



## RESEARCH ARTICLE

### ACTIVITY OF AN EXTRACT OF GRAPEFRUIT SEEDS (*CITRUS PARADISI*) ON THE GROWTH OF *CANDIDA ALBICANS*

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#### ABSTRACT

With the aim of complementing the efforts of modern medicine against viral, bacterial, parasitic and fungal diseases, the plant extract of hydroalcoholic grapefruit seed extract (*Citrus paradisi*) was tested on the *in vitro* growth of *Candida albicans*. Antifungal tests were carried out on Sabouraud medium, to which the plant extracts were incorporated using the double dilution method in inclined tubes. Results showed that *Candida albicans* was sensitive to the 70% hydroethanolic extract of *Citrus paradisi* in a dose-dependent manner. The hydroethanolic extract may be a source for the development of Traditional Improved Medicines (TIMs) against skin mycosis.

## INTRODUCTION

Plants have always been a source of medicines. Even today, a majority of the world's population, particularly in developing countries, relies on traditional herbal remedies (1). Flora has already contributed enormously to the discovery of many of the active principles used in the preparation of numerous medicines, and continues to do so. Indeed, the modern pharmaceutical industry itself continues to draw on the diversity of secondary plant metabolites to find new molecules with biologically active properties(2),(3). Unfortunately, despite all the efforts made by the medical profession, infectious diseases have been on the increase in recent years. In terms of mycoses, candidiasis is a very frequent infection in subjects living with HIV. Susceptibility to antifungal agents is declining in certain *Candida* strains, due to drug pressure, mutation phenomena and the sharp rise in opportunistic infections (4). With the aim of helping people to treat themselves cheaply and safely, research has begun into the pharmacological activity of various medicinal plant extracts. Such is the case with *Citrus paradisi*, a rutaceae on candida. Grapefruit seed extract (*Citrus paradisi*) has enjoyed phenomenal success in the United States, being used both curatively and preventively in men to treat a wide range of ailments (5),(6),(7).

## MATERIALS

### Biological material

- The plant material is a plant powder obtained from grapefruit (*Citrus paradisi*) seeds (Figure 1). These samples were collected in Soubre region (Ivory Coast) in december 2023.

**Germ tested:** The germ tested was *Candida albicans*, which was inoculated into sabouraud agar commonly used for fungal growth.

## METHODS

**Harvesting and conditioning plant material:** Grapefruit (*Citrus paradisi*) seeds were harvested, washed and dried in the sun at room temperature (25-30°C) for two weeks in the laboratory. The dried seeds were then ground to powder using an electric grinder.

### Preparation of ethanolic extract 70%.

A quantity of 100 grams of grapefruit seed powder was dissolved in a mixture of 1000mL solvent consisting of 700ml



Figure 1. Grapefruit tree (*Citrus paradisi*)

