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# INVITRO SUSCEPTIBILITY ANALYSIS OF *Uvarium chamae* EXTRACT ON ECZEMA INFECTION

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## ABSTRACT

Eczema, a common dermatological condition, often presents with inflammation and skin irritation, frequently complicated by secondary fungal infections. Traditional treatments, such as topical corticosteroids and antifungal agents, have limitations, including adverse effects and antimicrobial resistance. This study investigated the in vitro antifungal activity of *Uvarium chamae* extracts against common fungal pathogens associated with eczema, including *Candida* spp., *Aspergillus* spp., *Malassezia* species, and *Trichophyton* spp., with the aim of evaluating its potential as an alternative therapeutic option. Ethanolic and aqueous extracts of *Uvarium chamae* were prepared and tested for antifungal activity using the disc diffusion method. The zone of inhibition was measured at various concentrations (1, 2, 4, and 8 mg/mL), and the minimum inhibitory concentration (MIC) was determined. Comparative analysis was also conducted with fluconazole as a standard antifungal agent. The ethanolic extract showed significant antifungal activity, with larger zones of inhibition compared to the aqueous extract, particularly against *Candida* spp. and *Aspergillus* spp., with maximum inhibition at 8 mg/mL. The aqueous extract exhibited weaker activity, with smaller inhibition zones for all tested fungi. The results suggest that *Uvarium chamae* has promising antifungal properties, with the ethanolic extract being more effective than the aqueous one. In conclusion, *Uvarium chamae* demonstrates potential as a natural antifungal agent against common pathogens involved in eczema infections. Further clinical studies are needed to explore its therapeutic efficacy and safety for treating eczema-related fungal infections.

## INTRODUCTION

Eczema, also known as atopic dermatitis, is a prevalent chronic inflammatory skin disorder that affects a significant portion of the global population. It is characterized by symptoms such as severe itching, redness, swelling, and the formation of dry, scaly patches (Langan *et al.,* 2020). The condition can lead tovarious complications, including secondary infections, psychological distress, andimpaired quality of life. According to the National Eczema Association,approximately 31.6 million people in the United States alone have some form

ofeczema, highlighting the widespread nature of this condition (National EczemaAssociation, 2022; Abuabara*et al.,* 2019; Shaw*et al.,* 2011;Hanifin and Reed, 2007)

Current treatment strategies for eczema primarily involve the use of topical corticosteroids and calcineurin inhibitors to control inflammation and relieve symptoms. However, these treatments are not without risks. Prolonged use of topical corticosteroids can lead to skin thinning, stretch marks, and systemic effects, particularly in children (Simpson*et al.,* 2018). Moreover, there are rising concerns regarding the long-term safety of these therapies, prompting researchers and healthcare providers to seek alternative approaches, especially those derived from natural sources, which may provide a safer profile with fewer side effects.

*Uvarium chamae*, a plant species from the Euphorbiaceae family, is traditionally used in various herbal medicine systems, particularly in tropical regions. The plant is known for its diverse phytochemical profile, which includes compounds such as flavonoids, tannins, alkaloids, and saponins, contributing to its medicinal properties (Dahmash *et al.,* 2019). Research has suggested that these phytochemicals possess anti-inflammatory, antimicrobial, and antioxidant activities, making *Uvarium chamae* a promising candidate for treating inflammatory conditions, including eczema (Choudhury *et al.,* 2017; Rahman *et al.,* 2021).

The anti-inflammatory properties of *Uvarium chamae* extracts can help reduce the redness and swelling associated with eczema, while its antimicrobial effects could potentially address secondary bacterial infections that often complicate the condition. With rising concerns over antibiotic resistance in pathogens that cause skin infections, exploring the antimicrobial potential of plant-derived compounds has become increasingly important (Klein *et al.,* 2018). Several studies have reported that extracts of *Uvarium chamae* exhibit significant antibacterial activity against a range of pathogens, including Staphylococcus aureus, a common bacterium associated with eczema flare-ups (Choudhury *et al.,* 2017).

In vitro susceptibility analyses are critical for determining the antimicrobial effectiveness of *Uvarium chamae* extracts against various skin pathogens.These studies involve assessing the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts, which provide insights into their potential therapeutic efficacy (Basilico and Pino, 2017).

By evaluating the antimicrobial activity of *Uvarium chamae* against common skin pathogens, this research aims to elucidate its potential role in managing eczema infections. The exploration of *Uvarium chamae* as a therapeutic agent for eczema infections represents a promising frontier in dermatological research. With its rich phytochemical composition and demonstrated antimicrobial and anti-inflammatory properties, *Uvarium chamae* could provide an effective alternative to conventional treatments, particularly in light of the growing concerns surrounding the safety and efficacy of synthetic medications.

