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RESEARCH ARTICLE

Development and Efficacy Evaluation of a Natural Antifungal Gel for Athlete's Foot: A Synergistic Approach with Tea Tree Oil, Oregano Oil, and Apple Cider Vinegar

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ABSTRACT

Athlete's foot (Tinea pedis) is a prevalent superficial fungal infection caused by dermatophytes, such as *Trichophyton rubrum* and *Trichophyton mentagrophytes*. The growing resistance to synthetic antifungal agents and concerns regarding their long-term use have prompted interest in developing alternative therapies utilizing natural products. This study aimed to formulate and evaluate a natural antifungal gel incorporating tea tree oil, oregano oil, and apple cider vinegar, known for their potent antimicrobial and anti-inflammatory properties. The gel was prepared using Carbopol 934P as the base and optimized for pH, viscosity, and consistency. Qualitative phytochemical screening confirmed the presence of key secondary metabolites, including flavonoids, phenolics, terpenoids, and saponins, which are potentially responsible for antifungal activity. The antifungal efficacy of the gel was assessed *in vitro* using agar well diffusion and broth microdilution methods against clinical isolates of *T. rubrum* and *T. mentagrophytes*. Minimum Inhibitory Concentration and Minimum Fungicidal Concentration values were determined. Further characterization included pH measurement, spreadability, washability, and stability studies under ICH-recommended conditions over 90 days. The results demonstrated that the natural antifungal gel possesses promising physicochemical characteristics and potential antifungal activity suitable for topical use. This formulation provides a safe and natural alternative for managing athlete's foot and could serve as a foundation for future clinical studies and product development.

Keywords: Apple cider vinegar, athlete's foot, natural antifungal gel, oregano oil, tea tree oil

INTRODUCTION

Athlete's foot, also known as tinea pedis, is a common superficial fungal infection affecting the keratinized tissue of the feet, particularly the interdigital spaces, plantar surface, and sometimes the toenails. It is primarily caused by dermatophytes, such as *Trichophyton rubrum* and *Trichophyton* mentagrophytes, organisms that thrive in warm and moist environments and utilize keratin as a nutrient source.[1] The condition is highly prevalent worldwide, with millions of cases reported annually, making it one of the most widespread fungal infections of the skin.[2] Although not life-threatening, athlete's foot significantly impacts the quality of life of affected individuals, leading to itching, burning sensations, scaling, and recurrent secondary bacterial infections if left untreated.[3]

Conventional treatment approaches for athlete's foot rely on synthetic antifungal agents, such as azoles, allylamines, and other fungicidal preparations.[4] While these medications are effective in inhibiting fungal growth and reducing infection, their use is frequently associated with limitations, including skin irritation, systemic toxicity, high recurrence rates, and the emergence of antifungal resistance.[5] These drawbacks highlight the need for alternative strategies, particularly those derived from natural

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bioactive substances that provide both efficacy and safety.[6] Increasing consumer preference for plant-based remedies has also encouraged research into phytotherapeutics as sustainable options for fungal management.[7]

Among natural antifungal agents, essential oils have gained considerable attention due to their broad-spectrum antimicrobial activity, minimal side effects, and multifunctional therapeutic potential. Tea tree oil, derived from *Melaleuca alternifolia*, is especially notable for its antifungal efficacy. Its primary active constituent, terpinen-4-ol, disrupts fungal cell membrane integrity, leading to increased permeability and eventual cell death.[8] Numerous studies have demonstrated the effectiveness of tea tree oil against dermatophytes, making it a promising candidate for the topical treatment of athlete's foot.[9]

Oregano oil, another potent natural remedy, contains high concentrations of phenolic compounds, such as carvacrol and thymol, which exhibit strong antifungal activity.[10] These compounds interfere with fungal cell wall synthesis, impair enzymatic activity, and enhance oxidative stress in fungal cells, thereby inhibiting their growth and survival. In addition to its antifungal effects, oregano oil has demonstrated antioxidant and anti-inflammatory properties, which can aid in reducing the irritation and discomfort associated with tinea pedis.[11]

Apple cider vinegar (ACV), long recognized in traditional medicine, also holds potential as an antifungal adjunct. Its acidic nature creates an unfavorable environment for fungal proliferation, while its bioactive components, such as acetic acid and polyphenols, contribute to microbial inhibition.[12] Moreover, ACV helps restore the skin's natural pH balance, enhancing its barrier function and potentially reducing the risk of recurrent infection. When combined with essential oils, it may exert synergistic effects, improving the overall antifungal activity of the formulation.

