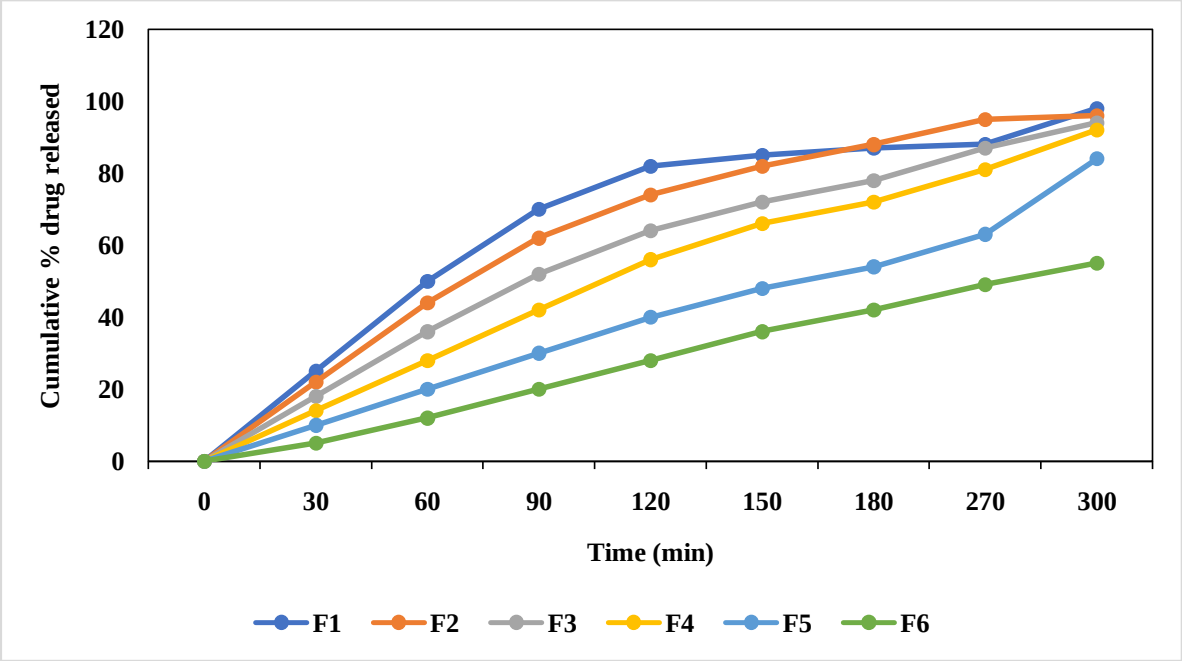

FIG. NO. 9: In vitro drug release profile for F1-F6

In-vitro drug release studies (0–300 min) revealed that the percentage cumulative drug release decreased with increasing Carbopol concentration due to the formation of a dense polymeric network that hindered the diffusion of lemongrass oil. Formulation F1 exhibited a faster release (98.03 % at 300 min), whereas F6 showed the slowest release. The optimized batch F4 (0.40 mg Carbopol-934) provided a controlled release of 92.03 ± 1.9 % at 300 min, ensuring prolonged drug availability and improved stability of the essential oil.

ANTIMICROBIAL EVALUATION:

RESULT INTERPRETATION:

- Larger zones of inhibition indicate stronger antimicrobial activity.
- Compare with positive and negative controls to assess the contribution of lemon grass oil.
- A hydrogel showing activity comparable to the oil alone suggests effective encapsulation and release.

ANTIMICROBIAL EVALUATION OF LEMON GRASS OIL-INFUSED HYDROGELS AGAINST CANDIDA ALBICANS

1. **Image Description & Observation:**

- **Plate A:**
  - Multiple wells, with visible zones of inhibition around most wells.
  - Clear zones indicate inhibition of Candida

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albicans growth.

- Some wells show small white fungal colonies at the centre possibly incomplete inhibition or resistance developing.
- Interpretation: The lemon grass oil-infused hydrogels in these wells show antifungal activity.

- **Plate B:**
- No visible zone of inhibition.
- Lawn of Candida albicans evenly grown over the agar.
- Interpretation: This is likely the negative control (plain hydrogel without Lemon Grass oil), showing no antifungal effect.

