3.0. MATERIALS AND METHODS

3.1. Materials used for the study

3.1.1. Chemicals

1. Terbinafine hydrochloride    Aurobindo Pharma, Hyderabad.
2. Emulsifying wax - IP          ILE Company, Chennai.
4. Propylene glycol - IP        E.Merck(India) ltd, Mumbai.
7. Liquid paraffin - IP         Qualigens fine chemicals, Mumbai.
9. Hard paraffin - IP           E.Merck(India) ltd, Mumbai.
11. Sodium hydroxide - IP       Qualigens fine chemicals, Mumbai.
13. Carbopol 940 gel base       ILE company, Chennai.
14. Terbinafine hydrochloride cream Novartis Pharma, Bangalore.
### 3.1.2. Equipments

1. **HPLC**  
   Shimadzu LC-10A.

2. **UV Spectrometer**  
   Perkin-Elmer -Lamda 35.

3. **Stability chamber**  
   Thermolab.

4. **Infra red spectroscopy**  
   Perkin-Elmer.

5. **Centrifuge 8C**  
   Remi equipments.

6. **Rheometer-LV DV III +**  
   Brookfield Viscometer.

7. **TAXTplus Texture analyzer**  
   Stable Microsystems.

8. **Electronic balance**  
   Sartorious.

9. **Digital pH meter**  
   Systronics.

10. **Magnetic stirrer**  
    Remi equipments.

11. **Zeta potential apparatus**  
    Malveron.

12. **Franz diffusion cell.**  
    Fabricated by us.
3.2. Drug Profile (Colin Dollery et al 1999)

Terbinafine hydrochloride

Terbinafine hydrochloride is a synthetic allylamine antifungal which is fungicidal against dermatophytes, mold fungi, and some yeasts.

Chemistry

Molecular Formula

C\textsubscript{21}H\textsubscript{25}N.HCl

Molecular weight (free base)

327.9 (291.4)

Chemical name

(E)-(6.6-Dimethyl-2-hepten-ynyl)-N-methyl-1-naphthalene methanamine hydrochloride.
Properties

Terbinafine is a slightly bitter, off-white, odorless allylamine derivative that is obtained by chemical synthesis.

Pharmacology

Terbinafine hydrochloride is a non-competitive inhibitor of squalene epoxidase, the enzyme that converts squalene to squalene epoxide during ergosterol biosynthesis. The primary site of action as with most other systemically useful agents, is therefore fungal cell membrane.

The fungicidal effect of terbinafine may also be caused in part by the decreased levels of ergosterol in the cell. Ergosterol is an essential component of fungal cell membrane (eg. Cholesterol in mammalian cells) and fungi are dependent for cell wall integrity on sterol biosynthesis.

Structural activity relationship of Terbinafine hydrochloride
Characteristics of functional groups present in Terbinafine

Aromatic hydrocarbon - Hydrophobic, increases penetration of skin.

Alkene - Hydrophobic, increases penetration of skin.

Alkane - Hydrophobic, increases penetration of skin.

Alkyne - Hydrophobic, increases penetration of skin.

Tertiary amine - Hydrophilic, Hydrophobic, decreases Penetration of skin.

Pharmacokinetics

The preferred method of assay of terbinafine is high pressure liquid chromatography (HPLC) with ultraviolet detection, although bioassays have also been described. HPLC is far more sensitive than bioassay, with a detection limit of 50 mg/litre in plasma and 300mg/litre in urine. The lower limit of detection by bioassay is approximately. HPLC has the added advantage that it permits quantification of the numerous metabolites of Terbinafine that may be found in the body fluids. Extraction methods have been devised for plasma, milk, and urine as well as stratum corneum and various other body substances.

Concentration – effect relationship

There is a no direct evidence of a relationship between plasma concentration and therapeutic success, but efficacy clearly depends on accumulation in infected tissue. Measurement of plasma levels is unnecessary.
**Metabolism**

Terbinafine undergoes extensive biotransformation in the liver, with the production of about 15 metabolites. Major points of the metabolic attack are: N-demethylation of the central nitrogen atom, oxidation of the methyl groups, and arene oxide formation with hydrolysis to the corresponding dihydrodiol. The dimethyl and carboxy metabolites are less lipophilic than the parent compound and lack useful antifungal activity. Metabolism may be potentiated by drugs that induce hepatic enzymes (e.g. rifampin) and conversely, may be reduced by compounds such as cimetidine that inhibit cytochrome P450. Terbinafine has no significant inhibitory effect on the P450 system, having little or no effect on the metabolism of antipyrine in vivo or that of cyclosporine, tolbutamide, ethoxycoumarin, or ethinyl estradiol in vitro. About 80% of the dose is recoverable from urine with the remainder in feces. None of the parent compound is detectable in urine and excretion is essentially complete within 72 hours.

**Pharmaceutics**

Terbinafine is available as Terbinafine hydrochloride from Novartis pharma for oral and topical administration.
3.3. Excipients Profile

3.3.1. Carbopol

**Chemical Name:** Carbomer

**Empirical Formula and Molecular Weight**

Carbomers are synthetic high-molecular-weight polymers of acrylic acid that are crosslinked with either allyl sucrose or allyl ethers of pentaerythritol. They contain between 56% and 68% of carboxylic acid (COOH) groups calculated on the dry basis. The molecular weight of carbomer resins is theoretically estimated as from $7 \times 10^5$ to $4 \times 10^9$.

