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Antifungal Effectiveness of a Combination of Bajakah (*Uncaria acida*) and Bandotan (*Ageratum conyzoides*) Extracts in Mice with Onychomycosis Infected by *Trichophyton rubrum*

Efektivitas Antijamur Kombinasi Ekstrak Bajakah *(Uncaria acida)* dan Bandotan *(Ageratum conyzoides)* pada Mencit dengan Onikomikosis yang Terinfeksi *Trichophyton rubrum*

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Abstract

Onychomycosis is a fungal infection of the nail predominantly caused by *Trichophyton rubrum* and requires safer alternative therapies compared to synthetic antifungal agents. The combination of plant extracts may produce synergistic effects through interactions among bioactive compounds with complementary antifungal mechanisms. This study aimed to evaluate the effectiveness of a combined extract of Bajakah root (*Uncaria acida*) and Bandotan leaf (*Ageratum conyzoides*) as a topical therapy in *T. rubrum*–induced Balb/c mice. The selection of this combination was based on the presence of flavonoids, tannins, alkaloids, terpenoids, and saponins in both plants, which are theoretically associated with antifungal activity. Extracts were obtained by 96% ethanol maceration and formulated into ointments at concentrations of 25%, 50%, 75%, and 100%. Clinical parameters observed included periungual swelling, lesion diameter, nail discoloration, and nail brittleness. Phytochemical analysis confirmed the presence of major bioactive secondary metabolites in both extracts. The results demonstrated clinical improvement in all treatment groups, with the most pronounced effects observed at concentrations of 75–100%. Kruskal–Wallis analysis revealed significant differences among treatments ($p < 0.05$) for all parameters, which were further confirmed by Dunn's post hoc test. Overall, the combined extracts of *U. acida* and *A. conyzoides* effectively reduced the severity of *T. rubrum*–induced onychomycosis and show potential as a natural antifungal candidate derived from local plant resources.

***Keywords*: Ageratum conyzoides, Uncaria acida, Mice, Onychomycosis, Trichophyton rubrum.**

Abstrak

Onikomikosis merupakan infeksi jamur pada kuku yang umumnya disebabkan oleh *Trichophyton rubrum* dan memerlukan alternatif terapi yang lebih aman dibandingkan antijamur sintetis. Kombinasi ekstrak tanaman berpotensi menghasilkan efek sinergis melalui interaksi berbagai senyawa bioaktif dengan mekanisme antijamur yang saling melengkapi. Penelitian ini bertujuan mengevaluasi efektivitas kombinasi ekstrak akar Bajakah (*Uncaria acida*) dan daun Bandotan (*Ageratum conyzoides*) sebagai terapi topikal pada mencit Balb/c yang diinduksi infeksi *T. rubrum*. Pemilihan kombinasi didasarkan pada kandungan flavonoid, tanin, alkaloid, terpenoid, dan saponin dari kedua tanaman yang secara teoritis berkontribusi terhadap aktivitas antijamur. Ekstrak diperoleh melalui maserasi etanol 96% dan diformulasikan dalam bentuk salep dengan konsentrasi 25%, 50%, 75%, dan 100%. Parameter klinis yang diamati meliputi pembengkakan periungual, diameter lesi, perubahan warna kuku, dan kerapuhan kuku. Analisis fitokimia menunjukkan bahwa kedua ekstrak mengandung senyawa bioaktif utama yang berperan dalam aktivitas antijamur. Hasil pengamatan klinis menunjukkan perbaikan pada seluruh kelompok perlakuan, dengan efek paling nyata pada konsentrasi 75–100%. Uji Kruskal–Wallis menunjukkan perbedaan yang signifikan antarperlakuan ($p < 0,05$) pada seluruh parameter, yang dikonfirmasi dengan uji post hoc Dunn. Secara keseluruhan, kombinasi ekstrak Bajakah dan Bandotan menunjukkan efektivitas dalam menurunkan keparahan onikomikosis akibat *T. rubrum* dan berpotensi dikembangkan sebagai kandidat antijamur alami berbasis bahan lokal.

Kata Kunci: Bandotan, Bajakah, Mencit, Onikomikosis, Trichophyton rubrum.