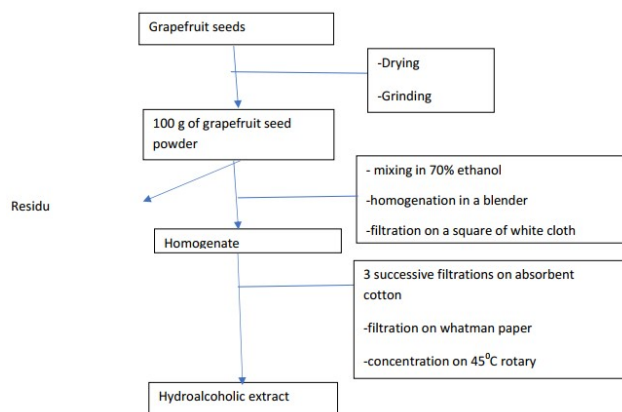


Figure 2. Hydroalcoholic extraction method

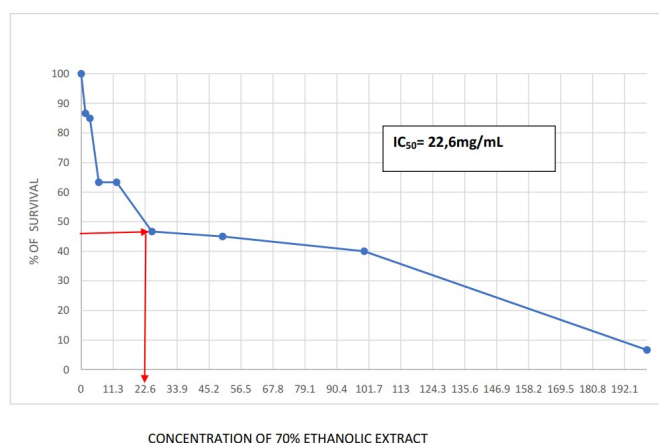


Figure 3. Growth inhibition curve for *Candida albicans* as a function of 70% dry ethanolic extract concentration

ethanol and 300mL distilled water, then homogenized in a Blender at room temperature (1),(8),(9). The resulting homogenate was first wrung out in a square of white cloth. It was then successively filtered through absorbent cotton. The filtrate obtained was oven-dried at 50°C for 48 h (10),(11) to give the 70% hydroethanol extract. The mass of extract obtained was stored in clean, dry, sterile urine dishes, then kept in a cool, dry place. 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 are autoclaved at 121°C for 15 minutes and then tilted with the pellet at room temperature to allow cooling and solidification of the agar.

**Inoculum preparation:** A germ oese was homogenized in 10 mL of sterile distilled water ( $10^0$  concentration). A second suspension ( $10^{-1}$ ) was prepared by taking 1mL from the  $10^0$  suspension and adding it to the 9 mL of distilled water to obtain a final volume of 10 mL. This will be used for the various tests.

**Antifungal tests in the presence of plant extract:** 10  $\mu$ L 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 48 h with *Candida albicans*(12);(13).

## RESULTS

The results of the various tests are shown in figure 3, which is the antifungigram chart. The appearance of the cultures is shown in figure 4. The sterility control tube, which contains no germs, 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.

## DISCUSSION

Given the hype surrounding grapefruit seed extract and the therapeutic promises it brings, it seems necessary to bring a critical sense to bear on its action and use. First of all, the scientific literature evoking the antimicrobial, immunostimulant and antioxidant activities of these extracts is not yet available (14). It is also richer in vitamin C and flavonoids, both in the seeds and in the juice (USDA, 2014). However, the most widespread route to industrial valorization remains the extraction of essences and essential oils, which can be used as an alternative to synthetic fungicides (5). The appearance of the cultures is shown in figure 8. The sterility control tube, which contains no germs, 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 70% grapefruit seed extract tested was more or less active and inhibited the in vitro growth of *Candida albicans*. There was a progressive drop in colony numbers as extract concentrations increased in the experimental tubes. The curve shows a decreasing trend. The concentration range chosen is between 100 mg/mL and 0.78 mg/mL. The Minimum Fungicidal Concentration (MFC) value is above 100 mg/mL with a concentration for 50% inhibition (IC<sub>50</sub>) value equal to 22.6 mg/mL.

This performance is inferior to that of the 70% hydroalcoholic extract of *Terminalia ivorensis* on *Candida albicans* with a Minimum Fungicidal Concentration (MFC) value of 90 µg/mL. (15) La fraction F1 de *Mitracarpus scaber*, une Rubiacée, a donné une valeur CMF de 150 mg/mL sur *Candida albicans* et une concentration pour une inhibition de 50 % (CI50) de 3 mg/mL (16). Similarly, the work of (17) with hydroalcoholic extract of *Enantioplycarpa*, an anonaceous plant, was more active, based on antifungal parameter values (CMF= 3.125 mg/mL and IC50=1.50 mg/mL). Work by (18) using dried fruits of *Solanum anguivi*, a Solanaceae, gave antifungal parameter values (CMF= 200 mg/mL and IC50=12 ;5 mg/mL) still on *Candida albicans*.

## CONCLUSION

This study shows that hydroethanol extract of grapefruit seeds effectively inhibits the *in vitro* growth of *Candida albicans*.

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## CONFLICTS OF INTEREST

The author declares no conflict of interest regarding the publication of this article.

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