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Short Communication

# In Vitro Evaluation Of Anti-Fungal Activity Of Homoeopathic Medicine Hydrocotyle Asiatica: A Homoeopathic Perspective

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## I N F O

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## A B S T R A C T

*Background of the study: Hydrocotyle asiatica* is a homoeopathic remedy that effectively treats skin disorders with thickening, scale exfoliation, itching, and redness. Numerous studies conducted worldwide have confirmed the remedy's antifungal efficacy**.**

*Aim:* To evaluate the antifungal properties of the homoeopathic preparation of *Hydrocotyle asiatica* using an in vitro analysis.

*Materials and Methods-* In-Vitro Analysis: The disc diffusion method was employed to ascertain the antifungal activity. The zone of inhibition and mean inhibitory concentration were also calculated.

*Results: Hydrocotyle asiatica* showed significant antifungal activity against Trichophyton rubrum in the in vitro analysis.

*Conclusion: Hydrocotyle asiatica* is having significant antifungal activity, with Trichophyton rubrum showing maximum sensitivity to potencies θ, 3 C and 200 C.

**Keywords:** Homoeopathy; *Hydrocotyle asiatica*; In vitro study; Zone of Inhibition; Mean Inhibitory Concentration

## Introduction

Herbal medicines have been extensively employed as efficacious treatments for the prevention and management of various health issues throughout nearly all known cultures for centuries. In certain countries, the populations continue to rely on botanical medicines to meet their healthcare requirements.[1] *Hydrocotyle asiatica* (synonym *Centella asiatica*), often known as Indian pennywort, belongs to the Apiaceae family (formerly known as Umbelliferae). A useful therapeutic herb in both the Old and New Worlds, Hydrocotyle is a perennial creeper with a subtle aroma. It may be found all across the world's tropical and subtropical areas. *Centella asiatica* is commonly used in India to treat skin disorders, heal wounds, and revitalise nerves and brain cells. Numerous reports have asserted its potential for antioxidant, antimicrobial, cytotoxic, neuroprotective, and other activities. These activities are essentially closely linked to the characteristics and mode of action of the plant's bioactive constituents, which include flavonoids, other phenolic compounds, triterpenic acid, and triterpenic saponin.[2] *Hydrocotyle*


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*asiatica* is a homoeopathic remedy with marked action on skin conditions associated with thickening, exfoliation of scales, itching and redness. Numerous studies carried out globally have demonstrated the remedy's antifungal activity [3]; nevertheless, homoeopathy has not yet been examined in this regard. This study aimed to investigate the antifungal effects of the homoeopathic preparation of *Hydrocotyle asiatica* by means of an in vitro analysis.

## Materials And Methods

### Study Design

An in vitro study was conducted with *Hydrocotyle asiatica* in various potencies, one positive control, and one vehicle control.

### Study Settings

An in vitro study was conducted at the research centre of Sarada Krishna Homoeopathic Medical College, Kulasekharam.

### Study Duration

1-month duration for in vitro study.

### Methods

- After getting approval from the ethical committee, the study was conducted at the research centre of Sarada Krishna Homoeopathic Medical College, Kulasekharam.
- Microorganism Used: Trichophyton rubrum
- Standard strains of Trichophyton rubrum were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.
- Trichophyton rubrum cultures were prepared with the help of authorities from the institute.

### Culture Maintenance

- The stock culture was maintained on Sabouraud Dextrose Agar medium at 40°C. SDA is a selective medium for fungal culture, slightly acidic, and used primarily for the isolation of dermatophytes, yeasts, and other fungi.
- The strains were maintained and cultivated in petri dishes containing Sabouraud agar, incubating and subculturing for 15 days at 28°C and maintained as a mother culture.

### Method Of Antifungal Assay- Disc Diffusion Method

In vitro antifungal activity was determined by using Sabouraud Dextrose Agar, obtained from Hi Media Ltd, Mumbai. Inoculum suspensions were prepared in Sabouraud dextrose broth by inoculating a small scrap of culture aseptically taken from the mother culture and incubated for 15 days at 28°C in a shaking incubator.