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Introduction

Onychomycosis, a fungal infection of the nails most commonly caused by *T. rubrum*, is one of the most prevalent skin diseases worldwide [1]. According to data from the World Health Organization (WHO), the global prevalence of onychomycosis averages 5.5%, with higher incidence rates observed among individuals with weakened immune systems or certain conditions such as diabetes [2,3]. In Indonesia, the prevalence of onychomycosis has been reported to reach 13–15%, with an increasing trend attributed to tropical environmental conditions that favor the growth of pathogenic fungi. Epidemiological studies indicate that *T. rubrum* is the primary causative agent in 44.9% of onychomycosis cases globally, particularly affecting toenails [4]. This infection is characterized by discoloration, nail thickening, and brittleness, and is often accompanied by localized inflammation. The management of onychomycosis remains challenging due to its chronic nature, high recurrence rates, and slow response to treatment [5,6]

Synthetic antifungal agents such as griseofulvin, terbinafine, and itraconazole are widely used in the treatment of onychomycosis. However, long-term use of these drugs carries the risk of systemic adverse effects, including hepatotoxicity, gastrointestinal disturbances, and the development of resistance in certain fungal strains. Therefore, research into natural products as alternative antifungal therapies is of great importance [7].

Bajakah (*Uncaria acida*), an endemic plant of Kalimantan that is well known in traditional Dayak medicine, is known to contain bioactive compounds such as flavonoids, tannins, and phenolic compounds with antimicrobial, antioxidant, and anti-inflammatory activities [8-10]. Several studies have demonstrated that Bajakah extracts also possess antifungal potential. A study by [11] ound that ethanolic extracts of Bajakah were able to inhibit the growth of *Candida albicans* through mechanisms involving cell membrane disruption and reduced hyphal viability. In addition, [12] reported the fungistatic activity of Bajakah extracts against *Aspergillus niger*, indicating its broad potential in the management of fungal infections.

Meanwhile, Bandotan (*Ageratum conyzoides*) is a weed plant that has been shown to possess antifungal, antibacterial, and anti-inflammatory activities, attributed to the presence of bioactive compounds such as alkaloids, coumarins, and saponins [13,14] . [15] demonstrated that Bandotan extracts were able to inhibit the growth of *T. rubrum* and *T. mentagrophytes* through mechanisms involving disruption of cell wall integrity. [16] reported that the flavonoid fraction of Bandotan exhibited significant effectiveness in reducing dermatophyte fungal colonization in animal models.

The combination of Bajakah and Bandotan extracts has the potential to exert synergistic effects in inhibiting the growth of dermatophytic fungi, particularly *T. rubrum*. However, the combined use of these two plants has not been extensively evaluated scientifically in the context of onychomycosis treatment, especially in animal models. Therefore, this study was conducted to evaluate the effectiveness of a combination of Bajakah and Bandotan extracts as a topical therapy in female mice induced with onychomycosis, with the aim of developing a safe, affordable herbal alternative with potential for phytopharmaceutical development [17,18].

The novelty of this study lies in the scientific exploration of the effectiveness of a combination of Bajakah root (*Uncaria acida*) and Bandotan leaf (*Ageratum conyzoides*) extracts in treating onychomycosis caused by *T. rubrum*. Although each plant has been reported to exhibit antifungal activity against various pathogens, no studies have specifically evaluated their combined efficacy against dermatophytic fungi responsible for nail infections. The combination approach using two local plants from Central Kalimantan, rich in bioactive compounds such as flavonoids, saponins, tannins, and phenolic compounds, provides added value due to its potential to produce synergistic antifungal and anti-inflammatory effects.

Experimental Section

Materials and Apparatus

The materials used in this study included 96% ethanol, distilled water, petroleum jelly (ointment base), *Trichophyton rubrum* culture grown on Potato Dextrose Agar (PDA), 70% and 96% alcohol, liquid soap, disinfectant (Lysol), sterile gauze, sterile cotton swabs, Petri dishes, aluminum foil, ketoconazole 2% (positive control), liquid paraffin, ointment jars (sterile), and labeling materials.