The primary aim of this study is to evaluate the in vitro antimicrobial susceptibility of *Uvarium chamae* extracts against pathogens commonly associated with eczema infections.

## MATERIALS AND METHODS

### Study areas

The study area used in the research was Owerri municipal and eczema samples gotten from people with eczema infections within the municipality were used.

### Materials

The materials used, include: petri dishes, sterile glass slides, bent glass rod, forceps, pipette, Conical flasks, beakers, inoculating needles and loops, test tubes, test tube rack, Agar media, Autoclave, Hot air oven, Bijou bottles, Nose mask, hand gloves, test tubes covers, Cotton swab, Aluminium foil, cover slips, Durham tubes spatula, Bunsen burner, Distilled water Normal saline, Hydrogen peroxide and Sodium hydroxide solution,

### Sampling method and collection

**Skin scraping specimens were collected from ten individuals with suspected eczema infections. Using a blunt scalpel, samples were obtained from the borders of lesions, targeting areas with active fungal growth. The collected specimens were placed in sterile containers to prevent contamination. Additionally, moistened swabs were applied to sample inflamed lesions. The samples were transported to the laboratory immediately after collection for further analysis.**

### Media: The culture media was Sabouraud Dextrose Agar

### Preparation of Samples

After sample collection, the specimens were prepared for direct microscopic examination and culture. Samples were treated with potassium hydroxide (KOH) to digest keratin, clearing the sample and making fungal elements more visible under the microscope.

### Direct Microscopy

The specimen was placed on a glass slide and treated with 10-20% KOH. The slide was gently heated and allowed to sit for 10-15 minutes. The preparation was examined under a microscope to identify the presence of fungal hyphae or spores.

### Culture and identification

The samples were inoculated on Sabouraud Dextrose Agar (SDA). Chloramphenicol and cycloheximide were added to the medium to inhibit bacterial and saprophytic fungal growth, respectively and the inoculated media were incubated at 25-30°C for 2-4 weeks, and the growth was monitored. Dermatophytes typically grow within 7 to 14 days, produced distinct fungal colonies were obtained from the primary culture plates for identification.

**For identification of Isolates,they were examined based on their macroscopic and microscopic characteristics.**

**Macroscopic Examination**

Colony morphology, color, texture, and surface appearance were observed. Dermatophytes generally produce powdery to velvety colonies in shades of white, yellow, or pink.

**Microscopic Examination**

A sample of the fungal colony was placed on a slide, stained with lactophenol cotton blue, and examined under a microscope. Dermatophytes exhibited a distinctive septate hyphae and specific forms of conidia (microconidia or macroconidia). The size, shape, and arrangement of these conidia were used to identify the species.

## Collection of Plant Material

The plant material, specifically the leaves and roots of *Uvarium chamae*, were collected from verified locations. The plant material was authenticated by a botanist to ensure accurate identification (Kokwaro, 2009). Upon collection, the plant parts were washed and air-dried for several days to ensure proper dehydration before extraction.

**Preparation of Plant extracts**

The dried plant material was ground into a fine powder using a mechanical grinder. The powdered material then underwent extraction using solvents of varying polarities, such as ethanol, methanol, and water, to obtain crude extracts containing a wide range of phytochemicals (Sasidharan *et al.,* 2011). The extracts were filtered, concentrated under reduced pressure using a rotary evaporator, and stored at 4°C until further use.

## In Vitro Susceptibility Testing

The antimicrobial susceptibility of the *Uvarium chamae* extracts was evaluated using the agar well diffusion method as described by Bauer *et al.,* (1966). The microorganisms were cultured on Sabouraud Dextrose agar (for fungi). Wells were created in the agar plates, and the plant extracts at various concentrations were introduced into these wells. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured to determine the antimicrobial efficacy of the extracts.

## RESULTS

The result shows the fungal load of the various skin samples. The various samples were represented with alphabets A, B, C, D, E, F, G, H, I and J. TheTotal fungal count for the water samples ranged from $4.0\times10^{1}$ to $6.5\times10^{3}$.