**Structural Formula**

![Acrylic acid monomer unit in carbomer resins](image)

Acrylic acid monomer unit in carbomer resins

**Functional category**

Bioadhesive; emulsifying agent; release-modifying agent; suspending agent; tablet binder; viscosity-increasing agent.
Applications in Pharmaceutical formulation

Carbomers are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity-increasing agents. Formulations include creams, gels, and ointments for use in ophthalmic, rectal, and topical preparations. (Tamburic S et al 1995).

Description

Carbomers are white-colored, ‘fluffy’, acidic, hygroscopic powders with a slight characteristic odor.

Density (bulk)

1.76–2.08 g/cm³

Density (tapped)

1.4 g/cm³

Melting point

The decomposition occurs within 30 minutes at 260°C.

Solubility

Soluble in water and, after neutralization, in ethanol (95%) and glycerin.

Safety

Carbomers are used extensively in nonparenteral products, particularly topical liquid and semisolid preparations.
3.3.2. Glyceryl monostearate

**Chemical Name:** Octadecanoic acid, monoester with 1,2,3-propanetriol

**Empirical Formula and Molecular Weight:** $C_{21}H_{42}O_4$ and 358.6

**Structural formula**

![Structural formula image]

**Functional category**

Emollient; emulsifying agent; solubilizing agent; stabilizing agent; sustained-release ingredient; tablet and capsule lubricant.

**Applications in Pharmaceutical formulation**

The many varieties of glyceryl monostearate are used as nonionic emulsifiers, stabilizers, emollients, and plasticizers in a variety of food, pharmaceutical, and cosmetic applications.

**Description**

Glyceryl monostearate is a white to cream-colored, wax like solid in the form of beads, flakes, or powder. It is waxy to the touch and has a slight fatty odor and taste.
Melting point

55–60°C

Solubility

Soluble in hot ethanol, ether, chloroform, hot acetone, mineral oil, and fixed oils. Practically insoluble in water, but may be dispersed in water with the aid of a small amount of soap or other surfactant.

Safety

Glyceryl monostearate is widely used in cosmetics, foods, and oral and topical pharmaceutical formulations and is generally considered as a nontoxic and nonirritant material.

3.3.3. Paraffin

Chemical Name: Paraffin

Empirical Formula and Molecular Weight

Paraffin is a purified mixture of solid saturated hydrocarbons having the general formula \( \text{C}_n\text{H}_{2n+2} \), and is obtained from petroleum or shale oil.
**Functional Category**

Ointment base; stiffening agent.

**Applications in Pharmaceutical formulation**

Paraffin is mainly used in topical pharmaceutical formulations as a component of creams and ointments. In ointments, it may be used to increase the melting point of a formulation or to add stiffness.

**Description**

Paraffin is an odorless and tasteless, translucent, colorless, or white solid. It feels slightly greasy to the touch and may show a brittle fracture.

**Melting point**

The various grades with different specified melting ranges are commercially available.

**Solubility**

Soluble in chloroform, ether, volatile oils, and most warm fixed oils; slightly soluble in ethanol; practically insoluble in acetone, ethanol (95%), and water. Paraffin can be mixed with most waxes if melted and cooled.

**Safety**

Paraffin is generally regarded as an essentially nontoxic and nonirritant material when used in topical ointments and as a coating material for tablets and capsules.
3.3.4. Mineral Oil (Liquid paraffin)

Chemical Name: Mineral oil

Empirical Formula and Molecular Weight

Mineral oil is a mixture of refined liquid saturated aliphatic ($C_{14–C_{18}}$) and cyclic hydrocarbons obtained from petroleum.

Functional Category

Emollient; lubricant; oleaginous vehicle; solvent.

Applications in Pharmaceutical formulation

Mineral oil is used primarily as an excipient in topical pharmaceutical formulations, where its emollient properties are exploited as an ingredient in ointment bases.

Description

Mineral oil is a transparent, colorless, viscous oily liquid, without fluorescence in daylight.

Boiling point

$> 360^\circ C$

Solubility

Practically insoluble in ethanol (95%), glycerin, and water; soluble in acetone, benzene, chloroform, carbon disulfide, ether, and petroleum ether. Miscible with volatile oils and fixed oils, with the exception of castor oil.
Safety

Mineral oil is used as an excipient in a wide variety of pharmaceutical formulations.

3.3.5. Propylene Glycol

Chemical Name: 1, 2-Propanediol

Functional Category

Antimicrobial preservative; disinfectant; humectant; plasticizer; solvent; stabilizer for vitamins; water-miscible cosolvent.

Structural Formula

![Propylene Glycol Structural Formula]

Applications in Pharmaceutical formulation

Propylene glycol has become widely used as a solvent, extractant, and preservative in a variety of parenteral and nonparenteral pharmaceutical formulations. It is a better general solvent than glycerin and dissolves a wide variety of materials, such as corticosteroids, phenols, sulfa drugs, barbiturates, vitamins (A and D), most alkaloids, and many local anesthetics.