The apparatus comprised an autoclave, test tubes, beakers, Erlenmeyer flasks, spatula, hot plate, magnetic stirrer, micropipettes, droppers, Bunsen burner, incubator, digital analytical balance, microtubes, laminar airflow cabinet, vortex mixer, nail file, vernier caliper (accuracy 0.01 mm), and a smartphone camera for documentation.

Extraction and Formulation

Dried Bajakah roots (*Uncaria acida*) and Bandotan leaves (*Ageratum conyzoides*) were individually ground into fine powders and extracted separately using the maceration method with 96% ethanol. Each plant material was processed independently to obtain crude extracts. The resulting filtrates were concentrated by evaporation to yield viscous extracts, which were then subjected to phytochemical evaluation. Qualitative identification of flavonoid compounds in *Uncaria acida* and flavonoid and tannin compounds in *Ageratum conyzoides* was conducted based on laboratory analyses performed at the UPT Laboratorium Herbal Materia Medica Batu. Meanwhile, the presence of other secondary metabolites, including alkaloids, saponins, terpenoids, and phenolic compounds, was determined based on previously published phytochemical studies of both plant species, serving as supporting references.

For formulation, the two crude extracts were combined at a fixed weight ratio of 1:1 (*Uncaria acida* : *Ageratum conyzoides*). This ratio was selected based on the comparable antifungal potential and complementary phytochemical characteristics of both plants, as reported in previous studies, with the aim of maximizing potential synergistic interactions between their bioactive constituents. The combined extracts were then formulated into topical ointments using petroleum jelly and liquid paraffin as the ointment base.

The ointments were prepared at concentrations of 25%, 50%, 75%, and 100% (w/w), with the total weight of each formulation standardized at 10 g. A 25% (w/w) formulation contained 2.5 g of the combined extract, 6.4 g of petroleum jelly, and 1.1 g of liquid paraffin. The 50% (w/w) ointment consisted of 5.0 g of the combined extract, 4.3 g of petroleum jelly, and 0.7 g of liquid paraffin. The 75% (w/w) formulation comprised 7.5 g of the combined extract, 2.14 g of petroleum jelly, and 0.36 g of liquid paraffin. The 100% (w/w) formulation contained 10.0 g of the combined extract without the addition of an ointment base. A negative control was prepared using sterile distilled water, while a 2% ketoconazole ointment served as the positive control. All ointments were homogenized thoroughly to ensure uniform consistency prior to topical application in the animal experiments.

Animal Model and Ethical Approval

This study employed a true experimental design using a posttest-only control group design. The experiment was conducted at the Microbiology Laboratory and Animal House, Faculty of Tarbiyah and Teacher Training, UIN Palangka Raya, Central Kalimantan, Indonesia.

A total of 24 female BALB/c mice were used as experimental animals to evaluate the effectiveness of a topical ointment containing a combination of Bajakah root (*Uncaria acida*) extract and Bandotan leaf (*Ageratum conyzoides*) extract. All experimental procedures involving animals were performed in accordance with institutional guidelines for animal care and use.

Induction of Onychomycosis and *Trichophyton rubrum* Inoculation

Trichophyton rubrum was cultured on Potato Dextrose Agar (PDA) and incubated at 28–30 °C for 7–10 days until mature fungal colonies were obtained. Conidia were harvested by adding sterile distilled water containing 0.1% Tween 80 to the agar surface, followed by gentle scraping of the colonies. The suspension was

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filtered through sterile gauze to remove hyphal fragments, and the fungal concentration was adjusted to approximately 1 × 10^6 CFU/mL by comparison with a McFarland standard.

Prior to inoculation, the nails of the mice were cleansed with 70% alcohol and allowed to dry. Mild mechanical abrasion of the nail surface was performed using a sterile nail file to disrupt the keratin layer and facilitate fungal penetration without causing bleeding or deep tissue injury. Subsequently, 20 µL of the *T. rubrum* suspension was applied topically to the abraded nail surface once daily for three consecutive days.

After inoculation, the nail area was left uncovered to allow fungal colonization. On the first day of observation, the mice nails already exhibited early signs of onychomycosis, which subsequently progressed into a clearly established infection. The development of onychomycosis was characterized by consistent clinical manifestations, including periungual swelling, erythema, nail discoloration, surface roughness, and increased nail fragility. Clinical observations were conducted continuously over a 14-day period. Only animals exhibiting clear signs of infection were included in the treatment phase.

