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RESEARCH ARTICLE

Formulation and Evaluation of an Antifungal Gel for Tinea Corporis by Using *Achyranthes aspera* Leaf Extract

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Abstract

Achyranthes aspera, a medicinal plant traditionally used in Sri Lanka, exhibits antidermatophytic properties. This study aimed to formulate and evaluate topical herbal gels incorporating *A. aspera* leaf extract for activity against *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Gels were formulated with different concentrations of *A. aspera* leaf extract: 1% (A), 3% (B), and 5% (C). Physicochemical properties, including pH, viscosity, and spreadability, and antifungal activity against clinical isolates of *T. rubrum* and *T. mentagrophytes* were evaluated using agar well diffusion, with ketoconazole as a positive control and 5% DMSO as a negative control. Stability was monitored over 15 days by assessing changes in physicochemical properties. All formulations were green, homogeneous, opaque, and smooth, with a semi-solid consistency; they maintained a skin-compatible pH (6.5–6.9) and stable physicochemical characteristics throughout the 15 days. All gels exhibited antifungal activity, with formulation C displaying the highest potency, producing zones of inhibition of 23.33 mm against *T. rubrum* and 24.33 mm against *T. mentagrophytes*. In comparison, the positive control yielded inhibition zones of 25.66 mm and 29.00 mm, respectively. In conclusion, the *A. aspera* leaf extract gel demonstrates moderate antifungal activity, particularly the 5% formulation, suggesting its potential as an alternative topical therapy for *Tinea corporis*. Further preclinical, clinical, and long-term stability studies are warranted.

Keywords: *Achyranthes aspera* leaves extract; Herbal gel; Tinea corporis; *Trichophyton mentagrophytes; Trichophyton rubrum*

INTRODUCTION

Disease is a common threat to the world; infectious diseases commonly have a huge impact on world health and cause a high range of morbidity and mortality throughout the world (Havlickova & Friedrich, 2008). Tinea corporis is a common fungal disease, known as "ringworm" (Kaushik et al., 2015; Lakshmipathy & Kannabiran, 2010; Leung et al., 2020). It is caused by dermatophytes, including *Trichophyton rubrum*, *Trichophyton mentagrophytes,* and *Microsporum canis* (Shy, 2007). According to the WHO, *T. rubrum*, *T. mentagrophytes*, and *M. canis* are responsible for 80%, 35%, and 14% of tinea corporis worldwide, respectively (Degreef, 2008).

A recent study conducted in Sri Lanka has revealed that *T. mentagrophytes* has become the most common causative organism of dermatophytosis, surpassing *T. rubrum* (Jegadeesan et al., 2017; Madarasingha et al., 2022). Current treatment options for tinea corporis include both topical and systemic antifungal agents. Topical therapy is generally effective and provides satisfactory outcomes for managing tinea corporis. In many cases, topical treatment is sufficient to cure the infection, but if a large surface area is affected, systemic treatment is needed (Gupta et al., 2017). Currently, Allylamines (Terbinafine, Naftifine 1%, Butifine 1%), Azoles (Clotrimazole 1%, econazole 1%, Miconazole 1%, Oxiconazole 1%, Sertaconazole 1%, Luliconazole 1%, Eberconazole 1%), Amorolfine 0.25%, and Amphotericin B (1mg) 0.1% are commonly used for topical treatment (Sahoo & Mahajan, 2016). The use of these conventional medications is related to several side effects, such as skin irritation and allergy; moreover, prolonged usage can lead to drug resistance (Degreef & DeDoncker, 1994; Martinez-Rossi et al., 2008). On the other hand, studies have shown that plant-based products have fewer side effects, less cost, and are equally effective as their conventional counterparts (Lopes et.al, 2016; Singh & Samanta, 2022).