Description

Propylene glycol is a clear, colorless, viscous, practically odorless liquid with a sweet, slightly acrid taste resembling that of glycerin.
Boiling point

188°C

Solubility

Miscible with acetone, chloroform, ethanol (95%), glycerin, and water; soluble at 1 in 6 parts of ether.

Safety

Propylene glycol is used in a wide variety of pharmaceutical formulations and is generally regarded as a relatively nontoxic material. It is also used extensively in foods and cosmetics.

3.3.6. Methyl paraben

Chemical Name: Methyl-4-hydroxybenzoate.

Functional Category

Antimicrobial preservative.

Structural Formula

\[
\begin{array}{c}
\text{O} \\
\text{OCH}_3 \\
\text{C} \\
\text{OH}
\end{array}
\]
Applications in Pharmaceutical formulation

Methyl paraben is widely used as an antimicrobial preservative in cosmetics, food products, and pharmaceutical formulations. In cosmetics, methyl paraben is the most frequently used antimicrobial preservative. (Decker RL et al 1987).

Functional Category

Antimicrobial activity.

Methyl paraben exhibits antimicrobial activity of pH 4–8. Preservative efficacy decreases with increasing pH owing to the formation of the phenolate anion. Parabens are more active against yeasts and molds than against bacteria. They are also more active against Gram-positive bacteria than against Gram-negative bacteria.

Description

Methyl paraben occurs as colorless crystals or a white crystalline powder. It is odorless or almost odorless.

Melting point

125–128°C

Safety

Methyl paraben and other parabens are widely used as antimicrobial preservatives in cosmetics and oral and topical pharmaceutical formulations.
3.3.7. Propyl paraben

Chemical Name: Propyl 4-hydroxybenzoate

Empirical Formula and Molecular Weight

\[ C_{10}H_{12}O_3 \] 180.20

Functional Category

Antimicrobial preservative.

Structural Formula

![Structural formula of Propyl paraben]

Applications in Pharmaceutical formulation

Propylparaben is widely used as an antimicrobial preservative in cosmetics, food products, and pharmaceutical formulations.

It may be used alone, in combination with other paraben esters, or with other antimicrobial agents. It is one of the most frequently used preservatives in cosmetics.
**Description**

Propyl paraben occurs as a white, crystalline, odorless.

**Boiling point**

295°C

**Functional Category**

Antimicrobial activity

Propyl paraben exhibits antimicrobial activity between pH 4–8. Preservative efficacy decreases with increasing pH owing to the formation of the phenolate anion. Parabens are more active against yeasts and molds than against bacteria.

**Safety**

Propyl paraben and other parabens are widely used as antimicrobial preservatives in cosmetics, food products, and oral and topical pharmaceutical formulations.
3.4. Methods adopted in the study

3.4.1. Determination of physical characteristics

The following physical characteristic studies were performed for the drug.

- Description

The drug is observed visually for its color and nature.

- Solubility (Bahl BS et al 1994)

The room temperature was noted and the temperature was adjusted to 20°C.

100 ml of solvent was taken in a clean and dry beaker. To this solvent, Terbinafine hydrochloride was added slowly with mechanical stirring. Terbinafine hydrochloride was added continuously till a portion remains undissolved. This indicated saturation. This solution was filtered and the filtrate was collected. The first 20 ml of the filtrate was rejected and then 10ml of filtrate was pipetted out and transferred into a tarred china dish and weighed to find out weight of 10ml of the solution. The solution was evaporated by heating in a controlled manner (temperature slightly more or equal to boiling point of solvent). Towards final stages, controlled evaporation was done and cooled to room temperature. The china dish is weighed again to find out the residue. The residue was weighed. The weights were substituted in the following formula to
get the amount of solvent required to dissolve 1 gram of solute at the given temperature.

\[
\frac{\text{Weight of 10ml of saturated solution of drug in solvent in grams}}{-} = \frac{\text{Weight of residue obtained in grams}}{
\]

Weight of residue in grams

The experiment was repeated thrice and the average value is reported.

- **Partition Coefficient** (H.A Libermann, et al 2004)

100 mgs of Terbinafine hydrochloride was weighed and it was transferred to 250 ml separating funnel containing 25 ml of water and 25 ml of n-octanol. The funnel was shaken frequently for 30 minutes. The funnel was shaken frequently for 30 min., then the two phases allowed to separate collection of the octanol and water phases separately. Ten milliliters each of the phases were taken up in separate tarred china dishes and the solvents were evaporated. The weights of the residues after complete evaporation were found out. The partition coefficient of the drug was found out by using the formula

\[
p = \frac{\text{the amount of drug in 10ml of octanol solution}}{\text{the amount of drug in 10 ml of water solution}}
\]
• **Melting point determination** (Bahl BS et al 1994)

The melting point of the compound is the physical property used to identify or to check the purity of the compound. The determination was done by capillary method. The Terbinafine hydrochloride was placed on a clean surface. A capillary tube open at one end and closed at another end was taken. The open end of the capillary tube was pushed in to the compound to fill the drug into the capillary tube. The capillary melting point tube was placed in the melting temperature apparatus. The temperature of the chamber was slowly raised. The sample was continuously observed. The melting range of the sample was recorded when the sample started melting till all the sample goes in to liquid form.

