IN VITRO GROWTH OF TRICHOPHYTON RUBRUM IN THE PRESENCE OF 80% ETHANOLIC EXTRACT OF GRAPEFRUIT PEPINS AS ANTIFUNGAL AGENT

Abstract

Introduction: Many modern antifungal drugs have limits in terms of germ resistance, and to remedy this, plant compounds are being explored to find new more effective principles.

Aim: With the aim of complementing the efforts of modern medicine against viral, bacterial, parasitic and fungal diseases, the plant extract of 80% hydroalcoholic grapefruit seed extract of *Citrus paradisi* was tested on the *in vitro* growth of *Trichophyton rubrum*.

Method: Antifungal tests were carried out on Sabouraud medium, to which the plant extracts were incorporated using the double dilution method in inclined tubes. A 10 µL volume of the *Trichophyton rubrum* suspension was inoculated on the culture medium contained in the test tubes.

Results: Results showed that *Trichophyton rubrum* was sensitive to the 80% hydroethanolic extract of *Citrus paradisi* in a dose-dependent manner.

Conclusion: The hydroethanolic extract may be a source for the development of Traditional Improved Medicines (TIM) against skin mycosis.

Key words: *Citrus paradisi*,80%hydroethanolicextract, mycosis, *Trichophyton rubrum*,grapefruit seed.

INTRODUCTION

The skin is vital to our health and well-being. As well as being our body's first line of defense against bacteria and viruses, healthy skin keeps our body fluids in balance and helps regulate

our body temperature. From a biological point of view, the skin is the body's main protection against external elements. It covers the entire surface of the body and is the first point of contact with the outside world **[1].**

Infectious skin diseases are on the increase. Candidiasis, cryptococcosis and aspergillosis are among the mycoses on the rise (**[2]**;**[3]**). The ineffectiveness of current treatments has led impoverished populations to turn to pharmacopoeia plants for their cures (**[4], [5]**, **[6]**). Indeed, the use of medicinal plants by populations has existed since the dawn of time. Over 80% of populations use plants for their primary health care (**[7], [8].**).

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Over 80% of the population use plants for primary health care (**[8]**). However, misuse of medicinal plants can lead to health problems such as kidney failure, heart disease and poisoning.

Numerous studies have shown that extracts *Mentha pulegium* ,*Marrubium vulgare* and *Teucrium polium* are aromatic and medicinal plants of the Lamiaceae family widely used in traditional medicine; their antimicrobial power has been demonstrated by several studies ([9];[10]).

To help people who use medicinal plants, work has been initiated to extract their active ingredients, verify their therapeutic virtues and give them a scientific basis. This is the case with *Citrus paradisi*, a rutaceae on *Trichophyton rubrum*.

MATERIAL

Biological material

The plant material is a plant powder obtained from the seeds of the grapefruit tree, the scientific name of which is *Citrus paradisi* (Figure 1). These samples were collected in Soubré in December 2023.

Figure 1:*Citrus paradisi*

- Germ tested

The germ tested was *Trichophyton rubrum*, which was inoculated insabouraud agar, commonly used for fungal growth.

METHOD

Harvesting and conditioning plant material

Citrus paradisi seeds were harvested, washed and sun-dried at room temperature (25-30°C) for two weeks in the laboratory. Then, using an electric grinder, the dried seeds were ground into powder.

Preparation of 80% ethanolic extract

A quantity of 100 grams of grapefruit seed powder was dissolved in a mixture of 1000mL solvent consisting of 800mL ethanol and 200mL distilled water, then homogenized in a Blender at room temperature using the method adapted from **[5]**. The resulting homogenate was first wrung out in a square of white cloth. It was then successively filtered through hydrophilic cotton. The filtrate obtained was oven-dried at 50°C for 48h (**[11], [12]**, **[13]**) to give the 80% hydroethanolic extract. The mass of extract obtained was stored in sterile, clean, dry urination dishes and then kept under heat and humidity protection.