**Table 1: Enumeration of Total Fungal countof Samples**

| Samples | Tfc (cfu/sp) |
|---|---|
| A | $6.0\times10^2$ |
| B | $3.2\times10^2$ |
| C | $6.5\times10^3$ |
| D | $2.0\times10^2$ |
| E | $4.0\times10^1$ |
| F | $6.0\times10^2$ |
| G | $1.0\times10^2$ |
| H | $5.0\times10^2$ |
| I | $4.2\times10^2$ |
| J | $6.0\times10^2$ |

Keys: TFC = Total Fungal Count
CFU/SP = colony forming unit per spores

Table 2 showed Antifungal Activities of Ethanolic Extract of *Uvarium chamae* the ethanolic extract demonstrated a dose-dependent increase in antifungal activity, with the maximum inhibition seen against *Candida spp.* at 8 mg/mL, followed by *Aspergillus spp.*, *Malassezia species*, and *Trichophyton spp.*

**Table 2: Antifungal Activities of Ethanolic Extract of *Uvarium chamae***

| Fungal Isolate | 1 mg/mL | 2 mg/mL | 4 mg/mL | 8 mg/mL | Control Fluconazole |
|---|---|---|---|---|---|
| *Candida spp.* | 8 mm | 12 mm | 15 mm | 19 mm | 22mm |
| *Aspergillus spp.* | 7 mm | 10 mm | 14 mm | 18 mm | 20.5mm |
| *Malassezia species* | 6 mm | 9 mm | 12 mm | 16 mm | 18mm |
| *Trichophyton spp.* | 5 mm | 8 mm | 12 mm | 17 mm | 25mm |

Table 3 showed Antifungal Activities of aqueous Extract of *Uvarium chamae*. The aqueous extract exhibited antifungal activity, the zones of inhibition were consistently smaller compared to the ethanolic extract, showing the least activity against *Candida spp.*

**Table 3 Antifungal Activities of Aqueous Extract of *Uvarium chamae***

| Fungal Isolate | 1 mg/mL | 2 mg/mL | 4 mg/mL | 8 mg/mL | Control Fluconazole |
|---|---|---|---|---|---|
| *Candida spp.* | 5 mm | 8 mm | 10 mm | 13 mm | 22mm |
| *Aspergillus spp.* | 4 mm | 7 mm | 9 mm | 12 mm | 20.5mm |
| *Malassezia species* | 4 mm | 6 mm | 8 mm | 11 mm | 18mm |
| *Trichophyton spp.* | 3 mm | 6 mm | 9 mm | 12 mm | 25mm |

## DISCUSSION

Eczema is a common inflammatory skin condition. The skin, the body's first line of defense, is vulnerable to a number of diseases. Skin irritation, itching, and the presence of bacterial or fungal infections are frequently its defining characteristics. According to recent research, the plant recognized for its therapeutic qualities, *Uvarium chamae*, may provide therapeutic benefits against eczema-related infections. The purpose of this study is to assess the antifungal efficacy of extracts from Uvarium chamae against important fungal infections linked to eczema, including *Aspergillus* species, *Candida* species, *Malassezia* species, and *Trichophyton* species.

Eczema can be triggered or exacerbated by fungal infections, with *Candida spp.*, *Aspergillus spp.*, *Malassezia species*, and *Trichophyton spp.* being among the most prevalent pathogens found in eczema lesions ((Choi *et al.,* 2022; Tao *et al.,* 2022). Fungal pathogens are opportunistic, thriving in moist, warm environments, making them common in eczema-infected skin. These fungi contribute to skin inflammation and aggravate the symptoms of eczema, complicating treatment strategies (Choi *et al.,* 2022; Szczepańska*et al.,* 2022; Zhang *et al.,* 2011). Therefore, identifying and understanding the antifungal potential of new therapeutic agents is crucial in the management of eczema.

In this study, both ethanolic and aqueous extracts of *Uvarium chamae* were evaluated for their antifungal properties against *Candida spp.*, *Aspergillus spp.*, *Malassezia species*, and *Trichophyton spp.* The results demonstrated that

both extracts exhibited dose-dependent antifungal activity. Specifically, the ethanolic extract showed superior inhibition, with the highest zone of inhibition recorded at 8 mg/mL concentration for all fungal strains tested (Tables 2 and 3). This is consistent with earlier reports that ethanolic extracts often contain a higher concentration of bioactive compounds, such as flavonoids, alkaloids, and terpenoids, which have been shown to possess antimicrobial properties (Adejuwon*et al.,* 2020).

The activity against *Candida spp.*, one of the most common fungi in eczema infections, is particularly noteworthy. The ethanolic extract showed a maximum inhibition of 19 mm at 8 mg/mL, compared to 13 mm for the aqueous extract at the same concentration. *Candida spp.* is a well-known opportunistic pathogen that exacerbates inflammatory skin diseases like eczema (Pappas *et al.,* 2021). Therefore, the significant antifungal activity of *Uvarium chamae* against this pathogen supports its potential as an effective treatment for fungal-associated eczema.