Treatment Procedure and Clinical Evaluation

After confirmation of infection, the extract-based ointments were applied topically to the infected nails once daily according to the assigned treatment groups. The negative control group received sterile distilled water, while the positive control group was treated with 2% ketoconazole ointment.

Clinical evaluation of onychomycosis was conducted based on four parameters: periungual swelling, lesion diameter, nail discoloration, and nail fragility. To reduce subjectivity, operational definitions for each scoring parameter were established prior to observation. Periungual swelling was assessed visually using a standardized ordinal scale ranging from score 0 (no swelling) to score 4 (severe swelling characterized by marked erythema and thick nodular swelling). Lesion diameter was measured quantitatively using a vernier caliper with an accuracy of 0.01 mm, and a reduction in diameter was interpreted as an indicator of therapeutic effectiveness. Nail discoloration and nail fragility were evaluated using predefined visual and physical scoring criteria. All clinical assessments were conducted independently by two observers who were blinded to the treatment groups to minimize observer bias. Any discrepancies in scoring were resolved through discussion to reach a consensus.

Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test. As the data were not normally distributed, differences among treatment groups were analyzed using the Kruskal–Wallis test, followed by Dunn's post hoc test for pairwise comparisons. A p-value < 0.05 was considered statistically significant.

Results and Discussion

This section presents the experimental results obtained from phytochemical analysis, clinical observations, and statistical evaluation of the antifungal effectiveness of the combined extracts of *Uncaria acida* and *Ageratum conyzoides* against *Trichophyton rubrum*–induced onychomycosis in mice. The results are discussed comprehensively to interpret the biological significance of the findings and to address the research objectives by comparing them with previously reported studies.

Phytochemical profile of Uncaria acida and Ageratum conyzoides extracts

The qualitative and quantitative phytochemical profiles of Uncaria acida and Ageratum conyzoides extracts used in this experimental study are presented in Table 1.

Table 1. Results of Phytochemical Screening of *Uncaria acida* and *Ageratum conyzoides* Extracts [19-21]

		Identification of Phytochemical Compounds				
Sample	**Parameter**	**Flavonoids**	**Alkaloids**	**Tannins/Phenolics**	**Terpenoids**	**Saponins**
Uncaria acida	Qualitative	+	+	+	+	+
	Quantitative	28.5mg ± 1.42 QE/g	3.0–5.0 mg/g	15-25 mg TAE/g	2.0-4.0 mg/g	5.0-10.0 mg/g
Ageratum conyzoides	Qualitative	+	+	+	+	+
	Quantitative	36.63 ± 1.87 mg QE/g	5.0–8.0 mg/g	52.4 ± 2.96 mg TAE/g	3.0–6.0 mg/g	8.0–12.0 mg/g

Notes:
(+) = detected
(-) = not detected

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The qualitative and quantitative phytochemical profiles of Uncaria acida and Ageratum conyzoides extracts used in this experimental study are presented in Table 1. Both extracts qualitatively tested positive for flavonoids, alkaloids, tannins/phenolics, terpenoids, and saponins. Quantitative analysis revealed that U. acida contained flavonoids at a concentration of 28.5 mg QE/g, whereas A. conyzoides showed a higher flavonoid content of 36.63 mg QE/g. In addition, A. conyzoides exhibited a markedly higher tannin/phenolic content (52.4 mg TAE/g) compared to U. acida. The concentrations of alkaloids, terpenoids, and saponins in both extracts were consistent with the phytochemical characteristics typically reported for plants belonging to the Rubiaceae and Asteraceae families.

The presence of these secondary metabolites aligns with previous phytochemical studies, which reported that flavonoids, phenolics, alkaloids, terpenoids, and saponins contribute significantly to various biological activities, including antifungal, antioxidant, and anti-inflammatory effects [22,23]. Flavonoids are well-documented polyphenolic compounds capable of inhibiting fungal growth through multiple molecular mechanisms, such as disruption of fungal cell membranes, inhibition of hyphal formation, and induction of cytoplasmic leakage. Tannins and phenolic compounds function as strong antioxidants and are also known to precipitate fungal membrane proteins, thereby inhibiting fungal invasion and colonization of keratinized tissues, which are the primary targets of dermatophytes such as *T. rubrum* [24].

