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Development and *In Vitro* Evaluation of an Antifungal Ointment Formulated from *Erigeron floribundus* Leaf Extracts Against *Trichophyton rubrum*

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Abstract

Background: Dermatophytoses are superficial fungal infections affecting keratinized tissues and remain a significant public health concern, particularly in tropical regions. The emergence of antifungal resistance and limited access to conventional drugs underscore the need for cost-effective phytotherapeutic alternatives.

Objective: This study aimed to develop and evaluate the *in vitro* antifungal activity of a topical ointment formulated from ethanolic leaf extracts of *Erigeron floribundus* against *Trichophyton rubrum*.

Methods: Aqueous and ethanolic extracts were prepared by maceration (powder:solvent ratio 1:5 w/v) for 72 hours. Phytochemical screening included qualitative tests and quantitative determination of total polyphenols (Folin–Ciocalteu method) and flavonoids (aluminum chloride assay). Antifungal activity was evaluated by agar diffusion and broth microdilution methods (MIC and MFC, n = 3). Ointments containing 2% and 5% (w/w) ethanolic extract were prepared in a hydrophobic base (vaseline, lanolin, paraffin oil, glycerol, lecithin, vitamin E) and assessed for organoleptic properties, homogeneity, stability, and *in vitro* antifungal activity.

Results: Extraction yields were 4.43% (aqueous) and 4.89% (ethanolic). Phytochemical analysis revealed polyphenols, flavonoids, saponins, and glycosides in both extracts; alkaloids were present only in the ethanolic extract. The ethanolic extract showed superior antifungal activity (MIC = 1.87 ± 0.12 mg/mL; MFC = 6.25 ± 0.25 mg/mL) compared to the aqueous extract (MIC = 3.75 ± 0.18 mg/mL; MFC = 12.5 ± 0.35 mg/mL). The MFC/MIC ratio (≤4) indicated fungicidal activity. The 5% ointment produced an inhibition zone of 18 ± 0.5 mm against *T. rubrum*.

Conclusion: The ethanolic extract of *E. floribundus* demonstrated significant in vitro antifungal activity, retained efficacy in a topical ointment, and represents a promising candidate for phytotherapeutic management of dermatophytoses. Further *in vivo* studies and clinical trials are warranted .

Keywords: Dermatophytosis; Herbal medicine; Antifungal activity; Polyphenols; Ointment formulation; Cameroon

1. Introduction

Dermatophytoses are superficial fungal infections predominantly caused by species of *Trichophyton*, *Microsporum*, and *Epidermophyton*, with *Trichophyton rubrum* being the most prevalent worldwide [1]. Conventional antifungal agents, including azoles and allylamines, are effective but limited by increasing resistance, adverse effects, and high costs, especially in low-resource settings [1,2].

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Medicinal plants are an important source of antifungal compounds; over 80% of populations in developing countries rely on traditional remedies for primary healthcare [3,5,6]. *Erigeron floribundus* (Asteraceae) is traditionally used in African ethnomedicine for anti-inflammatory, antimicrobial, and wound-healing properties [4,7]. Few studies have evaluated its activity against dermatophytes, and no standardized topical formulation has been reported [4].

This study aimed to develop a topical ointment containing *E. floribundus* leaf extract and evaluate its *in vitro* antifungal activity against *T. rubrum* [8].

Figure 1 *Erigeron floribundus* plants [9]

2. Materials and Methods

2.1. Plant Material and Extraction

Fresh leaves of *E. floribundus* were collected in Yaounde, Cameroon, authenticated, washed, air-dried (25–30°C), and ground to a fine powder. 280 g of powder were macerated in distilled water and 95% ethanol (1:5 w/v) for 72 hours with intermittent shaking [6]. Filtrates were concentrated under reduced pressure at 70°C. Extraction yields were calculated:

$$\text{Yield (\%)} = \{\text{Weight of dry extract / Weight of plant powder}\} \times 100$$

2.2. Phytochemical Screening

- Qualitative tests included: Ferric chloride (phenolics), Dragendorff's reagent (alkaloids), frothing test (saponins), Keller–Kiliani test (glycosides) [7].
- Quantitative determination:
  - Total polyphenols: Folin–Ciocalteu assay, expressed as mg gallic acid equivalents/g dry matter (mg GAE/g)
  - Total flavonoids: Aluminum chloride colorimetric assay, expressed as mg quercetin equivalents/g dry matter (mg QE/g) [5,11]

2.3. Fungal Strain and Inoculum Preparation

T. rubrum was obtained from a clinical isolate and identified macroscopically and microscopically. Conidial suspensions were standardized to 0.5 McFarland (~1×10^6 spores/mL) [8].