The concept of synergism between natural agents is particularly important in addressing fungal infections, such as athlete's foot, where monotherapy often results in incomplete eradication and frequent relapses. By combining tea tree oil, oregano oil, and ACV, it is possible to harness complementary mechanisms of action that not only inhibit fungal growth but also alleviate associated inflammation and discomfort. This approach aligns with the growing interest in integrative and holistic therapies that utilize multiple natural agents to achieve enhanced therapeutic outcomes.

MATERIALS AND METHODS

Materials

The ingredients and materials used in the development of the natural antifungal gel were of analytical or therapeutic grade, sourced from reputed suppliers. These include natural actives (tea tree oil, oregano oil, and ACV), gel-forming agents, solvents, and microbiological media.

Table 1 below presents the list of all materials used in the formulation and evaluation of the antifungal gel, along with their sources and grades.

Extraction Procedure

1. Tea Tree Oil: Fresh *M. alternifolia* leaves were shade-dried, chopped, and subjected to steam distillation. The oil was separated in a Florentine receiver, dried with sodium sulfate, filtered, and stored in amber bottles at 4°C
2. Oregano Oil: Dried oregano leaves were distilled using a Clevenger apparatus. Separated oil was dried, filtered, and stored under refrigeration (4–8°C)

Table 1: Materials used in the study

S. No.	Material	Source	Grade
1	Tea Tree Oil	-	Extract
2	Oregano Oil	-	Extract
3	Apple Cider Vinegar	-	Extract
4	Carbopol 940	Loba Chemie, India	Analytical
5	Triethanolamine	Sigma-Aldrich	Analytical
6	Distilled Water	In-house distillation unit	Purified
7	Sabouraud Dextrose Agar	HiMedia Laboratories Pvt. Ltd., India	Laboratory
8	DMSO	Merck, India	Analytical
9	MTT Reagent	Sigma-Aldrich	Cell Culture
10	Trichophyton rubrum, T. mentagrophytes	MTCC, Chandigarh	Clinical

3. ACV: Chopped apples were fermented with sugar and yeast for alcohol production, followed by acetic acid fermentation with *Acetobacter*. The final vinegar was filtered, bottled, and stored under controlled conditions.

Phytochemical Screening of Natural Extracts

The extracts of tea tree oil, oregano oil, and ACV were subjected to standard qualitative phytochemical screening to identify the presence of major classes of secondary metabolites responsible for antimicrobial activity. All tests were conducted in triplicate, and observations were recorded based on color changes or precipitate formation.

Test for alkaloids (Mayer's test)

Procedure: 2 mL of the extract was acidified with 1% HCl and then a few drops of Mayer's reagent were added.

Test for flavonoids (Alkaline reagent test)

Procedure: 2 mL of extract was treated with a few drops of 10% NaOH solution.

Test for tannins (Ferric chloride test)

Procedure: 2 mL of extract was mixed with 2–3 drops of 5% $FeCl_3$.

Test for terpenoids (Salkowski's test)

Procedure: 2 mL of extract was mixed with 2 mL of chloroform and then 3 mL of concentrated H_2SO_4 was carefully added.

Test for phenolic compounds (Ferric chloride test)

Procedure: 1 mL of extract was mixed with 3–4 drops of 5% ferric chloride solution.

Test for saponins (Foam test)

Procedure: 2 mL of extract was diluted with 5 mL of distilled water and shaken vigorously.

Test for glycosides (Keller–Killiani test)

Procedure: 1 mL of extract was mixed with 2 mL of glacial acetic acid containing one drop of ferric chloride, followed by careful addition of 1 mL of concentrated sulfuric acid.