Antifungal susceptibility is assessed by employing the disc diffusion method on SDA media. To the sterilised Mueller-Hinton agar media, 10 ml of 72-hour-grown Trichophyton rubrum culture was inoculated and mixed well for uniform distribution. The media was then plated in sterile petri dishes to form a fungal lawn. 200 microlitres of homoeopathic dilutions and controls were impregnated into a sterile plain disc and placed carefully on the surface of the Sabouraud Dextrose Agar plate. *(Figure1)*

### Control

- Negative control is a homoeopathic placebo.
- Positive control is Griseofulvin-EM143-10ST.

The plates were then incubated at 28°C and were examined daily for the presence of fungal colonies. The growth inhibition areas were established after 72-96 h.*(Figure 2)*

### Determination Of MIC- Turbidometric Method

The Minimal Inhibitory Concentration (MIC) was a crucial parameter in antimicrobial testing, representing the lowest concentration of an antimicrobial agent required to inhibit the visible growth of a microorganism. This parameter was vital for determining the efficacy of antimicrobial agents and for guiding appropriate therapeutic strategies. In this study, the MIC was determined using the turbidometric method, which measured the turbidity or cloudiness of a microbial culture to infer microbial growth indirectly. Specifically, the study evaluated the antifungal effects of different potencies of Hydrocotyle asiatica against the fungal strain Trichophyton rubrum, with griseofulvin serving as the control.

### Sample Preparation

The initial step in this study involved the preparation of samples, including various potencies of *Hydrocotyle asiatica* (mother tincture, 3X, 3C, 6C, 12C, 30C, 200C) Griseofulvin, a standard antifungal drug, was utilised as a positive control. Each of these samples was tested at four concentrations: 0.0 ml, 0.2 ml, 0.4 ml, and 0.6 ml.

### Inoculum Preparation

The MIC was measured using the macro-broth dilution method, a well-established technique for antimicrobial susceptibility testing.[4] This method involved preparing a series of 9 sterile test tubes for each sample concentration. A 72-hour culture of Trichophyton rubrum, exhibiting an absorbance of 1.60 at 625 nm, was used as the inoculum. One millilitre of this fungal culture was added to each test tube containing the respective concentrations of the antimicrobial agents and the control.

### Incubation and Measurement

The inoculated test tubes were incubated at 28°C for 24 hours, an optimal temperature for fungal growth. Post-incubation, the optical density (OD) values at 625 nm

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were measured using a UV/Vis spectrophotometer.[5] The OD measurement provided a quantitative assessment of the turbidity in each sample, reflecting the extent of microbial growth. Turbidity, which resulted from microbial proliferation, indicated the presence and concentration of fungal cells; higher turbidity correlated with greater microbial growth.

## Determination of MIC

The MIC was determined as the lowest concentration of the antimicrobial agent that inhibited visible microbial growth, as evidenced by a significant reduction in turbidity compared to the control (Griseofulvin). This inhibition was reflected by a decrease in the absorbance reading from its initial value.[6] Specifically, the MIC corresponded to the concentration at which the OD value showed a marked decrease, indicating effective inhibition of Trichophyton rubrum growth.

## Data Collection

### In-Vitro Study

#### Zone of inhibition

Table 1 Zone of inhibition in mm of samples and controls

**Inference: (Table 1)**Trychophyton rubrum is more sensitive to *Hydrocotyle asiatica* Q, 3C and 200C

## Determination Of Mic

## Analysis And Statistical Methods

#### Interpretation Of The Results

The MIC results provide insights into the effectiveness of different potencies of *Hydrocotyle asiatica* against Trichophyton rubrum compared to the standard antifungal drug griseofulvin.*(Table 2)*

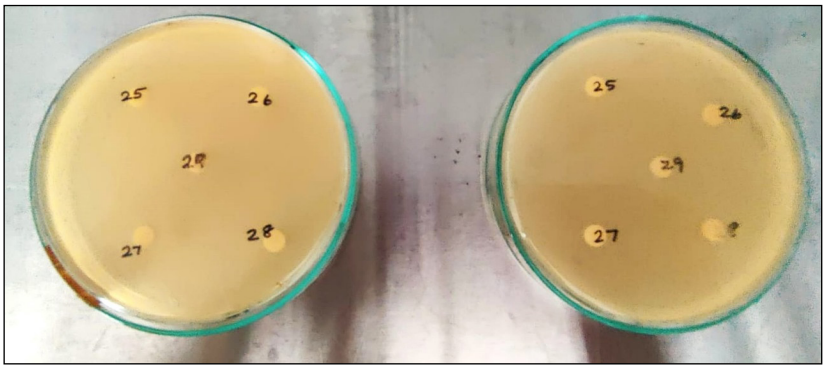


**Figure 1.Antifungal susceptibility test: using the disc diffusion method. Medicated and control discs placed on an agar plate with inoculum suspension**

