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Comparative Study of the Activity of *Citrus Sinensis* Barks on the *In Vitro* Growth of *Candida Albicans* and *Trichophyton Rubrum*

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ABSTRACT

The emergence as well as resistance of pathogenic microorganisms currently poses a particularly serious public health problem. The use of medicinal plants is therefore one of the most interesting avenues to explore. It is with this in mind that this work was carried out and aims to evaluate the activity of *citrus sinensis* bark on the growth of *Candida albicans* and *Trichophyton rubrum*. In this study, we proceeded to the hydroethanolic extraction at 70% of the bark of this plant and the extract was tested by the method of double dilution. The results showed that the two microbial strains are sensitive to the ethanolic extract of *Citrus sinensis* according to the dose-response relationship with an MIC value of 50mg/mL for *Candida albicans* and an MIC value greater than 50mg/mL for *Trichophyton rubrum*. The extract is fungistatic against *Candida albicans* but has weak inhibitory activity for *Trichophyton rubrum*.

Keywords: Antifungal activity- *Citrus sinensis* - mycosis

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INTRODUCTION

Infectious diseases are widespread in many parts of the world, particularly in developing countries. With the high incidence of Acquired Immunodeficiency Syndrome (AIDS) in many sub-Saharan African countries, opportunistic pathogens, along with other fungal, bacterial and parasitic infections, are among the biggest health problems[19]. In recent decades microbial diseases, transmitted by bacteria, viruses, fungi and other parasites, claim around 17 million victims worldwide. And Africa, which accounts for two-thirds of the burden of this mortality, is the continent that pays the highest price [9-17]. To cope with this situation, mankind has found and developed knowledge and practices to treat itself using natural products of plant, mineral or animal origin. This knowledge and techniques, known as pharmacopoeia and traditional medicine, have been passed down and enriched from generation to generation[8]. As a result, synthetic products have been developed from the chemical compounds present in these natural products, to create so-called conventional medicines. However, the use of these conventional drugs, notably antibiotics, is sometimes ineffective, due to the resistance developed by microorganisms and the manifestation of severe and even toxic side-effects in some cases [24-13-14-3]. In addition, the main difficulties associated with the treatment of these microbial diseases are the inaccessibility and high cost of these drugs. Indeed, in the treatment of infectious diseases, antibiotic prescriptions are often recommended. On the other hand, there are currently many chronically debilitating or life-threatening diseases that urgently require improved or new medical treatments. In view of the emergence of new diseases and growing resistance to existing drugs, it is therefore necessary to discover and develop innovative new drugs with reduced side-effects to combat these microbial infections [5-22]. Even today, medicinal plants have become a precious heritage for the survival of humanity. Indeed, the WHO recognizes that traditional, complementary and alternative medicine provide numerous benefits. Africa has a long history of traditional medicine and traditional health practitioners, who play an important role in people's health care. Also, some 80% of the world's population in developing countries, due to poverty and lack of access to modern medicine, depend essentially on traditional medicinal plants for their primary health care [5-22]. Medicinal plants therefore remain the most important source of molecules used in the composition of pharmaceutical drugs[16]. It therefore makes sense to continue or even intensify research in this direction, given that plants remain an almost inexhaustible source of biomolecules. This approach provides industry with a basis for innovation in the development of new drugs, which can be a source of cost savings. time in research and development processes[4]. Thus, from the wide range of medicinal plants used in

Côte d'Ivoire, our choice fell on Citrus sinensis (figure 1), a plant from the Rutaceae family. With this in mind, our study was carried out with the main aim of assessing the activity of *Citrus sinensis* bark on the growth of *Candida albicans* and *Trichophyton rubrum*.

Figure 1: ***Citrus sinensis***

MATERIALS AND METHOD

Biological material

Plant material

The plant material is a vegetable powder obtained from *Citrus sinensis* barks. The bark was harvested in February 2023 in Oumé (Côte d'Ivoire).

Fungal species tested

The microbiological material consisted of two (02) species of fungi, namely: *Candida albicans* and *Trichophyton rubrum* grown at the National Floristic Center of Abidjan.