In addition, alkaloids have been reported to interfere with ergosterol biosynthesis, a critical component of fungal cell membranes, while terpenoids exert membrane-disruptive effects due to their lipophilic properties. Saponins enhance membrane permeability by forming complexes with membrane sterols. The coexistence of these compounds suggests the potential for complementary or synergistic antifungal activity when the extracts are combined [25,26]. These phytochemical characteristics provide a molecular basis for interpreting the clinical outcomes observed in the animal model.

These findings are consistent with previous reports demonstrating that U. acida exhibits antibacterial and antifungal activities mediated by its flavonoid and terpenoid content [27]. Similarly, A. conyzoides has been reported to possess antifungal activity against Candida albicans and Microsporum gypseum, attributed to its high phenolic, flavonoid, and saponin contents [28]. Collectively, the phytochemical profiles presented in Table 1 provide a molecular basis for interpreting the clinical outcomes observed in the onychomycosis model presented in Table 2.

Table 2. Recapitulation of the Mean Clinical Parameters of Onychomycosis-Induced Mice Following Treatment with Bajakah (*Uncaria acida*) and Bandotan (*Ageratum conyzoides*) Extracts

Treatment	Swelling	Lesion Diameter	Nail Color	Nail Brittleness
P1	2,66	1,78	3,38	3,16
P2	2,5	1,07	1,69	1,38
P3	3	1,34	2,1	1,57
P4	2,91	1,34	2	1,72
P5	2,94	1,12	1,63	1,44
P6	2,59	1,06	1,72	1,41

Notes:
The assessment of periungual swelling, nail color changes, and nail brittleness was performed using a scoring scale of 1–4, with higher scores indicating greater severity.
P1 = negative control (aquadest)
P2 = positive control (*ketoconazole* 2%)
P3 = 25% treatment of combined Bajakah root and Bandotan leaf extracts (1:1)
P4 = 50% treatment of combined Bajakah root and Bandotan leaf extracts (1:1)
P5 = 75% treatment of combined Bajakah root and Bandotan leaf extracts (1:1)
P6 = 100% treatment of combined Bajakah root and Bandotan leaf extracts (1:1)

Clinical response of onychomycosis after extract treatment

The mean clinical scores for periungual swelling, lesion diameter, nail discoloration, and nail brittleness following treatment are summarized in Table 2. The negative control group (P1) exhibited the highest scores across all parameters, indicating progressive fungal infection in the absence of antifungal intervention. Conversely, the positive control group treated with ketoconazole 2% (P2) demonstrated the lowest scores, confirming its efficacy as a standard antifungal agent.

Mice treated with combined *U. acida* and *A. conyzoides* extracts (P3–P6) showed varying degrees of clinical improvement. Notably, higher extract concentrations (75% and 100%) resulted in more pronounced reductions in lesion diameter, nail discoloration, and nail brittleness, approaching the therapeutic effect

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observed in the positive control group. This concentration-dependent trend suggests that increased levels of bioactive metabolites enhance antifungal efficacy, supporting previous findings that phytochemical-rich extracts exhibit dose-responsive antifungal activity [29].

The observed reductions in periungual swelling and lesion diameter indicate effective suppression of fungal growth and associated inflammatory responses. These findings are consistent with prior reports describing the antifungal potential of *Uncaria* and *Ageratum* species against dermatophytes and other pathogenic fungi, mediated by their diverse secondary metabolites.