Preparation of Base Gel

A gel base was developed using Carbopol 940, a polymer well-suited for topical formulations due to its high viscosity, smooth texture, and excellent stability. After preparation of extracts, the antifungal actives were incorporated into a carbopol gel base. The quantities of each component in the final gel formulation are outlined in Table 2.

Procedure for Gel Preparation:

1. Dispersion of Polymer: 1 g of Carbopol 940 was gradually added to 98 mL of distilled water under continuous stirring with a magnetic stirrer until fully dispersed
2. Neutralization: 1 mL of Triethanolamine was added dropwise to adjust the pH to 6.0–6.5 and to convert the dispersion into a clear gel
3. Addition of Active Ingredients: After gel formation, tea tree oil (1.0 mL), oregano oil (0.5 mL), and ACV (0.5 mL) were added slowly with constant stirring for uniform distribution.

Evaluation and Phytochemical Screening of Extracts

pH measurement

The pH of the final gel formulation was measured using a digital pH meter (Eutech Instruments) calibrated with standard buffer solutions at pH 4.0 and 7.0. Triplicate readings were taken to ensure consistency.

Color and appearance

Color, transparency, and phase uniformity were evaluated visually under natural light. Homogeneity

Table 2: Composition of natural antifungal gel

Ingredient	Quantity
Tea Tree Oil	1.0 mL
Oregano Oil	0.5 mL
Apple Cider Vinegar	0.5 mL
Carbopol 940	1.0 g
Triethanolamine	1.0 mL
Distilled Water	q.s. to 100 mL

was assessed by gently rubbing the gel between the fingers to check for any grittiness or separation.

Washability

About 1 g of the gel was applied to a marked area of the forearm and allowed to dry for 10 minutes. The area was rinsed under running tap water without scrubbing. The ease of removal and residual film formation were noted qualitatively.

Antifungal Activity Test

Antifungal efficacy was tested using the agar well diffusion method against clinical strains of *T. rubrum* and *T. mentagrophytes*.

Culture media

Sabouraud Dextrose Agar (SDA) was prepared and sterilized. After cooling to ~45°C, plates were poured and allowed to solidify under sterile conditions.

Inoculum preparation

Fresh cultures of *T. rubrum* and *T. mentagrophytes* were maintained on SDA slants. The fungal suspensions were prepared in sterile saline and adjusted to 1×10^6 spores/mL using a hemocytometer.

Agar well diffusion assay

- Sterile SDA plates were inoculated with fungal suspension using sterile cotton swabs
- Wells (6 mm diameter) were punched into the agar
- 100 µL of gel formulation was introduced into each well
- The plates were incubated at 28°C for 48–72 h under aseptic conditions
- Zone diameters were measured post-incubation.

Consistency and Viscosity

Consistency test

1 g of gel was pressed between two glass plates, and the ease of spreading was noted manually. A qualitative observation was recorded based on tactile feedback.

Viscosity measurement

Viscosity was measured using a Brookfield viscometer with spindle No. 64 at 20 rpm at room temperature (25 ± 1°C). Triplicate readings were recorded to ensure reproducibility.

Spreadability and Extrudability Testing

Spreadability test (Glass slide method)

The spreadability of the gel was assessed using the slip and drag characteristics of the formulation.

- Apparatus: Two glass slides (10 × 20 cm), 500 g weight, stopwatch
- Procedure:
  About 1 g of the formulated gel was placed between two glass slides. A 500 g weight was gently placed on the upper slide to ensure uniform spreading and to remove air bubbles. The weight was removed, and the time required for the upper slide to slip off under its own weight was noted using a stopwatch.
- Calculation:

$$\text{Spreadability} = M \times L / T$$

where:

M = weight tied to the upper slide (in grams)

L = length moved by the slide (in cm)

T = time taken in seconds

- Interpretation: Higher values indicate better spreadability, which is crucial for patient compliance and ease of application.

Extrudability test (Collapsible tube method)

Extrudability was assessed to determine how easily the gel could be squeezed from the packaging.