Culture medium

The culture medium used was SABOURAUD**.**

Chemical products

Several products were used in the course of the work: For extractions, we used distilled water and ethanol as solvents. The various work surfaces were sanitized with bleach, and hands disinfected and washed with ethanol.

METHOD

Preparation of the hydroalcoholic extract

The crude extract was prepared from C. sinensis powder using the method of 23 (Figure 2). To prepare the ethanolic extract, 100 g of Citrus sinensis powder were homogenized in 1 L of 70% hydroethanolic solution using a blender. The resulting homogenate was first wrung out in a square of clean white cloth, then filtered three times on hydrophilic cotton and once on whatman

paper. The filtrate obtained was concentrated in an oven at a temperature of 50°C. The brown powder obtained is the ethanolic extract codified Xeth. This extraction was performed 5 times to calculate the yield.

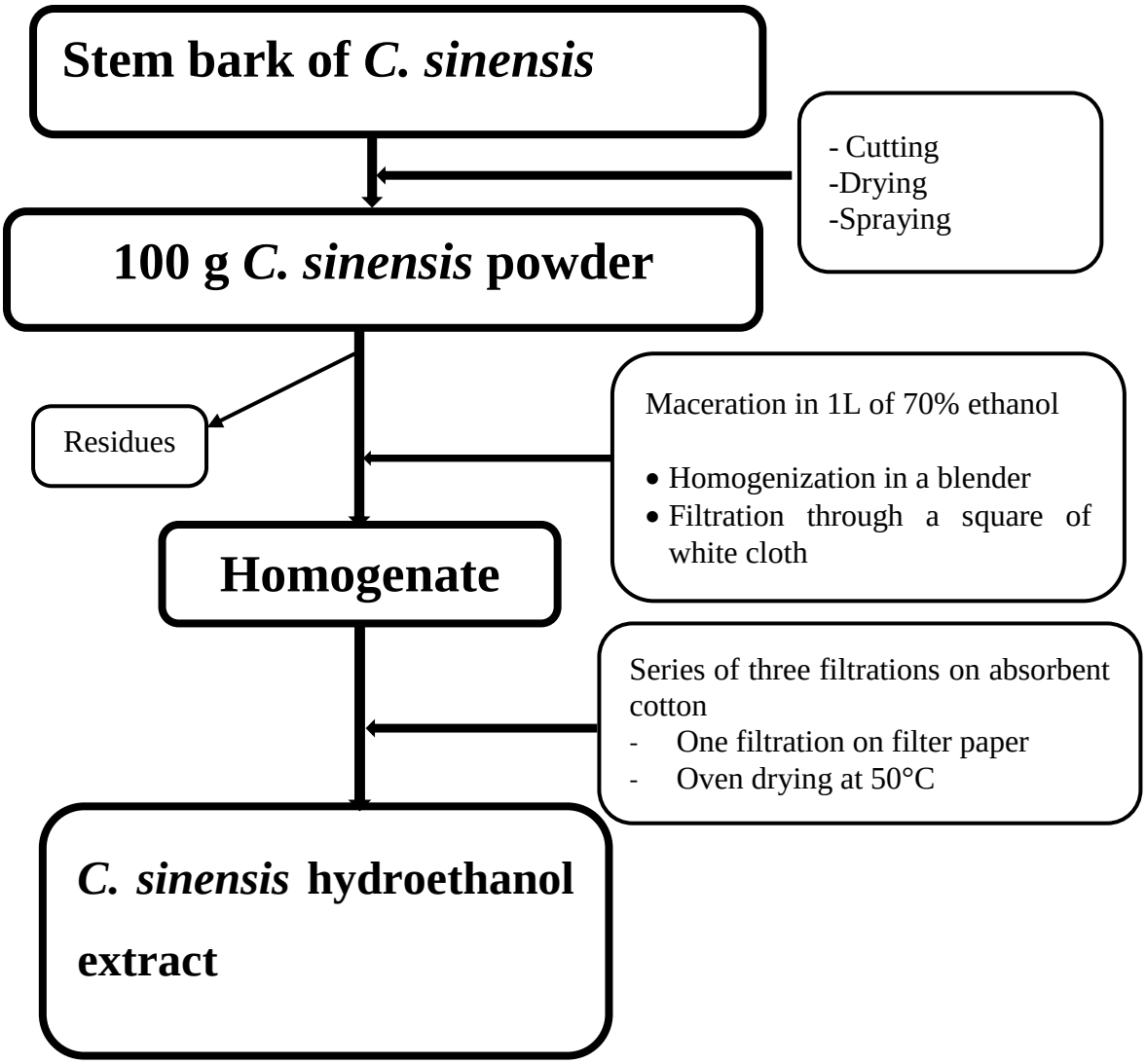

Figure 2: *C. sinensis* bark extraction method

Yield calculation

The yield is the extracted quantity produced from a relative quantity of the powder. It is expressed as a percentage and calculated according to the following formula:

R=100 x m / M.

R: extraction yield

m: mass of the extracted - M: mass of the powder used plant

Culture medium preparation

Sabouraud medium was prepared according to the manufacturer's instructions, i.e. 14.7g of powder homogenized in 350 mL of distilled water; this mixture was homogenized and then heated on a hot plate for 5 min. The various plant extracts were incorporated into Sabouraud agar using the double dilution method in inclined tubes[21].

For each plant extract, each series comprises ten test tubes. Eight of these test tubes contain the plant extracts. The other two tubes are regarded as control tubes, one without plant extract but without germs, to check the sterility of the culture medium, and the other without plant extract to check the growth of germs. Our concentration range varies from 50 to 0.39 mg/mL. After incorporation of the extracts into the eight test tubes, all ten tubes in each series were autoclaved at 121°C for 15 minutes and then tilted with small pellets at laboratory temperature to allow cooling and solidification of the agar[21].