Macroscopic observation of nail condition

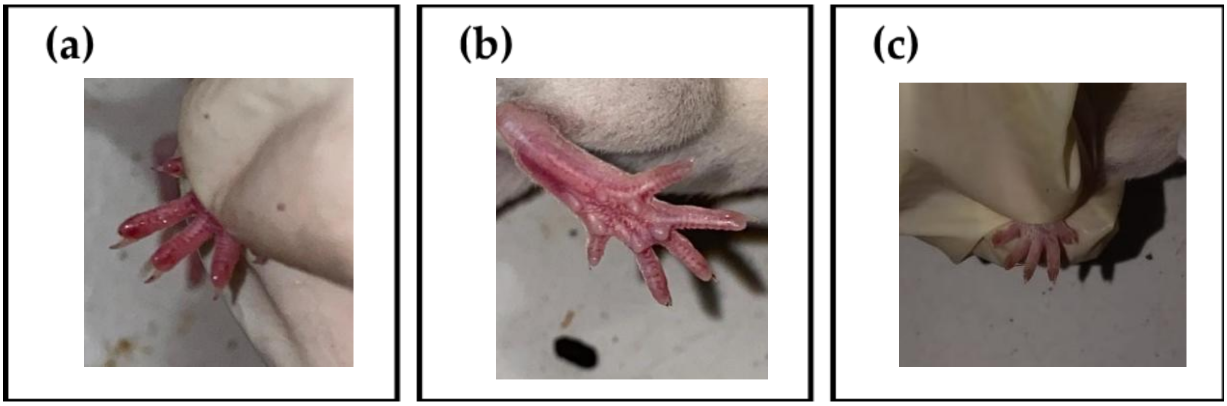

Figure 1. Representative macroscopic images of onychomycosis in mice infected with *Trichophyton rubrum*. (a) P6 group (100%) before treatment (day 1), showing typical signs of onychomycosis. (b) P6 group after 7 days of treatment, showing marked clinical improvement. (c) P3 group (25%) after 14 days of treatment, showing partial improvement with residual infection.

Representative macroscopic images illustrating the progression of onychomycosis and the treatment response are presented in Figure 1. As shown in Figure 1a (day 1), mice in the P6 group (100% extract concentration) exhibited typical clinical features of *Trichophyton rubrum* induced onychomycosis, including pronounced periungual swelling, erythema, nail discoloration, and surface roughness, indicating successful establishment of fungal infection.

In contrast, Figure 1b demonstrates the nail condition of the same group (P6) after 7 days of topical treatment, showing marked macroscopic improvement characterized by a clear reduction in swelling and erythema, as well as improvement in nail color and surface texture. Meanwhile, Figure 1c shows the nail condition of the P3 group (25% extract concentration) after 14 days of treatment, where only partial clinical recovery was observed and residual signs of infection remained, suggesting limited antifungal efficacy at lower extract concentration.

Overall, these representative images provide visual confirmation of the clinical scoring data, indicating that higher concentrations of the combined extracts of *Uncaria acida* and *Ageratum conyzoides* were more effective in suppressing fungal progression and restoring nail integrity in the onychomycosis mouse model.

Interpretation, limitations, and future perspectives

Onychomycosis is a chronic dermatophyte infection that is often difficult to treat due to prolonged therapy requirements and high recurrence rates. In the present study, topical application of a combined extract of Bajakah root (*U. acida*) and Bandotan leaf (*A. conyzoides*) produced significant clinical improvement in a murine onychomycosis model, particularly at higher extract concentrations. The therapeutic effects observed are likely associated with the complementary actions of flavonoids, phenolics, alkaloids, terpenoids, and saponins present in both extracts.

However, it is important to acknowledge that the proposed antifungal mechanisms in this study are inferred from phytochemical composition and clinical outcomes rather than supported by direct mechanistic evidence. This study did not include in vitro antifungal assays such as minimum inhibitory concentration (MIC) or minimum fungicidal concentration (MFC) determination, nor histopathological examination of nail tissue to directly confirm fungal clearance and tissue recovery.

Therefore, further studies are warranted to validate the proposed synergistic mechanisms through in vitro antifungal and checkerboard assays, as well as histological analyses of infected nail tissue. Such

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investigations would strengthen the evidence base and support the development of this herbal combination as a potential natural antifungal agent for the management of onychomycosis.

Statistical analysis of clinical parameters

Normality testing using Kolmogorov–Smirnov and Shapiro–Wilk tests revealed that all clinical parameters had p-values < 0.05, indicating non-normal data distribution. Therefore, nonparametric Kruskal-Wallis analysis was applied. The results of the Kruskal–Wallis test are presented in Table 3.