Preparation and incorporation of extracts into culture medium

Sabouraud culture medium was prepared according to the supplier's instructions. A quantity of 5.04 g of Sabouraud agar was homogenized in 120 mL of distilled water. For *in vitro* testing, the medium was poured into test tubes, into which the extract was incorporated. Plant extracts were incorporated into Sabouraud agar using the double dilution method in inclined tubes. Each series comprises 10 test tubes numbered from 1 to 8, and 2 control tubes (one coded TC, used as a control for germ growth; the other, TS, as a control for culture medium sterility). The concentration range varies from 100mg/mL to 0.78 mg/mL**.**After incorporation of the extract, all tubes were autoclaved at 121°C for 15 minutes and then inclined with the pellet for solidification.

For inoculum preparation, a germ oese was homogenized in 10 mL sterile distilled water (10° concentration). A second suspension (10^{-1}) was prepared by taking 1mL from the 10° suspension and adding it to the 9 mL of distilled water to give a final volume of 10 mL. The latter will be used for the various tests.

Antifungal tests in the presence of plant extract

10 µL of suspension 10^{-1} is sown in transverse streaks until exhaustion on tubes (1 to 8 and TC). This corresponds to 1000 seeded cells. The resulting cultures were incubated at 30°C for 5 days (**[14], [15]**, **[16]**).

RESULTS

The results of the various tests are shown in Figures 2 & 3, which respectively show the appearance of *Trichophyton rubrum* cultures as a function of the concentration of 80% ethanolic extract of *Citrus paradisi*, and the antifungal curve, which is the growth inhibition curve for *Trichophyton rubrum* as a function of the concentration of 80% ethanolic extract of *Citrus paradisi*. The appearance of the cultures is shown in figure 2. The sterility control tube, which contained no germs, proves the sterility of the medium used. The germ growth control tube showed not only the appearance, but also the maximum number of colonies obtained for normal

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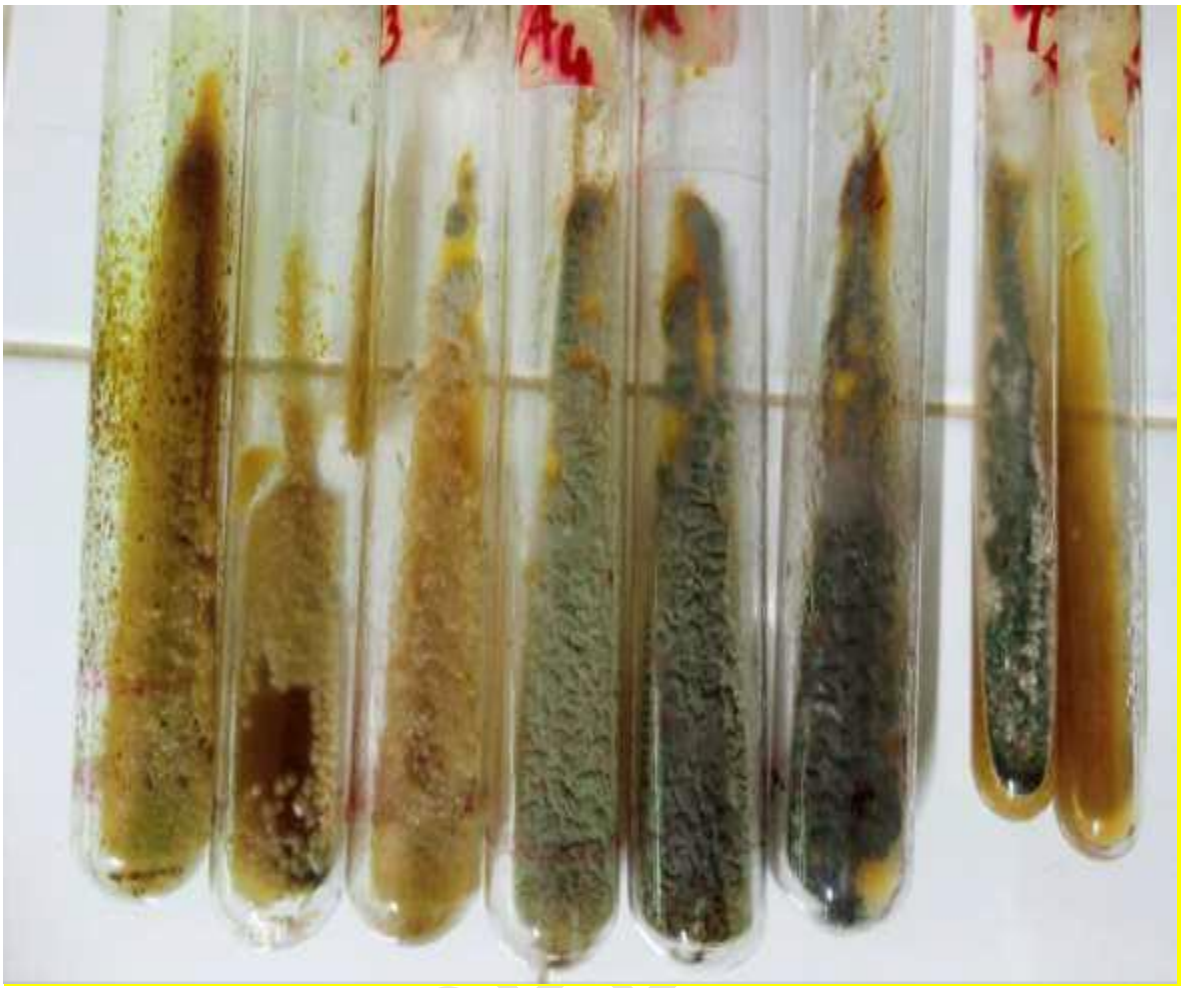