Topical gel is considered the best topical drug-delivery

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system for treating skin infections. Because there is no first-pass metabolism, it is easy to apply to infected areas, provides site-specific action, improves patient compliance, allows self-medication, and reduces gastrointestinal tract incompatibilities (Havlickova & Friedrich, 2008). Sri Lanka is a country rich in natural resources, with a wide variety of valuable herbs and plants. According to the literature, there are many plants traditionally used for ringworm in Sri Lanka and other countries, such as *Ocimum sanctum, Dodonaea viscosa* Roxb*, Rhinunthus nasutus*, *Cassia* species (*Senna auriculata, Senna tora, Senna alata*), *Allium sativum*, *Acalypa indica*, *Aloe vera*, and *Curcuma longa* (Anand et al., 2022; Farzana & Tharique, 2014; Kalaivanan et al., 2013; Meena & Ramaswamy, 2014; Singh et al., 2013). Particularly, studies had been conducted on an *Achyranthes aspera plant* against dermatophytes, showing anti-dermatophytes activity against *T. rubrum*, *T. mentagrophytes,* and *M. canis* (Anand et al., 2022; Farzana & Tharique, 2014; Kalaivanan et al., 2013; Londonkar et al., 2011; Meena & Ramaswamy, 2014; Singh et al., 2013)

A. aspera belongs to the family Amaranthaceae. It is commonly known as Prickly chaff- flower, it is also called Kartheba (Sinhala) and Nayuruvi (Tamil). It is an erect annual herb, available in many tropical countries. It has many pharmacological activities. Traditionally, it is used to treat asthmatic cough, rabies, renal dropsy, cholera, snakebite, influenza, gonorrhea, rheumatoid arthritis, hydrophobia, bronchitis, diarrhea, skin disease, and abdominal pain (Goyal et al., 2007; Raju et al., 2022; Sharma & Chaudhary, 2015).

Although *A. aspera* has been incorporated into various formulations, such as topical antibacterial agents, toothpaste, anti-acne creams, shingles ointments, and wound-healing herbal gels, no previous studies have investigated its formulation and evaluation as an antifungal gel for the management of tinea corporis. Patil et al., 2026; Swathy et al., 2023). Incorporating this bioactive extract into gels could lead to the development of novel, cost-effective, locally sourced antifungal products while also supporting the country's economic growth. Therefore, this study aimed to formulate and evaluate an antifungal gel for tinea corporis using *A. aspera* leaf extract.

MATERIALS AND METHODS

Chemical and reagents

Ethyl acetate, dimethyl sulfoxide (DMSO), Sabouraud Dextrose broth (SD broth), Carbopol 940, propylene glycol 400, methyl paraben, propyl paraben, triethanolamine, ketoconazole gel, desiccant (silica gel), normal saline, distilled water, sodium hypochlorite, agar, and 70% ethanol solution were used.

Preparation of leaf extract

Fresh *A. aspera* leaves were collected from an agricultural farm in the Kilinochchi district and authenticated at the National Herbarium, Department of National Botanic Gardens, Peradeniya. A 500 g powder of A. aspera leaves was extracted with 1500 mL of ethyl acetate using a Soxhlet apparatus (Das et al., 2010; Kalaivanan et al., 2013). The extraction was carried out for 6 cycles, and the solvent was evaporated by rotary evaporator at 50 °C. The extract was stored in a refrigerator at 4 °C for future use.

Ethical Considerations and Study Population

Ethical approval was granted by the Ethics Review Committee, Faculty of Medicine, University of Jaffna, during its 160th meeting held on 27th June 2024. Written informed consent was obtained from all participants (or their parents/guardians in the case of minors) presenting at the Dermatology Unit, Teaching Hospital, Jaffna, Sri Lanka, before enrollment.

The study population comprised 20 male and female patients diagnosed with *Tinea corporis* by a Consultant Dermatologist, Medical Officer, or Resident House Officer at the Dermatology Unit.

Sample Collection and Isolation of Fungal Isolates

Skin swabs were collected from the affected areas of the selected patients by trained medical personnel (Medical Officers or Resident House Officers) without disrupting routine clinical care.

Primary culturing of the collected skin swabs yielded isolated colonies of *Trichophyton rubrum* and *Trichophyton mentagrophytes*, which were subsequently processed for antifungal susceptibility testing.

Preparation of culture media

Microorganisms were grown on Sabouraud Dextrose Agar (SDA). 6.5 g of SDA was transferred into a 500 mL conical flask, and 100 mL of distilled water was added and mixed well. The mixture was sterilized by autoclave at 121 °C for 15 minutes with 15 Pa. The tubes were then cooled to produce slant agar and stored at 4 °C until use (Shakya, 2015).