**3.4.2. Selection of ointment bases**

Ointment bases should not produce irritation or sensitization of the skin, it should not retard wound healing. It should be smooth, and compatible with the skin and dermatological medicaments (Walter Lund. 1994).

A hydrocarbon base usually consists of soft paraffin (Vaseline, petroleum jelly or petrolatum) or a mixture of soft with hard paraffin to produce suitable consistency. Paraffin deposit greasy film on the skin and retards moisture loss (Jain NK 2006).

Absorption bases posses hydrophilic properties so they can soak up water to form water in oil emulsion. Absorption bases are similar to hydrocarbon bases, but they suppress the transepidermal water loss (Jain NK 2006).
Water soluble bases are high and low molecular weights of polyethylene glycols (macrogols). They do not hydrolyze, support mould growth or irritate the skin (Jain NK 2006).

In our study hydrocarbon bases were used for development of ointment. The ointment was prepared by fusion method.

The infusion method use is made of the differences in melting points of the ingredients to the determined the order of addition and avoid over heating of ingredients with lower melting points. Melting time is also shortened by grating waxy components, by stirring during melting and by lowering the dish as far as possible into the water bath so that the maximum surface was heated.

After melting, the ingredients should be stirred until the ointment is cold, taking care not to cause localized cooling, placing a dish on a cold surface (Carter SJ 2000).

By varying the proportions of ointment base and other excipients, various ointment formulations were made by permutation and combinations. Out of which three ointment formulations having suitable spreadability and consistency by physical observation were selected for further study.
3.4.3. Selection of cream base

The absorption type bases are anhydrous bases having capacity to absorb water resulting water in oil emulsion. The soap type bases include triethanolamine and sodium bicarbonate. The surfactant type cream containing ionic and non-ionic surfactants are used. (Gaud RS et al 2004)

Emulsification: Ingredients which are soluble in water is dissolved in water and it is heated to 70°C. Simultaneously bases are melted and maintain the temperature at 70°C. Then the aqueous phase is added to the oily phase with continuous trituration until creams are formed.

In our study emulsifying wax base was used for the development of Terbinafine hydrochloride cream. By varying the proportions of cream base and other excipients, various cream formulations were made by permutation and combinations. Out of which three cream formulations having suitable spreadability and consistency by physical observation were selected for further studies.

3.4.4. Selection of gel bases

Gelling agents generally used are synthetic macromolecules, such as carbomer-934, cellulose derivatives such as carboxy methyl cellulose, hydroxy carboxy methyl cellulose, and natural gums like tragacanth. (Ansel et al 2005).

Carbomers are high molecular weight water soluble polymers of acrylic acid cross linked allyl ether of sucrose and or pentaerythiol. Carbomer exists in various grades as per National Formulary. But Carbomer-940 yields high
viscosity between 40000 to 60000 cps with 0.5% aqueous dispersion (Ansel et al 2005).

Carbomer will produce gel in low concentration and for medicated gels 0.5% to 2% is used (Walter Lund. 1994).

In our study carbopol-940 was used for the development of Terbinafine hydrochloride gel. By varying the proportions of gel base and other excipients, various gel formulations were made by permutation and combinations. Out of which three gel formulations having suitable spreadability and consistency by physical observation were selected for further study.
3.5. Preparation of 1% Terbinafine hydrochloride ointments

Out of various formulation trials, three formulations of 1% Terbinafine hydrochloride ointments namely O1, O2 and O3 were selected which were having suitable spreadability and consistency and their formulae are given below.

Table 2. Formulae for development of 1% Terbinafine hydrochloride ointments

<table>
<thead>
<tr>
<th>S.no</th>
<th>Ingredients</th>
<th>Trials (All the ingredients in grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O1</td>
</tr>
<tr>
<td>1</td>
<td>Terbinafine hydrochloride</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>Liquid paraffin</td>
<td>14.0</td>
</tr>
<tr>
<td>3</td>
<td>Hard paraffin</td>
<td>9.0</td>
</tr>
<tr>
<td>4</td>
<td>Propylene glycol</td>
<td>12.0</td>
</tr>
<tr>
<td>5</td>
<td>Soft paraffin</td>
<td>64.0</td>
</tr>
</tbody>
</table>

The 1% Terbinafine hydrochloride ointment was prepared by fusion method.

First hard paraffin (50°C to 57°C) and soft paraffin (38°C to 56°C) were melted together in a china dish over a water bath and the liquid paraffin and propylene glycol containing Terbinafine hydrochloride were added and mixed well. The fluid mixture was taken away from the water bath stirred until cooled, avoiding aeration. The content was stirred effectively to avoid any crystallization.
Three batches of Terbinafine hydrochloride ointments were prepared as mentioned above and it was subjected to physical and chemical analysis.

Ointment of Terbinafine hydrochloride is not available in the market. Hence simple hydrocarbon ointment base IP abbreviated as RO was purchased and compared with three batches of prepared Terbinafine hydrochloride ointment for their physical analysis. The best formulation comparable with simple ointment base was chosen for further studies.