Microbiology tests

From young cultures of *Candida albicans* (48-hour incubation) and *Trichophyton rubrum* (5-day incubation), the inoculum was prepared as follows: A young colony of *Candida albicans* collected with a loop was homogenized in 10 mL of sterilized distilled water. This gave the mother suspension (10^o) concentrated to 106 cells/mL. From this suspension, a second suspension (10-1) was prepared by dilution to 1/10th of the first. It carries a load of 105 cells/mL. For each test tube in each series of ten extracts (except the sterility control tube for the culture medium), germs were cultured on the previously prepared media by inoculating 10 µL of suspension 10-1 in transverse streaks until exhausted. This corresponds to 1000 seeded cells. The resulting cultures were incubated at 30°C for 48 hours. Growth in the eight experimental tubes of each series was assessed as percentage survival calculated against 100% survival in the growth control tube[23].

RESULTS AND DISCUSSION

Yield of extract preparation

The hydroethanol extract gave a brown powder with a yield of 6.02%.

Test results

After 48 h of incubation, compared with the control, there was a progressive decrease in the number of *Candida albicans* and *Trichophyton rubrum* colonies as the extract concentration in the agar increased. These results are the statistical averages of the 5 tests per series of 10 tubes. Clear inhibition was observed at 50 mg/mL for *Candida albicans*. For *Trichophyton rubrum* , no inhibition was observed in our chosen concentration range (50 to 0.39 mg/mL). The experimental data are translated into sensitivity curves for both germs (Figure 3 & 4). For

Candida albicans, we obtained an MIC value of 50 mg/mL and an IC50 value of 1.7 mg/mL , while the MIC of *Trichophyton rubrum* was not within our chosen concentration range. The IC50 of *Trichophyton rubrum* was 15.5 mg/mL . The MFC of *Trichophyton rubrum* was not in our chosen range, so was above 50 mg/mL, and that of *Candida albicans* was also above 50 mg/mL.