Primary culture preparation

Skin swab samples were inoculated onto SDA media; microorganisms were grown aerobically on SDA and incubated at 30 °C for 10 days before use (Bartolomeu, 2012). The Grown fungus was used to confirm the presence of *T. rubrum* and *T. mentagrophytes* in skin swaps and was subcultured.

Confirmation of *T. rubrum* and *T. mentagrophytes* in primary culture

The presence of *T. rubrum* and *T. mentagrophytes* was confirmed by microscopic examination and colony morphology. Then, microscopic examination was

performed after staining with Lactophenol cotton blue. The stain was applied completely to cover the glass slide and left for 1 minute to react. Slides were observed under a light microscope at low and high power.

Preparation of gels

The quantities of chemicals required for gel preparation were tabulated in Table 1. 1 g of Carbopol 940 was dispersed in 50 mL of distilled water with continuous stirring and allowed to stand for 24 hours. 5 mL of distilled water was taken in a separate beaker. The required quantities of methyl paraben and propyl paraben were added to the water, which was then heated in a water bath until dissolved. The solution was allowed to cool. Extracts of 1 g, 3 g, and 5 g were mixed with the preservative to form formulations A, B, and C, respectively. After adding it to the Carbopol 940 mixture with continuous stirring, propylene glycol 400 was diluted in a small amount of distilled water and gradually incorporated into the mixture. The total volume was then adjusted to 100 g by adding the remaining distilled water. Upon continuous stirring, triethanolamine was added dropwise to the formulation to adjust the required pH (6.5-7) and obtain the gel at the required consistency. The same method was followed to prepare a blank gel without adding any *A. aspera* leaf extract (D) (Das et al., 2011).

Physicochemical Evaluation of Formulations

Organoleptic Properties and Homogeneity

Organoleptic properties, including color, clarity, odor, and general physical appearance, were visually evaluated (Giri et al., 2019). Homogeneity was assessed by applying a thin smear of the gel sample onto a clear glass slide and observing for phase separation or the presence of aggregates.

pH

The pH was measured using a calibrated digital pH meter (Eutech H700). Briefly, 1.0 g of the gel was accurately weighed, dispersed in 10 mL of distilled water, and allowed to stand for 2 h at room temperature. The pH of each formulation was measured in triplicate, and the mean value was recorded (Nand et al., 2012).

Viscosity

Viscosity was measured using a digital viscometer (Brookfield Ametek HB). A 20 mL sample of the prepared gel was transferred into a 25 mL beaker, and the viscometer spindle was immersed in the sample. Readings were taken in triplicate at room temperature (Nand et al., 2012; Shahtalebi et al., 2018).

Spreadability

Spreadability was evaluated using a wooden block apparatus fitted with a pulley, as described by Mutimer et al. (1956). Ground glass slides were used to evaluate the slip and drag characteristics of the gels. A 2.0 g sample of gel was placed onto a fixed ground glass slide and covered with a second glass slide equipped with a hook. A 1.0 kg weight was placed on top of the slides for 5 min to expel air and form a uniform film of gel. Excess gel was wiped from the edges, and a 50 g pan weight was attached via a string through the pulley to the upper slide. The time (T , in seconds) required for the upper slide to travel a distance of 7.5 cm (L) was recorded. A shorter time interval indicated greater spreadability.

Spreadability (S) was calculated using the following equation:

$$S = \frac{M \times L}{T}$$

where:

S = spreadability (g ·cm/s)

M = weight tied to the upper slide (g)

L = length moved by the glass slide (cm)

T = time taken to separate the slides (s)

All measurements were performed in triplicate (Das et al., 2011; Dwivedi & Gupta, 2012).