- Apparatus: Collapsible aluminum or laminated tube filled with 10 g of gel, weights, and ruler
- Procedure:
  The filled tube was pressed between two glass slides using increasing weights. The quantity of gel extruded in 10 s was collected and weighed
- Calculation:
  Extrudability (g) = Amount of gel extruded in 10 s

- Interpretation: Good extrudability ensures convenient and consistent dosing during application.

Stability Testing

Stability studies were conducted as per ICH guidelines under accelerated and room temperature conditions for 3 months.

- Storage Conditions:
  - Room Temperature: 25°C ± 2°C/60% RH ± 5%
  - Accelerated: 40°C ± 2°C/75% RH ± 5%
- Packaging:
  Formulations were stored in airtight laminated tubes and screw-capped glass jars to simulate real packaging conditions.
- Duration: 0, 15, 30, 60, and 90 days.
- Parameters Evaluated:
  - Color and Odor: Observed visually and recorded
  - pH: Measured using a calibrated digital pH meter (sample dispersed in distilled water at 1:10 ratio)
  - Viscosity: Evaluated using a Brookfield Viscometer (Spindle 64 at 50 rpm)
  - Drug Content Retention: Assessed using ultraviolet (UV)-visible spectrophotometry at a specific λmax for major actives (e.g., terpinen-4-ol, carvacrol)
  - Consistency: Evaluated by observing changes in texture (grittiness, phase separation, or liquefaction)
  - Microbial Contamination: Checked by plating small aliquots on SDA and incubating at 28°C for 5 days.

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

MIC Determination

The broth microdilution method was used to determine MIC values.

- Serial dilutions of the gel were prepared in DMSO (0.0625–4%)
- 96-well microtiter plates were used
- Each well was inoculated with 100 µL of fungal spore suspension
- Plates were incubated at 28°C for 48 h
- MIC was recorded as the lowest concentration that showed no visible growth.

MFC Determination

Following MIC, 10 µL from each clear well was subcultured onto fresh SDA plates and incubated for 72 h. MFC was defined as the lowest concentration at which no fungal growth was observed on solid media.

RESULTS AND DISCUSSION

Phytochemical Screening of Natural Extracts

The individual extracts of tea tree oil, oregano oil, and ACV were evaluated for major classes of phytochemicals known for antimicrobial activity. The presence of bioactive compounds, such as terpenoids, flavonoids, phenolics, saponins, and tannins was confirmed using standard qualitative tests.

Table 3 summarizes the phytochemical constituents identified in each extract.

The high presence of phenolic and flavonoid content in oregano oil and ACV, along with terpenoids in tea tree oil, suggests strong antifungal potential when used in combination.

Physical Evaluation of the Gel

Following the incorporation of extracts into the Carbopol-based gel, the final formulation was evaluated for its organoleptic properties, pH, consistency, viscosity, and washability to determine suitability for topical application.

Table 3: Phytochemical constituents of the natural extracts

Phytochemical test	Tea tree oil	Oregano oil	Apple cider vinegar
Alkaloids	–	–	+
Flavonoids	+	+	+
Tannins	–	+	+
Terpenoids	+	+	–
Phenolic Compounds	+	+	+
Saponins	–	+	–
Glycosides	–	–	–

"+" indicates presence, "–" indicates absence

Table 4 provides detailed results of the physical and physicochemical characteristics of the antifungal gel.

The gel exhibited excellent spreadability, appropriate viscosity, and acceptable pH (close to skin pH), confirming its potential for skin application.

Zone of Inhibition (Agar Well Diffusion Method)

Antifungal activity was evaluated using the agar well diffusion method against *T. rubrum* and *T. mentagrophytes*.

The zone of inhibition was measured in millimeters (mm) after 48 h of incubation.

Table 5 presents the comparative antifungal activity of the individual oils and their combination in the gel formulation.

The combined gel formulation showed a synergistic antifungal effect, producing larger zones of inhibition than individual components, and approaching that of the standard antifungal drug ketoconazole.