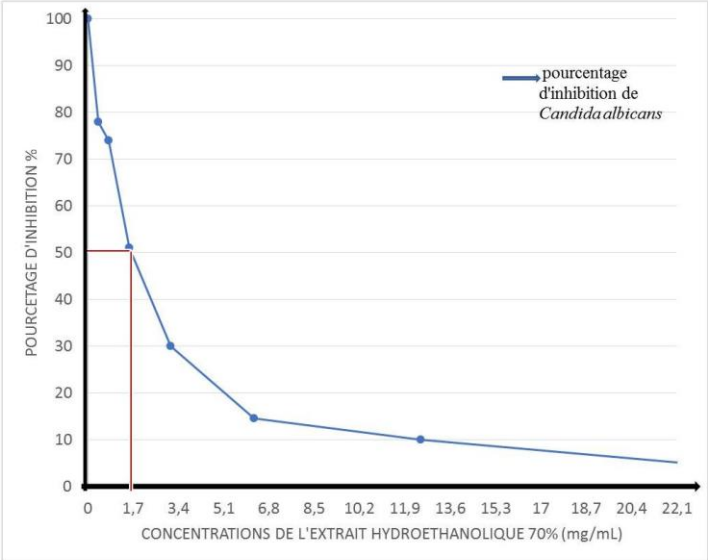

Figure 3: Susceptibility curve of *Candida albicans* to 70% ethanolic extract

Sterility testing of the tube corresponding to the MIC of *Candida albicans* by inoculating a sample taken from the surface of the agar in this tube onto new agar showed us that the tube containing the MIC, where no germ colonies were visible to the naked eye, did contain germs.

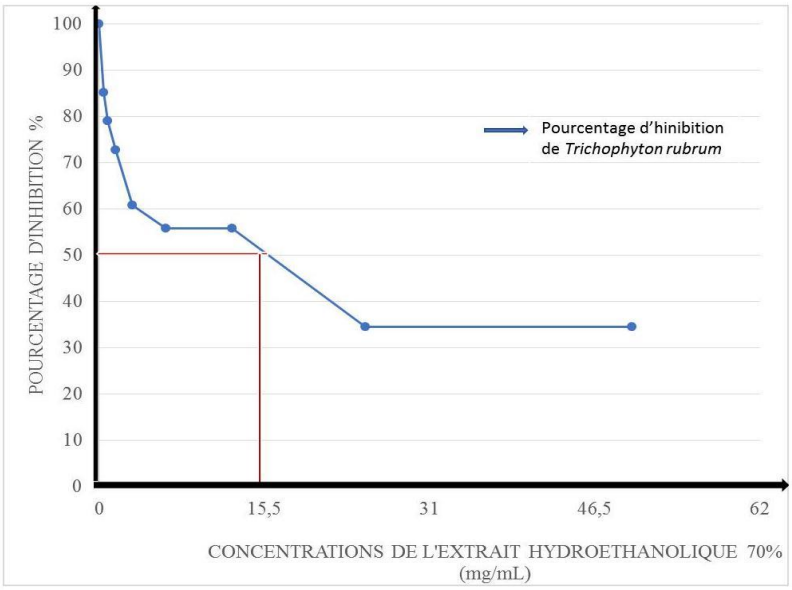

Figure 4: Susceptibility curve of *Trichophyton rubrum* to 70% ethanolic extract

DISCUSSION

Antimicrobial resistance is at the root of many therapeutic failures. This has led to new research, particularly in the hope of treating certain infectious diseases through the use of medicinal plants. The discovery of plants active against these germs could be beneficial to a country's public health.

This work was designed to determine the antimicrobial activity of *C. sinensis* bark on *Candida albicans* and *Trichophyton rubrum*. To this end, we prepared the 70% ethanolic extract, and tested its antifungal activity. Analysis of the results of the various tests showed that both microbial strains are sensitive to the ethanolic extract of Citrus sinensis. The results show a progressive decrease in colony numbers as extract concentrations are increased in the experimental tubes. We deduce that the strains studied are sensitive to the extract according to a dose-response relationship. However, the antifungal parameter values show that *Candida albicans* is more sensitive to the extract than *Trichophyton rubrum*. This is demonstrated by the antifungal parameter values; *Candida albicans* (CMF>50mg/mL; IC50=1.7mg/mL) and *Trichophyton rubrum* (CMF>>50mg/mL, IC50=15.5 mg/mL). Sterility testing of the tube corresponding to the MIC for *Candida albicans*, by inoculating a sample taken from the surface of the agar in this tube onto new agar, showed that the tube containing the MIC, where no colonies of germs were visible to the naked eye, did contain germs. This allows us to deduce that the 70% ethanolic extract has a fungistatic action on *Candida albicans*. In the light of these results and by comparison with other research, more or less similar results have been obtained on *Candida albicans* and *Trichophyton rubrum*. Work[11] on the antimycotic activity of extracts from the cork oak, Quercus suber, on *Trichophyton rubrum* and *Candida albicans*, showed that the best performance was obtained in the presence of Quercus suber bark extract, with MICs of between 50 and 12.5 mg/mL for *Trichophyton rubrum* and *Candida albicans* respectively, the latter being the most sensitive even at very high dilutions. This is in line with our results showing that *Candida albicans* is more sensitive to the 70% ethanolic extract than *Trichophyton rubrum*. The inhibition of *Candida albicans* growth in our case is less significant, compared with the results found by other authors such as those of[1], who tested the 96% alcoholic extract of MISCA-F3 on *Candida albicans* (CMF = 5.10 µg/ml), our extract is 1000 times less active than the alcoholic extract of MISCA-F3 on *Candida albicans* . Also, the 70% alcoholic extract of MISCA-F2 tested by[18] on Candida albicans (MFC =10.10 µg/mL) shows that the 70% alcoholic extract of MISCA -F2 is 1000 times more active than our extract; also, the author[15]who tested the 80% alcoholic extract of MISCA-F1 on Candida albicans (MFC = 15. 10 µg/mL), and the