3.6. Preparation of 1% Terbinafine hydrochloride creams

Out of various formulation trials, three formulations of 1% Terbinafine hydrochloride creams namely C1, C2 and C3 were selected which were having suitable spreadability and consistency and their formulae are given below.

Table.3. Formulae for development of 1% Terbinafine hydrochloride creams

<table>
<thead>
<tr>
<th>S.No</th>
<th>Ingredients</th>
<th>Trials (All the ingredients in grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C1</td>
</tr>
<tr>
<td>1</td>
<td>Terbinafine hydrochloride</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>Emulsifying wax</td>
<td>5.0</td>
</tr>
<tr>
<td>3</td>
<td>Glyceryl monostearate</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>Soft paraffin</td>
<td>10.0</td>
</tr>
<tr>
<td>5</td>
<td>Liquid paraffin</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>Propylene glycol</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>Methyl paraben</td>
<td>0.2</td>
</tr>
<tr>
<td>8</td>
<td>Propyl paraben</td>
<td>0.1</td>
</tr>
<tr>
<td>9</td>
<td>Purified water</td>
<td>68.7</td>
</tr>
</tbody>
</table>
Emulsifying wax, glyceryl monostearate and soft paraffin were melted together in a china dish over a water bath at a temperature of 70°C. Terbinafine hydrochloride was dissolved in propylene glycol and it was mixed with liquid paraffin. This mixture was added to the melted mixture and the temperature was maintained at 70°C. Simultaneously methyl paraben and propyl paraben were dissolved in freshly boiled and cooled water taken in a beaker and the temperature was maintained at 70°C. Then aqueous phase was added to the oily phase and stirred well. The fluid mixture was taken away from the water bath and stirred until cooled, avoiding aeration. The content was stirred effectively to avoid any crystallization.

Three batches of 1% Terbinafine hydrochloride creams were subject to physical and chemical analysis.

Terbinafine hydrochloride marketed cream is available and it is denoted as MC. Hence Terbinafine hydrochloride marketed cream was compared with three batches of prepared Terbinafine hydrochloride cream for their physical analysis. The best formulation comparable with the marketed sample was chosen for further studies.
3.7. Preparation of 1% Terbinafine hydrochloride gels

Out of various formulation trials, three formulations of 1% Terbinafine hydrochloride gels namely G1, G2 and G3 were selected which were having suitable spreadability and consistency and their formulae are given below.

Table 4. Formulae for development of 1% Terbinafine hydrochloride gels

<table>
<thead>
<tr>
<th>S.no</th>
<th>Ingredients</th>
<th>Trials (All the ingredients in grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>G1</td>
</tr>
<tr>
<td>1</td>
<td>Terbinafine hydrochloride</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>Carbopol-940</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>Sodium hydroxide</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>Propylene glycol</td>
<td>11.0</td>
</tr>
<tr>
<td>5</td>
<td>Methyl paraben</td>
<td>0.2</td>
</tr>
<tr>
<td>6</td>
<td>Propyl paraben</td>
<td>0.1</td>
</tr>
<tr>
<td>7</td>
<td>Purified water</td>
<td>85.2</td>
</tr>
</tbody>
</table>

Carbopol – 940 was dispersed in water at the 1000 rpm. Preservatives such as methyl paraben and propyl paraben were dissolved in freshly boiled and cooled water. Terbinafine hydrochloride was dissolved in propylene glycol and it was added to the preservative mixture and stirred well. Then aqueous solution was added to the carbopol-940 dispersion followed by the addition of sodium hydroxide solution. It was stirred vigorously until the formation of gels.
Three batches of 1% Terbinafine hydrochloride gels were prepared and subject to physical and chemical analysis.

Gel of Terbinafine hydrochloride is not available in the market. Hence carbopol-940 gel base abbreviated as RG was purchased and compared with three batches of prepared Terbinafine hydrochloride gels for their physical analysis. The best formulation comparable with the carbopol-940 gel base was chosen for further studies.

3.8. Physical analysis

The following physical analysis was carried out for all the 1% topical formulations of Terbinafine hydrochloride and compared with marketed product and with reference bases.

3.8.1. Organoleptic test  (Marquele et al)

The organoleptic features of the samples were examined at the same temperature, lighting and packaging condition to assess variation in appearance, phase separation and color.

3.8.2. pH measurements

One gram of each formulation was weighed and dispersed with 10 ml distilled water. After homogenization, the pH of the sample was measured with pH meter.
3.8.3. Rheological Properties

**Viscosity:** The viscosity was determined by using Brookfield viscometer (Model LVDV III). The viscometer is placed on the "Standby Mode". Samples were allowed to reach room temperature and the samples were shaken vigorously. Then the samples were filled in a 600-mL, low-form Griffin beaker at least 3/4 full of slurry from the sample bottle.

The spindle number 64 was selected. The spindle was inserted in to the appropriate beaker containing sample. Care was to be taken to avoid air bubbles since it may be trapped beneath the spindle. The beaker was moved in to the position beneath the viscometer and the spindle was attached so that the spindle remains submerged into the sample. The sample viscosity is measured 30° c. The reading was recorded in centipoises.