Table 1: Composition of gel formulations containing different concentrations of *Achyranthes aspera* leaf extract

Formulation	Extract w/w%)	Carbopol 940 w/w%)	Methyl paraben (w/w%)	Propyl paraben w/w%)	Propylene Glycol 400 (w/w%)	Distilled Water (ml)
A	1	1	0.2	0.02	10.35	Up to 100
B	3	1	0.2	0.02	10.35	Up to 100
C	5	1	0.2	0.02	10.35	Up to 100
D	-	1	0.2	0.02	10.35	Up to 100

In vitro antifungal activity evaluation of formulated gels

The antifungal effect of the formulated gels was evaluated against *T. rubrum* and *T. mentagrophytes* using the agar well diffusion method, with ketoconazole gel as the positive control and 5% DMSO as the negative control. 100 mg/mL concentrations of samples from formulations A, B, C, D, and the positive control (E) were prepared in a 5% DMSO solution. 100 µL of the fungal suspension (1-5 × 10^6 spores/mL) was spread onto the surface of the plate containing cooled culture medium. Wells of diameter 6 mm were punched aseptically using a sterile borer in each plate. 100 µL of the 100 mg/mL samples A, B, C, D, and E were added to each well. Plates were incubated at 30 °C for 72 h. The zone of inhibition around the wells was measured (Balouiri et al., 2016).

Stability of formulated gel

Stability studies were carried out by storing gel formulation in airtight, sterile closed containers at room temperature. pH, viscosity, spreadability, organoleptic characteristics, and homogeneity tests were carried out on days 0, 7, and 15.

Data analysis

Viscosity, pH, spreadability tests, and antifungal activity were done in triplicate, and the results were reported as mean ± standard deviation (SD). Statistical significance of differences in the physicochemical properties and zone of inhibition of the formulations was assessed using ANOVA, followed by Tukey's test, in the Statistical Package for the Social Sciences (SPSS) version 23. Differences between means were considered significant if P-values were lower than 0.05 ($p<0.05$). Organoleptic characteristics were solely based on visual inspection.

RESULTS

Extract Yield and Fungal Identification

The extraction of *Achyranthes aspera* leaves yielded 4.22% (w/w) crude extract. Clinical isolates of *Trichophyton rubrum* and *Trichophyton mentagrophytes* were identified based on macro- and microscopic characteristics. *T. rubrum* colonies exhibited a white, creamy, cottony, or powdery surface with a characteristic red pigment on the reverse side, and microscopic examination with lactophenol cotton blue staining revealed tear-shaped microconidia. *T. mentagrophytes* formed white-to-tan, cottony or powdery colonies, and exhibited clusters of microconidia alongside cigar-shaped macroconidia with terminal rat-tail filaments (Uthansingh et al., 2019).

Physicochemical properties of the gels and the stability of the gels

All the gel formulations were tested for homogeneity and organoleptic properties, including color, physical appearance, and odor. The results are shown in Table 2. The results showed that the prepared gels were homogeneous, opaque, and smooth in appearance. Formulation A was light green, while Formulations B and C were dark green. The characteristic odor of the formulations suggests the presence of active components, while the blank gel remained colorless and odorless. The homogeneity and organoleptic properties of all formulations remained unchanged throughout the stability evaluation period.

Table 2: Organoleptic properties, homogeneity, and physical stability of *Achyranthes aspera* gel formulations over 15 days at room temperature

Type of Formulation	Time (Days)	Homogeneity	Appearance	Colour	Odour
Formulation A	0 7 15	Homogenous	Opaque and smooth, semi-solid consistency	Light Green	Characteristic
Formulation B	0 7 15	Homogenous	Opaque and smooth, semi-solid consistency	Dark Green	Characteristic
Formulation C	0 7 15	Homogenous	Opaque and smooth, semi-solid consistency	Dark Green	Characteristic
Blank gel D	0 7 15	Homogenous	Transparent and smooth, semi-solid consistency	Colour less	Odourless

pH, viscosity, and spreadability evaluation

Physicochemical properties such as viscosity, spreadability, and pH were measured for the formulations at different time points. The results are illustrated in Table 3. The pH of the formulation on day 0 ranged from 6.49±0.01 to 6.89±0.01 and showed a slight reduction during the stability study period, ranging from 6.31±0.02 to 6.89±0.01. All the formulations showed significant differences. However, there was no significant difference across the different time durations for formulations B and D.