2.4. Antifungal Activity

- Agar diffusion: Kirby–Bauer disk diffusion on Sabouraud chloramphenicol agar; zones measured after 48 h at 25–28°C (n = 3).
- Broth microdilution: MIC and MFC determined by serial two-fold dilutions in Sabouraud broth according to CLSI M38-A2 standards [9]. Ketoconazole served as reference. MFC/MIC ratio determined fungicidal (≤4) or fungistatic (>4) activity.

2.5. Ointment Formulation

Ointments containing 2% and 5% (w/w) ethanolic extract were prepared in a hydrophobic base: vaseline 50%, lanolin 10%, paraffin oil 10%, glycerol 5%, lecithin 5%, vitamin E 0.5%, water q.s. to 100%. Formulations were evaluated for organoleptic properties, homogeneity, consistency, and phase stability [8].

2.6. Statistical Analysis

All experiments were performed in triplicate. Data are presented as mean ± SD. Differences between groups were analyzed using one-way ANOVA followed by Tukey's post hoc test; $p < 0.05$ was considered significant.

3. Results

3.1. Extraction Yield

- Aqueous extract: 4.43%
- Ethanolic extract: 4.89%

3.2. Phytochemical Composition

Both extracts contained polyphenols, flavonoids, saponins, and glycosides; alkaloids were present only in ethanolic extract. The highest concentrations of polyphenols and flavonoids were obtained with the ethanolic extract (table 1).

Table 1 Polyphenols and Flavonoids dosage

Extract	Total Polyphenols (mg GAE/g)	Total Flavonoids (mg QE/g)
Aqueous	312.5 ± 5.2	1150 ± 18
Ethanolic	522.8 ± 7.1	2080 ± 22

3.3. Antifungal Activity

Table 2 Antifungal activity

Extract	MIC (mg/mL)	MFC (mg/mL)	MFC/MIC
Aqueous	3.75 ± 0.18	12.5 ± 0.35	≤4
Ethanolic	1.87 ± 0.12	6.25 ± 0.25	≤4
Ketoconazole	0.0078 ± 0.0003	0.015 ± 0.001	2

The ethanolic extract exhibited superior antifungal activity ($p < 0.05$). Both extracts were fungicidal (MFC/MIC ≤4) (table 2).

3.4. Ointment Evaluation

Figure 2 Ointment formulated

Both 2% and 5% ointments were homogeneous, monophasic, and physically stable (figure 2). The 5% ointment exhibited an inhibition zone of 18 ± 0.5 mm against *T. rubrum*, comparable to griseofulvin control (20 ± 0.4 mm).

4. Discussion

The superior activity of the ethanolic extract is likely due to higher polyphenol and flavonoid content, which can disrupt fungal membranes and inhibit critical enzymatic pathways [5,11,12].

Although less potent than ketoconazole, the extract demonstrated fungicidal activity, supporting its potential as a natural antifungal agent [1,5,12]. Incorporation into an ointment base did not significantly reduce activity, indicating stability of active constituents [8].

Compared to other herbal antifungal formulations, the MICs obtained are within a therapeutically relevant range [11]. Limitations include *the in vitro* design, absence of dermal penetration data, and lack of toxicity assessment, which should be addressed in future studies.

5. Conclusion

The ethanolic extract of *Erigeron floribundus* possesses significant in vitro antifungal activity against *Trichophyton rubrum* and retains efficacy in a topical ointment formulation. Further *in vivo* studies, dermal irritation testing, stability assessments, and clinical trials are warranted to confirm its therapeutic potential.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

The authors alone are responsible for the content and writing of this manuscript.

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