![Figure 11. Brookfield viscometer](image)
3.8.4. Spreadability

Spreadability of the formulation was determined by an apparatus, suggested by multimeter et al, which was suitably modified in the laboratory and used for the study. It consists of a wooden block, which was provided by a pulley at one end. A rectangular ground glass plate (20cm x 20cm) was fixed on the block (Garg A et al 2002) (Margaret N et al 1946).

Two grams of the formulation was sandwiched between the ground fixed plates and another glass plate having the same dimensions of the fixed ground plate. The movable glass plate is provided with the hook. A 300gm weight was placed on the tip of two plates for five minutes to expel air and to provide a uniform film of the formulation between the plates. Excess of the formulation was scrapped off from the edges. The top plate was then subject to a pull of a 30 gms, initially with the help of a string attached to the hook and moves over the pulley. The time required by the top plate to move at a distance of 10 cms was noted. A shorter time interval indicates better spreadability.

The spreadability was calculated by using the formula

\[ s = \frac{m \times l}{t} \]

\( s = \) spreadability
\( m = \) weight tied to the upper side
\( l = \) length of the glass slides
\( t = \) time taken in seconds
In case the slide did not move with 30 gms, weight was increased gradually. In case the slide was moving fastly, the weight was decreased gradually.

3.8.5. Extrudability (Venkata ramana et al 2006)

The apparatus used to measure is extrudability apparatus. A closed collapsible tube containing formulation was pressed firmly at the crimped end by keeping weight. When the cap was removed, formulation extruded until the pressure dissipated. Weight in grams required to extrude a 0.5 cm ribbon of the formulation in 10 seconds was determined. The experiments were repeated thrice and the average value is reported.

3.8.6. Texture Profile Analysis (TPA)

![Texture analyzer](image)

**Figure.12.Texture analyzer**

The mechanical properties of each formulation were determined using a Texture analyzer (Model TA-XT 2, Stable Micro Systems, Surrey, UK).
Texture Profile Analysis (TPA) has been used to characterize the mechanical properties of pharmaceutical semisolid systems. This simple and rapid technique could provide information related to the mechanical parameters of the formulations as detailed below (Jones DS et al 1997).

**Evaluation of firmness, consistency and cohesiveness**

The samples were packed to a fixed height of 10.0mm with a trigger force of 30.0 gms with auto type trigger. The compression was done using 1cm diameter stainless steel probe and the probe was inserted twice into the formulation at a pre-test speed of 1.5mm/sec and test speed of 2.0mm/sec with a distance of 10.0mm/sec. The post test speed was maintained at 2.0mm/sec.

Data collection and calculation were performed using the XTRA Dimension software.

**Evaluation of greasiness** (Hardness and Stickiness)

The samples were packed to a fixed height of 10.0mm with a trigger force of 5.0 gms with auto type trigger. The compression was performed using a stainless steel probe of 1 cm diameter was compressed twice into the formulation at a pre-test speed of 2.0mm/sec and test speed of 1.0mm/sec with a distance of 2.0mm/sec. The post test speed was maintained at 2.0mm/sec.

Data collection and calculation were performed using the XTRA Dimension software.
3.8.7. Zeta potential measurement

The zeta potential was measured by using an electrophoresis cell or zeta meter in which the potential just required to make the particles move under a current is found out and the charge at the electro neutral region was measured.

The most common method for determining the zeta potential is the microelectrophoretic procedure in which the movements of individual particles under the influence of a known electric field are followed microscopically. The zeta potential can be calculated from the electrophoretic velocity of the particles using the Helmholtz-Smoluchowski equation

$$ U_p = V_p E = \zeta \varepsilon \eta $$

Where $U_p$ is the electrophoretic mobility, $V_p$ is the electrophoretic velocity, $E$ is the electric field strength, $\zeta$ is the zeta potential, $\varepsilon$ is the permittivity, and $\eta$ is the viscosity of the medium.

The 1% dispersions are located in the region between the pair of electrode. The pulse from the gated amplifier is applied across these electrodes, generating the electro kinetic sonic analysis sound waves. Voltage pulses rather than continuous sinusoids are applied to avoid interference from the sound wave emanating from the electrodes. The use of pulsed signals also avoids electrical heating of the suspension and the complications of multiple reflections of the waves at the electrodes and at the ends of the rods. The voltage pulse produced by the sound waves in the transducer on the right passes in to the signal processing electronics which measures the amplitude and phase.
of the sinusoidal component of the pulse. These data are passed through the computer where it is stored for subsequent processing.

**3.8.8. Primary skin irritation study**

The best formulations were tested for primary skin irritation using Draize patch test in rabbits. For this study six rabbits per group of both sexes were used. Each rabbits weighing 2.5 to 3.0kg were used. 0.5g of each formulation was applied on the hair free dorsum surface of the rabbit by uniform spreading within the area of 1inch². The skin was observed for 24 hrs and 48 hrs for any visible change such as erythema.

The evaluation was done by using the scale given by Draize (Patrick E et al 1989).

