COMPARATIVE STUDY OF THE ANTIFUNGAL ACTIVITY OF FRESH AND DRIED SOLANUM ANGUIVI FRUITS ON CANDIDA ALBICANS AND TRICHOPHYTON RUBRUM

Comment [1]: Replace with: A COMPARATIVE

Comment [2]: Replace with: ***Candida albicans*** **AND** ***Trichophyton rubrum***

Abstract

In Côte d'Ivoire, as elsewhere in Africa, Solanum anguivi Lam is widely used in traditional medicine to treat bacterial and fungal infections. Given the importance of this plant, the extracts 70%, 80% and 100% from fresh and dried fruits was evaluated on the in vitro growth of two isolates of pathogenic fungi (Candida albicans and Trichophyton rubrum), as well as its anti-free radical activity.

Antifungal tests were carried out by plating 1000 cells of each isolate on Sabouraud agar medium, using the double dilution method in inclined tubes. Both extracts were active on the different strains tested, according to a dose-response relationship based on the principle of the method used.

However, extracts from fresh fruit showed good activity on C. albicans, while the dried fresh fruit extracts were highly active on T. rubrum.

Key words: *Solanum anguivi Lam*, antifungal

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Comment [4]: Replace with: extracts of 70%, 80%, and 100% from fresh and dried fruits were evaluated for the in vitro growth of two isolates of pathogenic fungi (*Candida albicans* and *Trichophyton rubrum*), as well as their anti-free

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Introduction

The 20th century was marked by the development of various classes of synthetic molecules of plant origin, whose efficacy has made it possible to tackle diseases (malaria, HIV, cancer...); moreover, at national level, 54% of the population do not have adequate sanitation[1] would be exposed to water-borne diseases.

All these facts justify the efforts currently being made to develop traditional medicine and ensure its integration into modern national healthcare systems[2].

This approach makes it possible to select potentially active plants and significantly increase the number of discoveries of new active ingredients.

Comment [9]: Replace with: cancer, etc.); moreover, at the national level, 54% of the population that does not

Comment [10]: The author(s) should follow the journal in-text reference. Remove the superscript.Sanitation [1].
Do this throughout the manuscript.

This knowledge would not be possible without assessing their chemical composition, identifying the structure of the active principles responsible or not for the pharmacological properties exploited by traditional medicine, and confirming their biological activities. Medicinal plants represent a significant source of new medicines, especially as they have fewer side effects [3].

However, although medicine has evolved, certain diseases such as fungal infections have not yet been totally eradicated from our societies.

The aim of the present work is to evaluate the antifungal properties of the fruits of the Solanum anguivi Lam plant (Solonaceae) on Candida albicans and Trichophyton rubrum.

Comment [11]: Replace with: diseases, such as fungal infections,

Comment [12]: Italise. Do this where it is necessary.

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MATERIALS AND METHODS

MATERIALS

Biological material

Plant material

The plant material used was a powder obtained from the dried fruits of Solanum anguivi Lam (Figure 1). The plant was identified at the Centre National de Floristique (CNF) Université Félix Houphouët Boigny for herbarium number UCJ016884.

Figure 1: Plant of Solanum anguivi Lam

Fungal strains

The fungal material consisted of two strains of the fungi Candida albicans and Trichophyton rubrum. These fungi were obtained from the laboratory of the Centre National de Floristique, Université FELIX HOUPHOUËT-BOIGNY (Côte d'Ivoire).

METHODS

Harvesting and drying plant fruits

The fresh fruits were obtained from saleswomen in Abidjan and dried in the shade in a dry, airy place for about 7 days to avoid any reaction that might deteriorate or lead to the loss of the plant's active principles. After drying, we crushed the fruit using a blinder.

Comment [15]: Replace with: After drying, the fruit was crushed using a blinder.

Preparation of the various hydroalcoholic extracts

Hydroalcoholic extracts (ethanol 70% and 30% distilled water, ethanol 80% and 20% distilled water and ethanol 100%) of both dried and fresh fruit were prepared according to the method described by [4].

Comment [16]: Replace with: water, and

One hundred grams (100g) of fresh or dried fruit powder were macerated separately in one liter of solvent containing 30% distilled water and 70% alcohol, followed by homogenization using a blender. For the 80% extract, the same mass of fruit was taken and then macerated in 80% alcohol and 20% distilled water and homogenized with a blender. For the 100% extract, the same mass of fruit was used, macerated in a liter of alcohol and then homogenized using a blender. The various homogenates obtained were successively filtered twice on absorbent cotton and once on Whatman 3 mm filter paper. The filtrates obtained were dehydrated in an oven at 50°C to obtain a brown paste [4].