Viscosity of the formulations ranged from 8686.33±33.37 to 11066.33±16.54 cPs and gradually decreased over time, ranging from 8540.33±14.27 to 9975.00±17.80 cPs. Further, a significant difference was observed between the formulations at different time intervals.

Spreadability of the formulations ranged from 17.36±0.02 to 24.06±0.04 g.cm/s at day 0 and increased to 17.67±0.07 to 29.89±0.38 g.cm/s by the end of day 15. Significant differences were observed between the formulations; however, no significant differences were observed across different time intervals.

***In vitro* antifungal activity of prepared gels**

Antifungal activity was assessed using a well diffusion method against *T. rubrum* and *T. mentagrophytes*, and the results are shown in Figure 1. Mean of zone of inhibition of formulations A, B, C, and E showed 12.33±0.57 mm, 21.00±1.00 mm, 23.33± 0.57 mm, and 25.66±0.57 mm, respectively, against *T. rubrum*, while 14.66±0.57 mm, 21±1.00 mm, 24.33±0.57 mm, and 29±0.57 mm, respectively, against *T. mentagrophytes*. The negative control showed no zone of inhibition against both fungal strains. Statistical analysis indicated a significant difference between the formulations and the positive control for *T. rubrum* and *T. mentagrophytes* ($p<0.05$). The further positive control exhibited the largest zone of inhibition, followed by formulations C, B, and A against *T. rubrum* and *T. mentagrophytes,* respectively.

Table 3: pH, viscosity, and spreadability of *Achyranthes aspera* gel formulations measured at 0, 7, and 15 days of storage

	Days	Formulation A (Mean ± SD)	Formulation B (Mean ± SD)	Formulation C (Mean ± SD)	Blank gel (Mean ± SD)
pH	0	$6.89±0.01^{a,w}$	$6.49±0.01^{a,y}$	$6.82±0.02^{a,x}$	$6.50±0.01^{a,z}$
	7	$6.77±0.01^{b,w}$	$6.52±0.01^{a,y}$	$6.75±0.00^{b,x}$	$6.46±0.02^{a,z}$
	15	$6.75±0.01^{b,x}$	$6.48±0.02^{a,y}$	$6.75±0.01^{b,w}$	$6.31±0.02^{a,z}$
Viscosity (cP)	0	$9747.67±12.55^{a,x}$	$9039.00±18.67^{a,y}$	$8686.33±33.37^{a,z}$	$11066.33±16.54^{a,w}$
	7	$9427.67±43.61^{b,x}$	$8909.67±24.61^{b,y}$	$8539.33±45.64^{b,z}$	$10957.33±16.11^{b,w}$
	15	$9435.00±15.12^{b,x}$	$8557.00±24.54^{c,y}$	$8540.33±14.27^{b,y}$	$9975.00±17.80^{c,w}$
Spreadability (g.cm/s)	0	$19.30±0.05^{a,y}$	$21.66±0.12^{a,x}$	$24.06±0.04^{a,w}$	$17.36±0.02^{b,z}$
	7	$19.23±0.48^{a,y}$	$21.71±0.43^{a,x}$	$24.29±0.19^{a,w}$	$17.42±0.09^{b,z}$
	15	$19.92±0.69^{a,y}$	$29.89±0.38^{a,x}$	$24.50±0.28^{a,w}$	$17.67±0.07^{a,z}$

The values represented with different superscripts (a, b, c) in each column differ significantly ($p<0.05$); likewise, the values represented with different superscripts (w, x, y, z) in each row differ significantly ($p<0.05$)