Figure2 : Appearance of *Trichophyton rubrum* cultures as a function of concentrations of 80% ethanolic extract of *Citrusparadisi*

(Concentrations range from 400mg/mL to 3.125mg/mL from left to right)

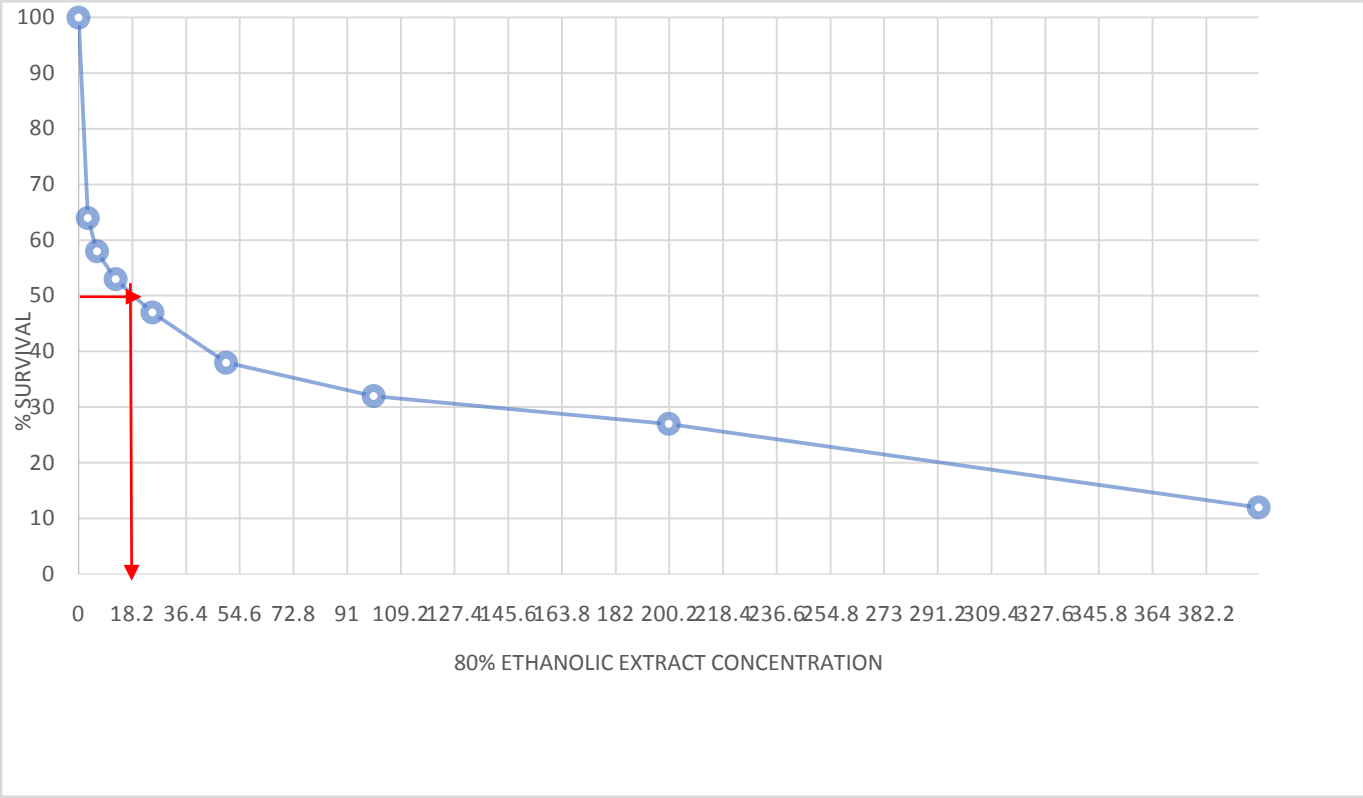

Figure 3: Growth inhibition curve for *Trichophytonrubrum* as a function of the concentration of 80% ethanolic extract of *Citrusparadisi*

DISCUSSION

Concentrations decrease from 400 mg/mL to 3.125 mg/mL with a geometric bond of reason ½. Our results show that ethanolic extract 80% of grapefruit seeds is more or less sensitive on the *in vitro* growth of *Trichophyton rubrum*. We were unable to identify the MFC (Minimum Fungal Concentration) value in the concentration range chosen for the study. However, the Inhibitory Concentration was determined.

Given the hype surrounding grapefruit seed extract and the therapeutic promises it holds, it seems necessary to take a critical look at its action and use.

It is also richer in vitamin C and flavonoids, both in the seeds and in the juice **[17]**. In terms of culture appearance, the germ-free sterility control tube proves the sterility of the medium used. The culture control tube shows not only the appearance, but also the maximum number of colonies obtained for normal germ growth. The antifungal chart is obtained from the colony count data in the various experimental