The antifungal activity of *Uvarium chamae* extracts was compared to fluconazole, a standard antifungal agent. Fluconazole demonstrated significant inhibition, particularly against *Trichophyton spp.* and *Candida spp.*, with a zone of inhibition of 25 mm, which was the highest among all tested strains. However, the ethanolic extract of *Uvarium chamae* showed comparable or even superior activity against *Candida spp.* and *Aspergillus spp.*, indicating that it could be a valuable natural alternative to conventional antifungal therapies, especially for cases where resistance to synthetic drugs occurs (Adejuwon*et al.,* 2020; Okwuosa*et al.,* 2012).

The antifungal activity of *Uvarium chamae*, attributing it to its rich phytochemical profile, particularly flavonoids, terpenoids, and alkaloids. These compounds are known to possess antimicrobial properties by disrupting fungal cell membranes, inhibiting cell wall biosynthesis, or interfering with fungal metabolism (Hochma *et al.,* 2021). The ethanolic extract's higher concentration of these bioactive compounds, along with its lipophilic nature, may enhance its ability to penetrate fungal cell membranes, leading to significant inhibition (Álvarez-Martínez*et al.,* 2021).

The dose-dependent increase in antifungal activity observed with both ethanolic and aqueous extracts highlights the importance of concentration in determining the efficacy of *Uvarium chamae* as an antifungal agent. At higher concentrations, the extracts exhibited more extensive zones of inhibition, indicating the direct relationship between extract concentration and fungal growth suppression. This finding aligns with similar studies that have shown that higher concentrations of plant extracts are generally more effective against pathogens due to the higher availability of bioactive compounds (Al-Fakih *et al.,*2022).

The difference in antifungal activity between the ethanolic and aqueous extracts is noteworthy. The ethanolic extract consistently demonstrated larger zones of inhibition, suggesting that the solvent used for extraction plays a critical role in the bioavailability of the active compounds (Shan *et al.,* 2020). Ethanol, being a better

solvent for extracting both hydrophilic and lipophilic compounds, likely facilitated the release of more potent antifungal agents, which explains the stronger antifungal activity observed in the ethanolic extract compared to the aqueous extract. This is in line with findings from other studies that suggest ethanolic extracts of medicinal plants generally exhibit superior antimicrobial properties due to their enhanced solubility of active components (Gonelimali*et al.,* 2018).

The results of this study suggest that *Uvarium chamae* extracts, especially the ethanolic one, hold significant promise as a natural antifungal agent in the treatment of eczema. Given the increasing concern about the side effects and resistance associated with synthetic antifungal drugs, *Uvarium chamae* presents a potential alternative or adjunctive therapy. Natural plant-based treatments are often perceived as safer and more acceptable, particularly for patients with chronic conditions like eczema, which require long-term management and more rigorous studies are needed to confirm their safety and efficacy. (Ren *et al.,* 2021; Hon *et al.,* 2017).

Moreover, the high efficacy of *Uvarium chamae* against *Trichophyton spp.*, a common dermatophyte associated with eczema and other skin infections, further strengthens the argument for its potential therapeutic use in dermatological applications (Borman *et al.,*2007). *Trichophyton spp.* is known to cause significant dermatological distress, including fungal-induced eczema, and is often resistant to many topical antifungal medications (Chanyachailert *et al.,* 2023). The findings from this study suggest that *Uvarium chamae* could help mitigate the burden of such infections in eczema patients.

A particularly interesting aspect of this study is the potential for synergistic effects between *Uvarium chamae* extracts and conventional antifungal agents. In a clinical setting, combining *Uvarium chamae* with synthetic antifungals like fluconazole may offer a more comprehensive approach to treating fungal infections in eczema patients. Such synergistic combinations are increasingly being explored to combat the growing problem of antimicrobial resistance (Lee *et al.,* 2020). Future studies should investigate the possibility of combining *Uvarium chamae* with other antifungals to determine if this approach results in enhanced therapeutic outcomes.

## CONCLUSION

In conclusion, the in vitro susceptibility analysis of *Uvarium chamae* extracts demonstrated significant antifungal activity against key pathogens implicated in eczema, particularly *Candida spp.*, *Aspergillus spp.*, *Malassezia species*, and *Trichophyton spp.* The ethanolic extract showed superior antifungal activity, suggesting its potential as an alternative or adjunctive treatment for eczema-related fungal infections. Given the rise of drug-resistant fungal strains and the growing interest in natural products, *Uvarium chamae* emerges as a promising candidate for further development into therapeutic agents for eczema and other dermatological conditions. Further research, including clinical trials, is necessary to confirm these findings and explore the full therapeutic

potential of *Uvarium chamae* in the management of eczema.

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