author6 who tested the 70% alcoholic extract of TEKAM 2 on Candida albicans (CMF =195µg/mL) also show that the 70% alcoholic extract of TEKAM 2 is also 1000 times more active on Candida albicans than our extract. A comparative analysis of our results with those of previous work carried out on *Candida albicans* by[7] showed that our extract was less active than the hydroalcoholic extracts of Entandrophragma angolense (CMF= 12.5 mg/mL), *Nesogordonia papaverifera* (CMF= 25 mg/mL), *Milicia excelsa* (CMF= 25 mg/mL), *Ceiba pentadra* (CMF= 50 mg/mL), *Entandrophragma cylindricum* (CMF= 50 mg/mL), *Guarea cedrata* (CMF= 100 mg/mL), *Khaya ivoirensis* (CMF= 100 mg/mL). Comparison of our results with those of[18] confirms that *Candida albicans* is a good indicator for assessing the antifungal parameters of plant extracts. *For Trichophyton rubrum*, some studies show that black cumin oil has good activity against this germ[2] The author[10] confirmed this hypothesis with ozonated olive oil (known as ozolive), which had good inhibitory activity against *Trichophyton rubrum* and *Candida albicans*. This is not the case with our extract, which has little activity against these germs. With regard to the antifungal activity of *Citrus sinensis*, several other studies carried out by[20] have shown that the essential oil of *Citrus sinensis* has an inhibitory effect on *Aspergillus niger*. In fact, the essential oil of fresh *Citrus sinensis* epicarp, extracted by hydrodistillation using a Clevenger-type apparatus, showed great antifungal power against *Aspergillus niger*. The authors[12] tested the effect of essential oil from fresh *Citrus sinensis* leaves and obtained satisfactory results on the pathogens *Candida albicans*, *Saccaharomyces cerevisia* and *Aspergillus niger* with inhibition zones of 19, 19 and 17 mm, respectively. This confirms the sensitivity of *Candida albicans* to Citrus sinensis extracts. All these results support the use of *Citrus sinensis* extracts as natural antifungal agents.

CONCLUSION

The study focused on the in vitro evaluation of the antifungal activity of the ethanolic extract of *Citrus sinensis* bark on the growth of *Candida albicans* and *Trichophyton rubrum*. The results showed that, using the concentration range from 50 to 0.39mg /mL, the MIC of *Candida albicans* is 50mg/mL, while that of *Trichophyton rubrum* is not in our concentration range. The MIC of *T. rubrum* is therefore higher than 50mg /ml. The FMC of *C. albicans* and *T. rubrum* are above 50mg/mL. Sterility testing of the tube corresponding to the *Candida albicans* MIC, by inoculating a sample taken from the surface of the agar in this tube onto new agar, showed that the tube containing the MIC, where no colonies of germs were visible to the naked eye, did contain germs. This suggests that the 70% ethanolic extract has a fungistatic action on *Candida albicans*, but a weak inhibitory activity for *T. rubrum*, since its MIC is greater than 50mg/mL.

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CONFLICTS OF INTEREST

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

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