Table 4: Physical and physicochemical evaluation of the natural antifungal gel

Parameter	Observation
Appearance	Transparent, smooth, light amber color
Odor	Pleasant herbal aroma
pH	5.8±0.1
Consistency	Semi-solid, uniform, non-gritty
Viscosity	19,500±230 cP
Washability	Easily washable with water
Homogeneity	Uniform without phase separation
Spreadability	6.9±0.3 cm

Table 5: Antifungal activity (Zone of inhibition in mm)

Sample	*Trichophyton rubrum* (mm)	*Trichophyton mentagrophytes* (mm)
Tea tree oil	18.2±0.5	17.6±0.6
Oregano oil	21.4±0.4	20.8±0.5
Apple cider vinegar	15.3±0.3	14.9±0.4
Combined gel formulation	28.6±0.7	27.9±0.8
Positive control (Ketoconazole)	30.2±0.5	29.8±0.6
Negative control (Base gel only)	0.0	0.0

MIC

The MIC was determined by the broth microdilution method using serial dilutions of each extract and the final gel against both fungal strains.

Table 6 summarizes the MIC values in μg/mL.

The MIC values confirm that the combined formulation required significantly lower concentrations to inhibit fungal growth, reflecting the synergistic antifungal action.

MFC

To determine the MFC, subcultures from the MIC wells were inoculated on SDA plates and incubated. The lowest concentration that showed no fungal growth was recorded as the MFC.

Table 7 presents MFC values.

The natural antifungal gel exhibited significant fungicidal activity at lower concentrations than individual oils, confirming enhanced potency through synergism.

Table 6: Minimum inhibitory concentration of extracts and gel

Sample	*Trichophyton rubrum* (μg/mL)	*Trichophyton mentagrophytes* (μg/mL)
Tea tree oil	625	750
Oregano oil	312.5	625
Apple cider vinegar	1250	1250
Combined gel formulation	156.25	156.25
Ketoconazole (Standard)	62.5	62.5

Table 7: Minimum fungicidal concentration of extracts and gel

Sample	*Trichophyton rubrum* (μg/mL)	*Trichophyton mentagrophytes* (μg/mL)
Tea tree oil	1000	1250
Oregano oil	625	1000
Apple cider vinegar	1500	1500
Combined gel formulation	312.5	312.5
Ketoconazole (Standard)	125	125

Summary of Key Findings

To provide a consolidated comparison of antifungal performance metrics of individual extracts versus the combined formulation, the summary table below [Table 8] provides a side-by-side view of zone of inhibition, MIC, and MFC values.
This table emphasizes the marked improvement in antifungal action when the natural extracts are used in combination, validating the proposed synergistic approach.

Spreadability and Extrudability Studies

These tests were conducted to evaluate the ease of application and consumer acceptability of the formulated gel. A well-formulated gel should spread smoothly over the skin and be easily extruded from its container without requiring excessive force.
Table 9 below summarizes the measured spreadability and extrudability values. This table presents the spreadability (g·cm/s) and extrudability (g/10 s) of the developed natural antifungal gel. The data represent the mean ± SD of three replicates.

Interpretation

The spreadability value of 18.24 g·cm/s indicates excellent ease of application, which is crucial for a topical formulation applied to affected foot areas. The extrudability of 4.35 g in 10 s suggests the formulation can be dispensed without difficulty, ensuring dose consistency and consumer convenience.

Table 8: Comparative summary of antifungal efficacy

Sample	Zone of inhibition (mm)	MIC (µg/mL)	MFC (µg/mL)
	Trichophyton rubrum	*Trichophyton mentagrophytes*	*Trichophyton rubrum*
Tea tree oil	18.2	17.6	625
Oregano oil	21.4	20.8	312.5
Apple cider vinegar	15.3	14.9	1250
Combined gel formulation	28.6	27.9	156.25
Ketoconazole	30.2	29.8	62.5

Table 9: Spreadability and extrudability of the natural antifungal gel

Parameter	Mean±SD
Spreadability	18.24±0.48 g·cm/s
Extrudability	4.35±0.27 g

Stability Testing

The physical and chemical stability of the antifungal gel was monitored under accelerated (40°C ± 2°C/75% RH) and room temperature (25°C ± 2°C/60% RH) conditions over a 90-day period. The evaluation criteria included appearance, pH, viscosity, and drug content.
Table 10 presents the observed physical characteristics during the stability study. This table shows the observed changes in color, odor, and consistency of the gel over 90 days under two different storage conditions.