Comment [17]: Replace with: litre

Comment [18]: Replace with: fruit was taken, macerated in 80% alcohol and 20% distilled water, and homogenized with

Comment [19]: Replace with: litre of alcohol, and

Preparation of fungal strain inocula

Inocula were prepared separately from young cultures of the 2 fungal germs to be tested. Cultures are 48 hours old for Candida albicans and 5 days old for Trichophyton rubrum. For each species of fungus, at least one or two well-isolated colonies were picked with a 2 mm

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loop and homogenized in 10 mL sterilized distilled water. This suspension gave a stock suspension rated 10°, with a load of 106 cells/mL. From suspension 10°, suspension 10^{-1} was prepared by 1/10th dilution, transferring 1 mL of suspension 10° into 9 mL of sterilized distilled water to give a final volume of 10 mL, containing 10^5 cells per mL [5,6,7,12,4]

Comment [21]: Replace with: rated at 10°

Comment [22]: Replace with: [4,5,6,7,12]

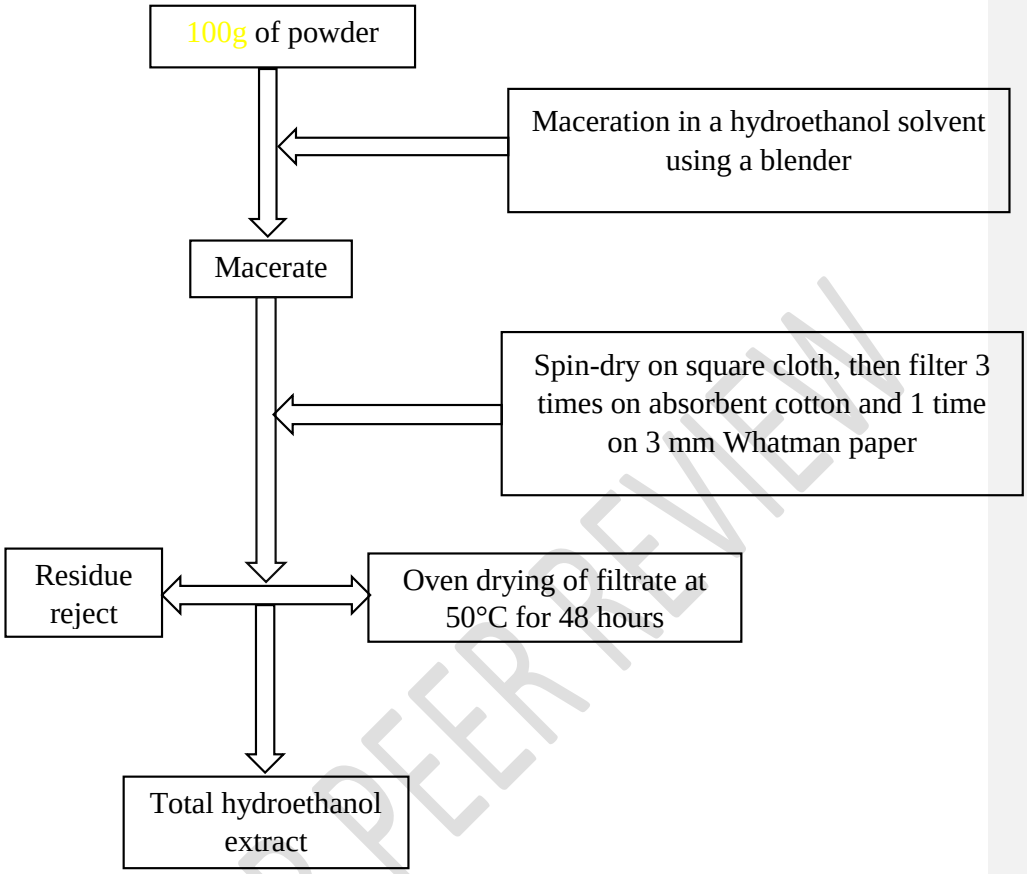

Figure 2 :Hydroethanol extraction diagram [3]

Preparation of Sabouraud agar

Sabouraud medium was prepared according to the supplier's instructions. A quantity of 42g of Sabouraud agar was homogenized in 1000 mL of distilled water. The mixture was stirred and heated on a heated magnetic stirrer (RSlab).

Double dilution test

To carry out the in vitro tests, the medium was poured into test tubes into which the extract was incorporated. This incorporation was carried out using the double dilution method in inclined tubes. For each plant extract, each series comprises 10 test tubes numbered from 1 to 10. The previously prepared medium was distributed among the 10 tubes of each series, with 20 mL in tube number. 1 and 10 mL in the other tubes. Thus, 4g for the Candida albicans test and 2g for Trichophyton rubrum were retained for all series of all extracts. This quantity was homogenized in tube n°1 containing 20 mL agar. Half the volume of this homogeneous mixture was transferred to tube n°2 containing 10 mL agar and homogenized. This operation was repeated for tube n°3 and so on by double dilution up to tube n°8.

Thus, the concentration range in these tubes varies from 200 to 1.5625 mg/mL for Candida albicans and 100 to 0.78125mg/mL for Trichophyton rubrum according to a geometric relationship of reason ½. In addition, tube 9 without plant extract is used as a control for germ growth (TC) and tube 10 without plant extract without germs is used as a control for culture medium sterility (TS). After incorporation of the extracts into the 8 test tubes, all 10 test tubes in each series were autoclaved at 121°C for 15 minutes and then tilted with small pellets at laboratory room temperature to facilitate cooling and solidification of the agar[9,10].