**Scale (Draize –Federal Hazardous Substance Act FHSA)**

0-No erythema

1-very slight erythema

2-Well- defined erythema

3-Moderate –to-severe erythema

4-Severe erythema
3.9. Chemical analysis

3.9.1. Preparation of standard graph by HPLC.

A stock solution of 1.0mg/ml of Terbinafine hydrochloride was prepared in a volumetric flask by dissolving 25mg in 25.0ml methanol. Appropriate amounts of stock solution were diluted with methanol yielding concentration of 10.0, 12.0, 14.0, 16.0, 18.0 and 20.0 μg/ml. Triplicate injection of each were made in HPLC and chromatograms were obtained.

Instrument : Shimadzu LC-10A  Model: LC-10AS

Detector : SPD-10A variable length detector (set as 254 nm)

Controller: SCL-10A with Rheodyne injection valve with 20 μl loop

Column : Shim-pack CLS-ODS (250mmx4mm, internal diameter: 5 μm, pore Diameter 100 A°)

Mobile Phase: Methanol: water (95:5v/v)

Flow rate : 1ml/minute

Chart speed : 0.5cm/min

Temperature : 19±1° C
3.9.2. Procedure for sample preparation

3.9.2.1. For 1% Terbinafine hydrochloride ointment (O3)

A quantity of the ointment containing 20.0mg of Terbinafine hydrochloride was warmed in a water bath at 50°C for 10 min. After 10 minutes 20ml of methanol was added and the Terbinafine hydrochloride was extracted by vigorous shaking with methanol. The crude extract of Terbinafine hydrochloride was extracted again by adding 10ml of methanol in a water bath at 50°C for 5 minutes with occasional shaking. This extract was added with 10ml of methanol with occasional stirring. The Terbinafine hydrochloride from the ointment base was extracted by warming and occasional stirring. The extracted Terbinafine hydrochloride solution was transferred to a 50.0ml volumetric flask and methanol was added to make up to the volume. After centrifugation (5 min at 1500 rpm), the dilution were made with methanol to give a final concentration of 16.0μg/ml.

3.9.2.2. For 1% Terbinafine hydrochloride cream (C3)

A quantity of the cream containing 20.0mg of Terbinafine hydrochloride was extracted by warming with 30ml of methanol in a water bath at 50°C for 10 minutes with occasional shaking. This solution was transferred to a 50.0ml volumetric flask and methanol added to make up to volume. After centrifugation 15 minutes at 1500 rpm, the dilutions were made with methanol to give a final concentration of 16.0 μg.
3.9.2.3. For 1% Terbinafine hydrochloride gel (G3)

A quantity of the gel containing 20.0mg of Terbinafine hydrochloride was extracted from carbopol by warming the sample with 15ml of methanol for 5 minutes followed by occasional stirring with methanol for 5-7 minutes.

To this extract 15ml of methanol was added and the Terbinafine hydrochloride was completely extracted from the polymer by vigorous shaking with methanol. Finally the extract was transferred to the 50 ml volumetric flask and the final volume was added to make up to the volume. After centrifugation (5 min at 1500 rpm), the dilutions were made with methanol to give a final concentration of 16.0 μg/ml. The injection was made six times.

3.9.3. Preparation of standard graph of Terbinafine hydrochloride by UV Spectrophotometry (Cardoso SC 1999)

The method was standardized with methanolic solution containing 1.0 mg of Terbinafine hydrochloride in 1ml. From this solution dilutions in the range of 0.2-5.0 μg/ml were made in methanol. Absorbencies of the resulting solution were measured at the wave length of maximum absorbance (224 nm) with methanol as blank. Compliance with Burger – Lambert Beer’s law was observed in the range of 0.8 – 2.8 μg/ml.
3.9.4. Validation (Cardoso, Schapoval.E.E.S et al 1999)

All the methods were validated by determination of the following operational characteristics linearity, range, precision, and accuracy, limit of detection and limit of quantification.

The linearity was correlated with the concentration of the standard Terbinafine hydrochloride at 254 nm by UV detection.\(n=6\).Precision express the closeness of agreement among various measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions it is usually expressed as the variance, standard deviation, or coefficient of variation (CV) of a series of measurements.

Accuracy expresses the agreement between the accepted value (either conventional true value or an accepted reference value) and the value found. Accuracy is reported as percent recovery of a known amount of analyte added to the sample.

3.9.5. Calculation

The drug content of the formulation was determined by referring to the calibration value. The reproducibility was validated by inter and intra-day precision in the range of 10-20µg/ml. The inter-day precision was evaluated by comparing the linear regressions of the four standard plots prepared on four different days over a two month period. The repeatability of the method was studied by assaying six samples of Topical formulations of Terbinafine hydrochloride during the same day under same experimental conditions.
3.10. In-vitro release studies by Franz diffusion cell

The previously prepared diffusion membrane was sandwiched between the receptor and donor chamber of the Franz cell. The receptor compartment was carefully filled with phosphate buffer of pH 7.4 and the cell was placed on the magnetic plate maintained at 37°C. A small teflon coated (3mm) magnetic bar was included in the receptor compartment such that stirring occurred throughout the duration of the experiment. After equilibrating for 30 minutes, at an appropriate time intervals a definite volume of the receptor solution was withdrawn and immediately replaced by equal volume of fresh receptor solution at the same temperature (37°C) as the receptor chamber. The experiments were conducted under unoccluded conditions for duration of 1, 2, 3, 6 and 12 hrs and drug content was analyzed by UV.Spectrophotometer at 224 nm (Akomeah FK et al 2007).