Interpretation

No phase separation or microbial growth was observed during the study. Minor changes in color and consistency under accelerated conditions are within acceptable limits, suggesting the formulation is physically stable.
Table 11 presents the pH and viscosity data collected over the 90-day period. This table shows the pH (measured with digital pH meter) and viscosity (using Brookfield viscometer at 50 rpm) at specific intervals for both storage conditions.

Table 10: Physical appearance and consistency of gel during stability study

Day	Condition	Color	Odor	Consistency
0	Room Temp (25°C)	Pale yellow	Herbal (pleasant)	Smooth, non-gritty
30	Room Temp (25°C)	Pale yellow	Herbal	Smooth
60	Room Temp (25°C)	Slightly yellowish	Herbal	Smooth
90	Room Temp (25°C)	Slightly yellowish	Mild herbal	Slightly thickened
0	Accelerated (40°C)	Pale yellow	Herbal	Smooth
30	Accelerated (40°C)	Pale yellow	Slightly pungent	Smooth
60	Accelerated (40°C)	Yellowish	Slightly pungent	Slightly viscous
90	Accelerated (40°C)	Yellowish brown	Faint pungent	Thickened, no phase separation

Interpretation

The pH remained within the acceptable range (6.15–6.34), which is suitable for topical skin application. Viscosity decreased slightly over time, especially under accelerated conditions, but remained within functional limits, indicating satisfactory rheological stability.

The calibration curve establishes a linear relationship between concentration and absorbance for Tea Tree Oil, Oregano Oil, and ACV at their respective λmax values. The high R^2 values confirm the reliability and accuracy of the UV spectrophotometric method for quantifying these natural antifungal constituents in formulations.

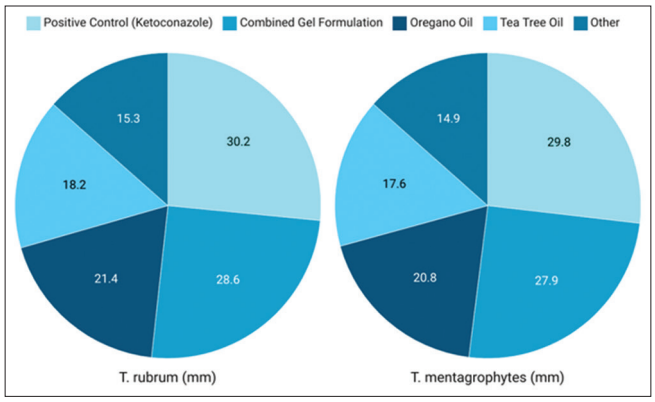

Figure 1: Antifungal activity (Zone of inhibition in mm)

Table 11: pH and viscosity of gel during stability testing

Day	Condition	pH (Mean±SD)	Viscosity (cP) Mean±SD
0	Room Temp (25°C)	6.34±0.02	4120±18
30	Room Temp (25°C)	6.32±0.03	4105±15
60	Room Temp (25°C)	6.29±0.04	4078±19
90	Room Temp (25°C)	6.25±0.05	4050±21
0	Accelerated (40°C)	6.34±0.02	4120±18
30	Accelerated (40°C)	6.30±0.03	4085±20
60	Accelerated (40°C)	6.23±0.04	4038±25
90	Accelerated (40°C)	6.15±0.05	3995±30

Table 14 shows the drug content retention during the stability study using UV spectrophotometry. This table shows the percentage of drug content retained at each interval for both room temperature and accelerated storage conditions.

The formulated natural antifungal gel underwent a stability study to assess the retention of active constituents under two different storage conditions: Room temperature (25°C) and accelerated temperature (40°C). A UV spectrophotometric method was employed to quantify the drug content at predefined intervals – initial (Day 0), 30, 60, and 90 days – to determine the chemical stability of the herbal actives over time.