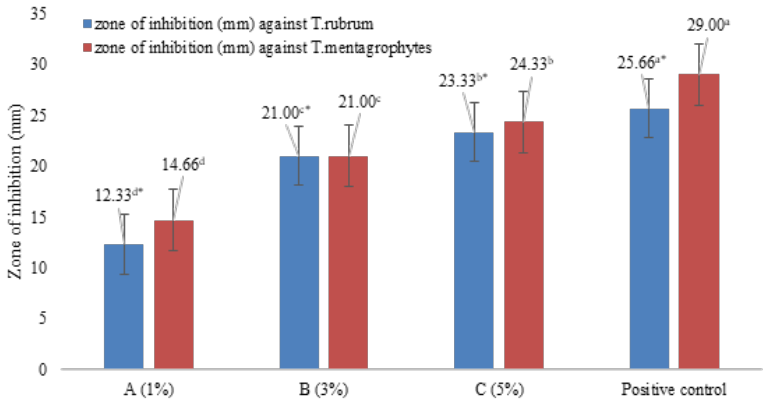

Figure 1. Antifungal activity of formulated gels and positive control against *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Values with different superscript letters containing asterisks (a^*,b^*,c^*,d^*) differ significantly ($p<0.05$) for *T. rubrum*. Values with different plain superscript letters (a,b,c,d) differ significantly ($p<0.05$) for *T. mentagrophytes*

DISCUSSION

Tinea corporis is a common fungal infection worldwide. The existing treatment for ringworm infection is showing relapses and side effects. Tinea corporis is not a life-threatening infection, but it has some negative impact on psychological, social, and economic aspects of human life. Studies have shown that plant-based products have fewer side effects, lower cost, and are as effective as their conventional counterparts. Plant-derived phytochemicals are showing promising potential for formulating antimicrobial agents (Kaushik et al., 2020). Tinea corporis is caused by dermatophytes. A recent study conducted in Sri Lanka revealed that *T. Mentagrophytes* and *T. rubrum* are the main causative organisms (Madarasingha et al., 2022).

In this study, the yield of *A. aspera* leaf extract was 4.22%, higher than that reported in previous studies (Goyal et al., 2007). Such variations in extraction yield may be attributed to differences in the geographical origin of plant material, which can lead to variations in phytochemical composition. Furthermore, methodological factors such as extraction technique and Soxhlet extraction duration may also contribute to the observed differences in yield (Das et al., 2010).

Several formulations incorporating *A. aspera* leaves or whole-plant extracts have been developed for various therapeutic applications, including topical antibacterial products, toothpaste, anti-acne creams, shingles ointments, and wound-healing gels (Karjkhede et al., 2025; Wagh et al., 2025). Despite its diverse pharmaceutical applications, no study has yet investigated the formulation and evaluation of an antifungal gel containing *A. aspera* for the treatment of tinea corporis. Previous studies have demonstrated that the ethyl acetate extract of *A. aspera* exhibits potent antidermatophytic activity against clinically important dermatophytes (Kalaivanan et al., 2013). This activity may be attributed to bioactive phytochemicals, including alkaloids, terpenoids, tannins, phenolic compounds, flavonoids, and saponins (Abhang et al., 2024). In particular, phenolic compounds, flavonoids, saponins, and terpenoids are known to exert antifungal effects by disrupting fungal cell membrane integrity, inhibiting spore germination, and interfering with fungal growth and metabolic processes (Martinez-Rossi et al., 2008). Therefore, the present study was undertaken to formulate and evaluate an antifungal gel containing the ethyl acetate extract of *A. aspera* as a potential topical treatment for tinea corporis. Based on these findings, the present study aimed to formulate and evaluate an antifungal gel incorporating the ethyl acetate extract of *A. aspera* as a potential topical treatment for tinea corporis. Different amounts of leaf extract, ranging from 1 to 5 grams, were incorporated into formulations to determine the optimal dose against fungal pathogens and to assess mixture stability during storage (Patil et al., 2026; Swathy et al., 2023). Prior research showed that the plant extract ranged from 1.5 to 5 grams. Carbopol was selected as the gelling agent owing to its biodegradability, biocompatibility, non-irritant nature, absence of systemic absorption, and favorable bioadhesive properties. Methyl paraben and propyl paraben were incorporated as preservatives due to their effectiveness and compatibility with the formulation's excipients. Propylene glycol was included as a permeation enhancer, consistent with previous studies demonstrating its ability to improve drug penetration through the skin. Triethanolamine (TEA) was used as a pH-adjusting agent and neutralizer to impart the desired gel consistency, stability, and homogeneity to the formulation (Kaushik et al., 2025).

Physicochemical parameters, including organoleptic characteristics, homogeneity, pH, viscosity, and spreadability, were assessed. The gels were visually homogeneous, with a uniform appearance and consistency, which is critical for ensuring the even distribution of active compounds and maximizing antifungal activity. Increasing extract concentration resulted in a color change from light green to dark green, attributed to the plant material's chlorophyll content.