Figure 13. Franz Diffusion cell

At least 6 replicates were performed for each sample including the control. Sink condition was maintained throughout the experiment and this may be defined as ensuring that the concentration of the penetrated in the
receptor does not exceed 20% of the saturated solubility of penetrate in the vehicle, in order to ensure an adequate drinking force for diffusion is maintained.

3.11. Stability study of the formulations

The best formulations were packed in a 10 gms collapsible tube and stored at 25°C ± 2°C. Samples from each formulations were withdrawn at definite time intervals namely 30, 90, 180, 270 and 360 days as per ICH guidelines (G.T Kulkarni et al 2004).

3.12. Compatibility studies by using Infrared Spectroscopy (IR)

The Drug excipient compatibility studies were performed using a potassium bromide pellet technique by Infrared spectroscopy was taken for the Terbinafine hydrochloride and for the three best topical formulations of 1% Terbinafine hydrochloride.
3.13. Clinical study methods

The clinical efficacies of the developed formulations were assessed by randomized single centre, open, controlled and comparative clinical studies in patients infected with tinea. The study was conducted at the Department of Dermatology, Sri Ramachandra Hospital, Sri Ramachandra University, Porur, Chennai, India under the supervision of Dermatologist. The study was approved by Institutional Ethics Committee of Sri Ramachandra University, Porur, Chennai, India. (IEC/07/JUN/58/28 dated 16.08.07).

The study was conducted according to protocol, applicable to US Food and Drug Administration code of Federal Regulations, the Declaration of Helsinki, and the International Conference on Harmonization Tripartite Guideline for Good Clinical Practice. Institutional Ethics Committee of Sri Ramachandra University approved the “informed consent form” and “study protocol”. Eligible patients provided written informed consent before study-related procedures were initiated.

The patients diagnosed by the dermatologist as dermatophytosis were selected for the study. The primary and secondary identification of dermatophytosis was examined by wood’s light examination and microscopical examination as discussed in the introduction chapter 1.3.5. All the patients had given written consent as per the protocol and the patients were informed properly about the study. The study was designed and conducted in four groups of patients infected with dermatophytosis. Each group was treated for three
weeks under the supervision of dermatologists with observation at the end of each week.

The formulations were applied twice daily for three weeks with an intermittent follow up at the end of each week. Before starting the treatment the infection site was observed and scores were recorded.

**Scores** (Lakshmi PK et al 2006)

5-Severe erythema
4-Marked erythema, possible edema
3-Moderate erythema, possible presence of edema
2-Mild erythema
1-Spotty erythema
0 –No erythema

- **Inclusion criteria**

Patients of all age groups and both the sexes with tinea infection involving less than 15% of body surface area were included.

- **Exclusion criteria**

Patients with known sensitivity to Terbinafine.

Patients with all other skin condition except dermatophytosis.

All patients who did not visit for the follow up for the second week of treatment.

Patients with tinea capitis. (Systemic administration of griseofulvin provided the first effective oral therapy for tinea capitis. Topical
treatment alone usually is ineffective and is not recommended for the management of tinea capitis) (Grace F Kao)

Patients under interlesional therapy or ultraviolet therapy.

Women in reproductive age group wishing to have children.

Pregnancy and lactating women.

❖ **BEFORE starting the therapy, the patients were educated on**

- Compliance.

- Regular follow-up.

- Possible adverse drug reactions.

❖ Group I (Control group): Patients with tinea infection on the normal skin were selected and prescribed with marketed 1% Terbinafine hydrochloride cream (MC) applied twice daily for three weeks.

❖ Group II: Patients with tinea infection on the wet skin were selected and prescribed with 1% Terbinafine hydrochloride gel (G3) applied twice daily for three weeks.

❖ Group III: Patients with tinea infection on the dry skin were selected and prescribed with 1% Terbinafine hydrochloride ointment (O3) applied twice daily for three weeks.

❖ Group IV: Patients with tinea infection on the normal skin were selected and prescribed with 1% Terbinafine hydrochloride cream (C3) applied twice daily for three weeks.
For each group results of pre dermal and post dermal scores were obtained after three weeks of treatment with topical preparations of 1% Terbinafine hydrochloride as described earlier. Comparative efficacy is assessed statistically by one way ANOVA technique.

Clinical efficacy studies

The study was approved by Institutional Ethics Committee of Sri Ramachandra University, Chennai. (Ref: IEC/07/JUN/58/28 dated 16.08.07)

Type of study : Open, single, centre, comparative.

Place of study : Dept.of Dermatology, Sri Ramachandra Hospital Porur,Chennai,India.

Type of Patients : Patients infected with tinea.

Eligible patients were provided with informed consent and singed.

No.of Patients used : 320 (both sexes)

Type of dosage form : Ointment, Cream, Gel, and Marketed Cream

Groups : Four groups.

Duration of study : 3 weeks application
3.14. Statistical analysis

The physiochemical properties of the Terbinafine hydrochloride formulations and the clinical efficacy studies were analyzed by the application of one-way analysis of variance (ANOVA) using SPSS-15 software. The multiple comparisons to elicit the significant difference between the various groups were performed by the statistical analysis.