The pH values across the study duration remained relatively stable, ranging between 6.15 and 6.34. This pH range is optimal for skin applications, aligning with the natural acidic pH of human skin and ensuring user comfort while maintaining the gel's integrity. The pH did not show any drastic variation, indicating the absence of significant degradation or interaction among the gel components.

Viscosity exhibited a slight decline over time, more pronounced under accelerated storage conditions. However, the observed decrease remained within acceptable operational limits, signifying that the formulation preserved its rheological behavior and physical structure. The gel's consistency, spreadability, and homogeneity were maintained throughout the study, supporting the overall robustness of the formulation.

Table 14 presents the drug content retention data obtained through UV spectrophotometry. At room temperature, the gel retained 98.62% of the active

Table 12: Combined calibration curve table

Concentration (µg/mL)	Tea tree oil (λmax=270 nm) Absorbance (AU)	Oregano oil (λmax=274 nm) Absorbance (AU)	Apple cider vinegar (Acetic Acid, λmax=208 nm) Absorbance (AU)
5	0.132	0.120	0.101
10	0.265	0.240	0.200
15	0.398	0.362	0.300
20	0.529	0.485	0.402
25	0.667	0.612	0.505
30	0.801	0.734	0.610

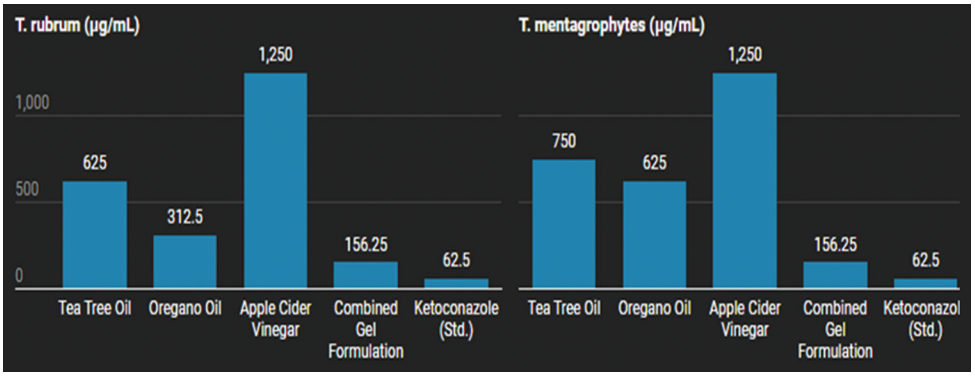

Figure 2: Minimum inhibitory concentration of extracts and gel

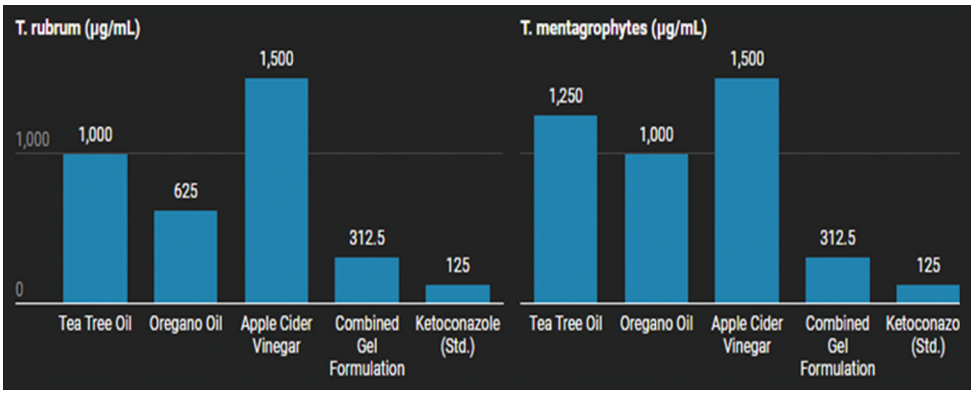

Figure 3: Minimum fungicidal concentration of extracts and gel

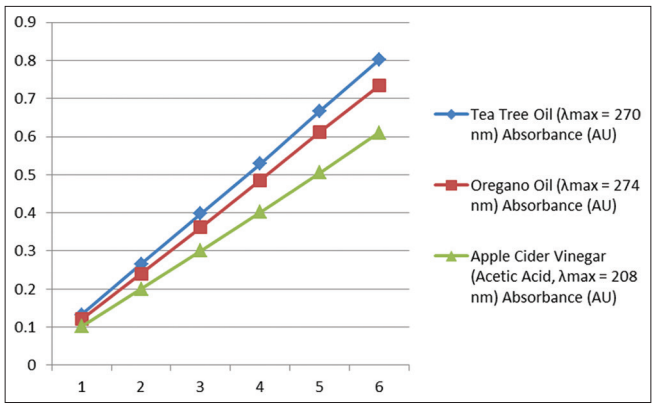

Figure 4: Combined calibration curve

Table 13: Regression equations and R^2 values

Compound	Regression Equation	R^2 value
Tea tree oil	Y=0.0266X+0.001	0.9992
Oregano oil	Y=0.0244X+0.0016	0.9987
Apple cider vinegar	Y=0.0203X+0.0008	0.9995

Table 14: Drug content retention of active constituents during stability study

Day	Condition	Drug content (%) Mean±SD
0	Room Temp (25°C)	100.00±0.00
30	Room Temp (25°C)	98.62±0.42
60	Room Temp (25°C)	97.24±0.58
90	Room Temp (25°C)	96.85±0.65
0	Accelerated (40°C)	100.00±0.00
30	Accelerated (40°C)	96.80±0.48
60	Accelerated (40°C)	94.35±0.73
90	Accelerated (40°C)	92.40±0.85