The pH values of all formulations remained within the skin-compatible range (pH 4–7), suggesting a low risk of skin irritation (Lukić et al., 2021). There were slight decreases in pH observed during the 15-day stability study period, yet all formulations remained within the acceptable range. Additionally, Karjkhede et al (2025). Reported that the gel formulation had a pH range of 5.5 to 6.5, consistent with this study's findings.

As reported by Genatrika et al. (2022), the viscosity of semisolid dosage forms typically falls within the acceptable range of 2,000–50,000 cPs. In the present study, the viscosity of the formulated gels ranged from 8,686.33 ± 33.37 to 11,066.33 ± 16.54 cPs, which is well within the recommended limits. Although a slight decrease in viscosity was observed during storage, it did not affect the consistency or overall stability of the gel formulations. This viscosity is comparable to that of the herbal gel containing a whole-plant water extract (Karjkhede et al., 2025).

Spreadability is inversely correlated with viscosity. The results indicated that Formulation C exhibited the highest spreadability, while the blank gel showed the lowest. Slight increases in spreadability during storage were attributed to minor reductions in viscosity and pH. These values were within the optimal range for semisolid topical gels, consistent with previous studies utilizing 1% Carbopol 940 as a gelling agent (Bhinge et al., 2017). It was noted in previous studies that decreased viscosity increased spreadability (Genatrika et al., 2022).

The antifungal efficacy of the formulations was evaluated using the agar well diffusion method, a standard technique widely used to screen antifungal activity (Genatrika et al., 2022). The results demonstrated that both test organisms were susceptible to the prepared formulations. Among them, Formulation C exhibited the strongest antifungal activity, followed by Formulation B, while Formulation

A showed moderate activity. As expected, the blank gel showed no antifungal effect, whereas the positive control demonstrated the highest inhibitory activity. These findings indicate that the antifungal activity of the formulations was dependent on the concentration of *A. aspera* extract. In a previous study (Genatrika et al., 2022), it was also shown that increasing the fraction of the active moiety yields better activity than a lower concentration of the active moiety. It shows that a higher concentration of the active ingredient in the formulation can be more effective when the number of bioactive compounds is higher, and that the available percentage for the antifungal effect is also higher. The blank gel failed to produce any zone of inhibition because there is no active moiety.

Limitations of this study include the use of a crude ethyl acetate extract of *A. aspera*, without fractionation or characterization of its active constituents, which may affect formulation standardization and reproducibility. Identification of *T. rubrum* and *T. mentagrophytes* was based solely on morphological and microscopic characteristics without molecular confirmation. In addition, antifungal activity was assessed using the agar well diffusion method, which may not accurately reflect the efficacy of semisolid formulations because of diffusion constraints. Furthermore, the 15-day stability study provided only preliminary data and was insufficient to establish the long-term stability and shelf life of the formulated gel.

CONCLUSION

Overall, all formulations demonstrated antifungal activity against *T. rubrum and T. mentagrophytes*. The observed increase in antifungal activity with increasing extract concentration is consistent with the expected concentration-dependent response and supports the reliability of the results. The physicochemical parameters of all formulations were within suitable ranges, and no significant organoleptic changes were observed during the stability study. When compared with standard gel, the findings suggest that the optimized herbal gel, particularly Formulation C, is effective against *T. rubrum* and *T. mentagrophytes*, the primary causative agents of Tinea corporis. In the future, stability of the gel formulation should be conducted under accelerated conditions and over a long-term period. Animal and human studies are needed to assess in vivo skin permeation, toxicity, and irritancy.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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AUTHOR CONTRIBUTION

Conceptualization: **C.R., S.S.;** Methodology: **C.R., L.S., T.K., S.S.,** TG; Investigation: **C.R., L.S., T.K.;** Statistical analysis: **C.R., L.S.;** Data interpretation: **C.R., S.S.;** Writing the first draft: **C.R., L.S., S.S.;** Manuscript editing and reviewing: **S.S.;** Project administration: **S.S.**

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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