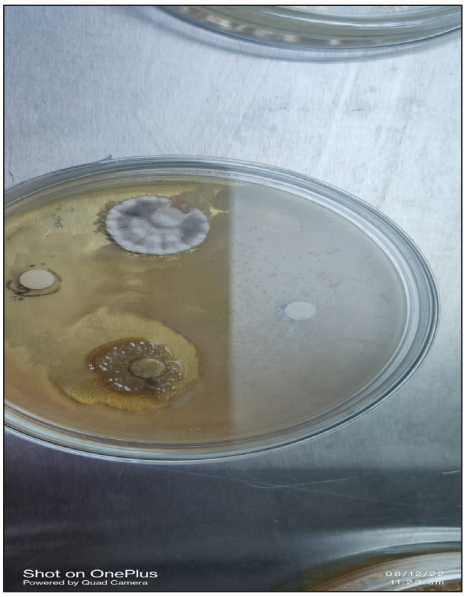


**Figure 2.Zone of Inhibition: the clear zones around the discs show the effectiveness of each antimicrobial agent against the test organism. A Fungal colony is seen over no inhibition zone.**

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**Table 1.Zone of inhibition in mm of samples and controls**

| Sample Code | Medicine Name | Zone Of Inhibition (Mm) |
|---|---|---|
| 20 | SBL *Hydrocotyle asiatica* MT | 10 mm |
| 22 | SBL *Hydrocotyle asiatica* 3 X | 6 mm (No inhibition) |
| 23 | SBL *Hydrocotyle asiatica* 3 C | 9 mm |
| 24 | SBL *Hydrocotyle asiatica* 6 C | 8 mm |
| 25 | SBL *Hydrocotyle asiatica* 12 C | 9 mm |
| 26 | SBL *Hydrocotyle asiatica* 30 C | 8 mm |
| 27 | SBL *Hydrocotyle asiatica* 200 C | 10 mm |
| 28 | SBL *Hydrocotyle asiatica* 1000 C | 9 mm |
| 29 | SBL *Hydrocotyle asiatica* 10 M | 8 mm |
| 30 | Dispensing alcohol (Placebo) | 7 mm |
| 31 | Griseofulvin-EM143-10ST | 12 mm |

**Table 2.Minimum Inhibitory Concentration**

| Sample Code | 0.0 ml | 0.2 ml | 0.4 ml | 0.6 ml | Griseofulvin |
|---|---|---|---|---|---|
| 20 (Mother tincture) | 1.62 | 1.68 | 0.92 | 0.6 | 1.16 |
| 22 (*Hydrocotyle asiatica* 3 X) | 1.64 | 1.36 | 1.12 | 0.89 | 0.92 |
| 23 (*Hydrocotyle asiatica* 3 C) | 1.61 | 1.34 | 0.77 | 0.14 | 1.14 |
| 24 (*Hydrocotyle asiatica* 6 C) | 1.67 | 1.41 | 1.32 | 1.14 | 1.13 |
| 25 (*Hydrocotyle asiatica* 12 C) | 1.68 | 1.42 | 0.78 | 0.28 | 1.12 |
| 26 (*Hydrocotyle asiatica* 30 C) | 1.62 | 0.96 | 0.74 | 0.56 | 1.10 |
| 27 (*Hydrocotyle asiatica* 200 C) | 1.64 | 1.22 | 1.18 | 1.13 | 0.89 |

## Mother Tincture (Sample Code 20)

- The MIC values decrease from 1.62 at 0.0 ml to 0.6 at 0.6 ml, indicating that higher concentrations of the mother tincture are more effective in inhibiting the growth of Trichophyton rubrum.
- The mother tincture shows comparable efficacy to griseofulvin at 0.6 ml concentration.

## Hydrocotyle asiatica 3 X (Sample Code 22)

- The MIC values range from 1.64 at 0.0 ml to 0.89 at 0.6 ml, indicating increased effectiveness with higher concentrations.
- At 0.6 ml, this potency is less effective than griseofulvin but still shows significant inhibition.