Table 3. Kruskal–Wallis Test Results for Each Onychomycosis Parameter

Parameter	H (Kruskal–Wallis)	df	p-value
Swelling	86.802	5	.000
Lesion Diameter	56.608	5	.000
Nail Brittleness	68.398	5	.000
Nail Color	65.152	5	.000

As shown in Table 3, statistically significant differences were observed among treatment groups for all evaluated clinical parameters ($p < 0.001$). The high Kruskal–Wallis H values for periungual swelling (86.802), lesion diameter (56.608), nail discoloration (65.152), and nail fragility (68.398) indicate that the treatments produced heterogeneous clinical responses in mice infected with *Trichophyton rubrum*. These findings confirm that the application of the combined extracts resulted in measurable and significant effects on the severity of onychomycosis[30]. To identify specific pairwise differences between treatment groups, a Dunn's post hoc test with Bonferroni correction was performed. The results of the significant pairwise comparisons are presented in Table 4.

Table 4. Dunn's Post Hoc Test Results for Pairwise Comparisons

Test Comparison	Adjusted p-value	Significance
P2 vs P1	< 0.001	Significant
P4 vs P1	0.017	Significant
P5 vs P1	< 0.001	Significant
P6 vs P1	< 0.001	Significant
P2 vs P3	0.002	Significant
P6 vs P3	0.003	Significant

The post hoc analysis demonstrated that all extract-treated groups at concentrations of 50%, 75%, and 100% differed significantly from the negative control (P1), indicating a clear antifungal effect of the combined extracts. In contrast, the 25% extract group (P3) showed limited efficacy and remained significantly different from the positive control (P2), suggesting insufficient antifungal activity at this concentration.

Notably, no statistically significant differences were observed between the higher extract concentrations (50–100%) and the positive control (2% ketoconazole), indicating that these formulations exhibited antifungal efficacy comparable to the standard antifungal treatment. Pairwise comparisons that did not reach statistical significance after Bonferroni adjustment are not shown in the table for clarity.

Integrative interpretation of antifungal efficacy

The integration of statistical outcomes with clinical observations and phytochemical analysis indicates that the combined extracts of *Uncaria acida* and *Ageratum conyzoides* exert strong antifungal effects against *T. rubrum*-induced onychomycosis in mice. The significant reductions in clinical severity at higher extract concentrations are consistent with the presence of flavonoids, tannins, and other polyphenolic compounds known to disrupt fungal cell walls and inhibit dermatophyte growth [31,32]. Importantly, the equivalence in therapeutic performance between the 50–100% extract formulations and ketoconazole highlights the potential of this plant-based combination as a natural alternative antifungal agent. These findings support further investigation into the formulation, safety, and clinical applicability of *U. acida* and *A. conyzoides* extracts for the treatment of onychomycosis [33].

Conclusions

This study demonstrates that the combined extracts of *Uncaria acida* and *Ageratum conyzoides* exhibit significant antifungal activity against *Trichophyton rubrum*–induced onychomycosis in a murine model. Phytochemical analysis confirmed the presence of key secondary metabolites, including flavonoids, alkaloids, tannins/phenolics, terpenoids, and saponins, which collectively provide a plausible biochemical basis for the observed antifungal effects. Clinically, topical application of the combined extracts resulted in significant reductions in periungual swelling, lesion diameter, nail discoloration, and nail brittleness compared to the negative control, with the most pronounced therapeutic responses observed at higher concentrations (75–100%). Statistical analysis confirmed significant differences across all evaluated clinical parameters, supporting the biological efficacy of the extract combination. These findings indicate that the synergistic interaction of Bajakah and Bandotan extracts has the potential to reduce the severity of onychomycosis and represents a promising candidate for the development of natural antifungal therapy.

Nevertheless, further investigations are required to strengthen translational relevance and safety. Future studies should include (i) acute and sub-acute topical toxicity assessments to ensure dermal safety, (ii) optimization of extract concentration and combination ratio to determine the most effective formulation, (iii) evaluation of antifungal efficacy in animal models with nail structures more comparable to humans, such as guinea pigs, and (iv) development of more applicable pharmaceutical formulations, including creams or film-forming solutions, to enhance clinical usability. Addressing these aspects will be essential for advancing this herbal combination toward preclinical and clinical application in onychomycosis management.

Conflict of Interest

The authors declare that there is no conflict of interest associated with this research. The results and interpretations presented in this manuscript were conducted and reported objectively without any influence from personal or external interests.

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