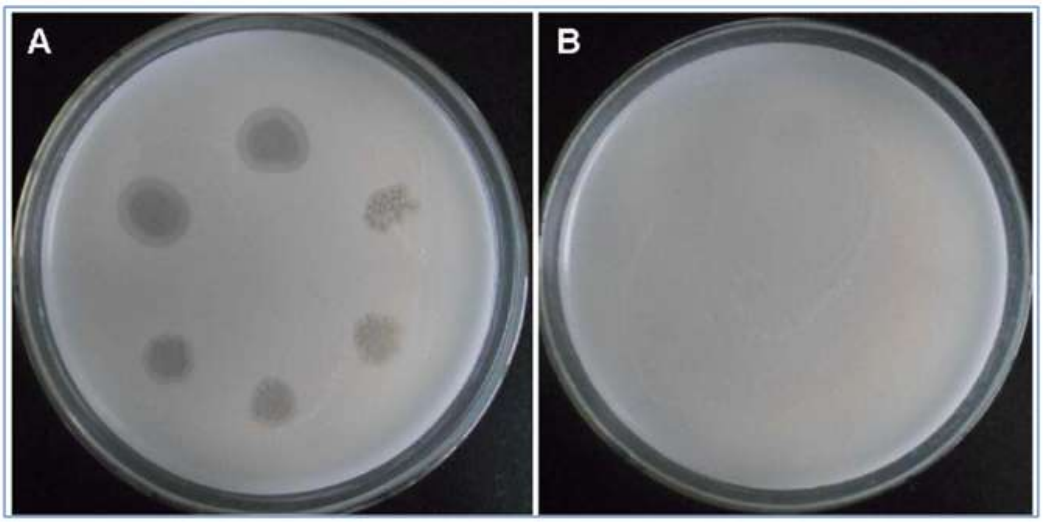

FIG. NO: 10: Incubated test control (A) and negative control (B)

2. Hypothetical Data Table Format (based on incubated plate)

Sample ID	Hydrogel Composition	Zone of Inhibition (mm)	Observation
A	Lemon grass oil hydrogel (high conc)	14 mm (maximum)	Clear zone, Effective inhibition
B	Plain hydrogel (No lemon grass oil)	0 mm	No inhibition zone, Full fungal growth

Note: Actual measurements are taken from the incubated plate using a calliper.

II. SUMMARY:

The present research work, entitled "Formulation and Evaluation of Lemongrass Oil Infused Hydrogel for the Treatment of Oral Candidiasis," aimed to develop a mucoadhesive oral hydrogel containing lemongrass oil, a natural antifungal agent, to provide sustained local delivery and enhanced therapeutic efficacy against Candida albicans.Preformulation studies including UV–visible spectrophotometric and FTIR analysis, confirmed the identity and compatibility of lemongrass oil with selected excipients such as Carbopol-934, propylene glycol, sodium benzoate, sodium citrate, peppermint oil, and triethanolamine. FTIR spectra showed no significant shifts or disappearance of major peaks, indicating the absence of chemical interactions between the drug and excipients.A series of formulations (F1–F6) was prepared by varying the concentration of Carbopol-934 (0.25–0.50 mg) to optimize gel characteristics. All formulations were smooth, homogeneous, and free from phase separation. The pH values ranged from 6.6 to 7.0, which is suitable for oral mucosal application and ensures minimal irritation.The drug content of all batches ranged from 87.25 ± 0.35% to 99.42 ± 0.55%, with formulation F4 (0.40 mg Carbopol) showing 98.12 ± 0.51%, confirming uniform drug distribution and high entrapment efficiency.Viscosity and swelling index increased proportionally with higher polymer concentration, demonstrating the role of Carbopol as a gelling and thickening agent. The optimized formulation (F4) exhibited a balanced viscosity (2950 ± 15 cps) and swelling index (155 ± 2%), ensuring sustained release and ease of application.Spreadability ranged from 5.6 to 6.0

cm, with F4 showing the highest (6.0 cm), indicating good flow and uniform spreading on the oral mucosa, which is essential for patient comfort and prolonged retention.

In-vitro drug release studies revealed that increasing Carbopol concentration reduced the drug release rate due to the formation of a dense gel matrix. Formulation F1 showed a rapid release of 98.03% at 300 minutes, whereas F6 exhibited the slowest (55%). The optimized formulation F4 provided a controlled release of 92.03 ± 1.9% at 300 minutes, which ensures prolonged drug availability at the target site.

Based on drug content, viscosity, spreadability, and in-vitro release behavior, F4 was identified as the optimized formulation. It offered a good balance between mechanical strength and bioadhesive properties while maintaining acceptable physical characteristics and patient usability.

III. CONCLUSION:

The study successfully developed a lemongrass oil-infused hydrogel suitable for oral application in the treatment of oral candidiasis. The selected polymer, Carbopol-934, effectively controlled the drug release and enhanced the retention of the formulation on the mucosal surface. Among all formulations, F4 (0.40 mg Carbopol-934) exhibited optimal pH, viscosity, swelling index, spreadability, drug content, and controlled drug release, confirming it as the optimized formulation. The prepared hydrogel demonstrated desirable physicochemical stability, non-irritant pH, and sustained antifungal release potential, suggesting that lemongrass oil-loaded hydrogels could serve as a promising, natural, and patient-friendly alternative for the local management of oral candidiasis. Further in-vitro antifungal studies and in-vivo evaluation on mucosal tissues are recommended to confirm its clinical efficacy and potential for commercial development.The lemon grass oil-infused hydrogels (Plate A) exhibit antifungal activity against Candida albicans, as shown by clear inhibition zones. The negative control (Plate B) confirms that the antifungal effect is due to the presence of lemon grass oil, not the hydrogel itself. This validates the potential of essential oil-loaded hydrogels for antifungal applications like wound dressings, personal care products, or drug delivery systems.