tubes. Generally speaking, the 80% ethanolic extract of grapefruit seeds tested is more or less active and inhibits the *in vitro* growth of *Trichophyton rubrum*. There was a progressive decrease in colony numbers as extract concentrations increased in the experimental tubes. The curve shows a decreasing trend.

The Minimum Fungicidal Concentration (MFC) value is over 400 mg/mL with a concentration for 50% inhibition (CI50) value equal to 18.2 mg/mL.

This performance is inferior to that of *Mitracarpusscaber* fraction1 on *Trichophyton rubrum*, with a Minimum Fungicidal Concentration (MFC) value of 12.5 g/mL and an IC_{50} value of 0.5 mg/mL **[18]**. Similarly, fraction1 of *Terminalia ivorensis*shown on *Trichophyton mentagrophytes* var. *interdigitale* with a Minimum Fungicidal Concentration (MFC) value of 97.5 g/mL and an IC_{50} value of 3.78 mg/mL **[14]**.

In addition, the work of **[16]** with ethanolic extract 80% of *Solanum anguivi*, a solanaceae plant, gave a better performance based on antifungal parameter values (50 mg/mL).

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CONCLUSION

This study shows that the 80% hydroethanol extract of grapefruit seeds effectively inhibits the *in vitro* growth of *Trichophyton rubrum*. As these results are crude, they could be improved by other techniques

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