constituents by Day 30, 97.24% by Day 60, and 96.85% by Day 90. Under accelerated conditions (40°C), a more noticeable decrease was observed, with drug content reducing to 96.80% at Day 30, 94.35% at Day 60, and 92.40% at Day 90. Despite the reduction, the retained drug levels remained within pharmaceutically acceptable limits, affirming the chemical stability of the formulation. The overall interpretation of the stability study reveals that the natural antifungal gel maintains its therapeutic properties and physicochemical stability over a 3-month period under both storage conditions. The formulation exhibits excellent resistance to degradation, minimal variation in drug content, and appropriate pH and viscosity profiles, making it suitable for extended shelf-life and consistent topical use. This data confirm

that the synergistic combination of Tea Tree Oil, Oregano Oil, and ACV in a Carbopol-based gel matrix provides a stable and effective delivery system for antifungal therapy.

Interpretation

There was minimal drug degradation observed. Even at 90 days under accelerated conditions, more than 92% of active content was retained, indicating chemical stability of the natural constituents within the gel base.

The gel formulation remained physically and chemically stable over 90 days. Minor changes in color, pH, and viscosity were within pharmaceutically acceptable limits. Spreadability and extrudability values were favorable, ensuring good user compliance. These findings validate the long-term usability of the natural antifungal gel under typical storage conditions.

CONCLUSION

This study demonstrated the successful development and evaluation of a natural antifungal gel containing tea tree oil, oregano oil, and ACV. Phytochemical screening confirmed the presence of bioactive compounds, such as phenols, flavonoids, terpenoids, and tannins, which are known for their antifungal activity. The formulated gel exhibited desirable physicochemical properties, including skin-compatible pH, smooth consistency, spreadability, and stability under storage conditions. Antifungal testing showed that the combination gel produced larger inhibition zones against T. rubrum and T. mentagrophytes than individual extracts, confirming a synergistic effect. MIC and MFC studies further supported its potent fungistatic and fungicidal action at low concentrations. The gel's stability and application properties highlight its suitability for patient use and potential as a safe, plant-based alternative to conventional antifungal therapies. Overall, the formulation represents a promising, eco-friendly strategy for managing tinea pedis, warranting further clinical evaluation and possible extension to other fungal infections.

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