## Hydrocotyle asiatica 3 C (Sample Code 23):

- The MIC values decrease from 1.61 at 0.0 ml to 0.14 at 0.6 ml, showing a substantial increase in effectiveness at higher concentrations.
- This potency is more effective than griseofulvin at 0.4 ml and 0.6 ml concentrations.

## Hydrocotyle asiatica 6 C (Sample Code 24):

- The MIC values range from 1.67 at 0.0 ml to 1.14 at 0.6 ml, showing moderate effectiveness.
- The potency is less effective compared to griseofulvin but shows similar effectiveness at 0.6 ml.

## Hydrocotyle asiatica 12 C (Sample Code 25):

- The MIC values range from 1.68 at 0.0 ml to 0.28 at 0.6 ml, indicating increased effectiveness with higher concentrations.
- At 0.6 ml, this potency is less effective than griseofulvin but shows significant inhibition.

## Hydrocotyle asiatica 30 C (Sample Code 26):

- The MIC values decrease from 1.62 at 0.0 ml to 0.56 at 0.6 ml, showing moderate effectiveness.
- This potency is less effective compared to griseofulvin but shows similar effectiveness at 0.6 ml.

## Hydrocotyle asiatica 200 C (Sample Code 27):

- The MIC values range from 1.64 at 0.0 ml to 1.13 at 0.6 ml, showing moderate effectiveness.

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- The potency is less effective compared to griseofulvin but shows similar effectiveness at 0.6 ml.

## Statistical Methods

- All MIC determinations were repeated twice.
- Comparisons of the influence of incubation temperature, incubation time, and tested media were performed using the Wilcoxon (Mann-Whitney) test.
- The MIC was defined as the lowest drug concentration at which 80% growth inhibition was observed, while the control (Griseofulvin) required 100% inhibition.

## Ethical Consideration

This study involved only in vitro experiments and did not include human participants or animal subjects. As such, ethical approval was not required. All laboratory procedures were conducted in compliance with institutional biosafety and good laboratory practice (GLP) guidelines. Microbial strains were obtained from authenticated sources, and all materials were handled according to relevant safety protocols.

## Discussion

- The study demonstrates that *Hydrocotyle asiatica*, particularly in potencies of Q, 3 CH, and 200 CH, shows significant antifungal activity against Trichophyton rubrum. The disc diffusion method revealed measurable zones of inhibition, indicating the sensitivity of the fungus to the homoeopathic medicine.
- The MIC determination using the turbidometric method provided a reliable measure of the antifungal efficacy of various potencies of Hydrocotyle asiatica against Trichophyton rubrum. The results suggested that Hydrocotyle asiatica 3 C exhibited the most significant antifungal activity at the 0.6 ml concentration. This potency showed a marked reduction in turbidity, indicating strong inhibition of microbial growth.
- The comparison with griseofulvin served as a benchmark, demonstrating that several potencies of Hydrocotyle asiatica had comparable or superior inhibitory effects at specific concentrations. These findings were essential for further research and potential clinical applications of *Hydrocotyle asiatica* as an antifungal agent.

## Limitations of the study

Although the study demonstrated notable antifungal activity of Hydrocotyle asiatica under in vitro conditions, these results may not fully reflect the complex interactions within a living organism; therefore, clinical trials are essential to validate these findings.

## Conclusion

The research indicates that Hydrocotyle asiatica, especially in Q, 3C, and 200C potencies, exhibits notable antifungal effects against Trichophyton rubrum. Using the disc diffusion method, clear zones of inhibition were observed, suggesting the fungus is responsive to the homoeopathic treatment. Further research, including clinical trials, would be beneficial to validate these findings and explore the practical applications of homoeopathic medicines in treating fungal infections. The adherence to methodologies recommended by recent publications ensures the robustness and relevance of this study in the current scientific landscape.

**Author's Contribution:** S K S :Conceptualized and designed the study, supervised sample collection, microbiological processing and fungal identification, performed data interpretation and critically revised the manuscript for important intellectual content. M A K R: Assisted in study design and methodology; contributed to data collection, statistical analysis and interpretation and drafted the initial manuscript.

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**Declaration of Generative AI and AI-Assisted Technologies in the Writing Process:**None

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