Antifungal tests

For each series, with the exception of the sterility control tube, 10 µL of suspension 10^{-1} (concentration 10^5 cells/mL) was inoculated in transverse streaks (until exhaustion), giving 1000 inoculated cells. The resulting cultures were incubated at 37°C for 48 hours for Candida albicans and 5 days for Trichophyton rubrum [5,6,8,4,11].

At the end of the incubation period, colonies were counted by estimation against the TC [8,4].

RESULTS AND DISCUSSION

Comment [23]: Replace with: tubes, into

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Comment [25]: Replace with: 4g for the *Candida albicans* test and 2g for *Trichophyton rubrum* were retained for all series of extracts.

Comment [26]: Replace with: n°2, containing 10 mL of agar, and

Comment [27]: Replace with: 0.78125mg/mL

Comment [28]: Replace with: *rubrum*, according

Comment [29]: Replace with: (TC), and

RESULTS

Extraction yields

Yields obtained from dried fruit at alcohol concentrations of 70%, 80% and 100% were 14.55%, 21.54% and 14.18% respectively, while those for fresh fruit at the same alcohol percentages were 8.22%, 13.36% and 10.25%.

These different yields obtained are linked to the affinity that the secondary metabolites contained in S. anguivi fruits have for the solvents used. According to [12], these differences could also be explained by the chemical nature (intrinsic) of the plant used, and above all the particle size in the plant powder as well as the solvent diffusion coefficient.

Indeed, the maceration extraction method, where alcohol and/or water, the solvents most recommended for extracting the maximum number of compounds, have the advantage of being easily eliminated, has a considerable influence on yields[13]. In addition, solvents containing alcohols are able to increase the permeability of cell walls, facilitating the extraction of a greater number of polar molecules of medium and low polarity[14]. Moreover, according to[15], the secondary metabolite content of a given plant influences its yield; due to the existence of a link with adverse climatic and collection conditions such as high temperatures, duration of solar exposure, soil type and growing season. In addition, the organ analyzed, the region, the harvest date and the degree of ripeness.

Antifungal activity of different extracts on Candida albicans

A study of the action of S. anguivi Lam with different extracts on the in vitro growth of Candida albicans showed that, after 48 hours incubation, there was a progressive decrease in the number of colonies in the experimental tubes, compared with the growth control tube, as the concentration of extract in the tubes increased.

In the sterility control tube, it was noted that no germs were present. This is a clear indication of the sterility of the culture medium used, and shows that the manipulations were carried out under ideal aseptic conditions.

The results showed that the extract inhibited the in vitro growth of the fungal strain, according to a dose-response relationship enabling minimum fungicidal concentrations (FMC) and minimum inhibitory concentrations (MIC) to be determined.

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Antifungal parameter values for fresh fruit extracts

MIC at 70%= 100 mg/mL FMC > 200 mg/mL, therefore the extract is fungistatic

MIC at 80%= 100 mg/mL FMC > 200 mg/mL, therefore the extract is fungistatic

MIC at 100% = 100 mg/mL FMC > 200 mg/mL, i.e. extract is fungistatic

Antifungal parameter values for dried fruit extracts

MIC at 70% = 200 mg/mL FMC = 200 mg/mL, therefore the extract is fungicidal

MIC at 80% = 100 mg/mL FMC = 200 mg/mL, making the extract fungicidal

MIC at 100% = 100 mg/mL FMC > 200 mg/mL, i.e. extract is fungistatic

With fresh fruit extracts, the MIC remains unchanged (100 mg/mL) and fungistatic, while with dry extract, at 70/30 and 80/20, the MFC = 200 mg/mL, giving fungicidal activity on Candida albicans. It is therefore clear that for all these extracts, the type of extraction influences the various antifungal parameters.

Figure 2 shows the six activity curves obtained from colony count data in experimental tubes for which fungal germ growth was evaluated in percentage survival determined in relation to 100% survival in the growth control tube. It allows comparison of extract activity for different extracts studied. The steeper the slope of a sensitivity curve, the more active the extract and the more sensitive the strain. In this figure, we can see that all the curves have a decreasing trend, with slopes of varying steepness.

The activity curves for 70/30 and 80/20 dry extracts show a steep slope, while the other curves show a shallow slope. These six activity curves were used to determine inhibitory concentrations for 50% germ survival (IC50).

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With regard to the performance of the extracts on the sprouts tested, FMC values varied according to the condition of the fruit and the type of extract. However, the difference in activity was at IC50 level. The ranking of extracts from most to least active is as follows

as follows: 100% alcohol, 80% alcohol and finally 70% alcohol with fresh fruit, which is very active, and 70% alcohol, 100% alcohol and 80% alcohol with dried fruit, which is less active.

IC50 values for fresh fruit extracts

IC50 at 70% = 11 mg/mL

CI50 at 80% = 10.6 mg/mL

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IC50 at 100% = 10.3 mg/mL

IC50 values for extracts from dried fruit

CI50 at 70% = 12.5 mg/mL

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CI50 at 80% = 25 mg/mL

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IC50 at 100% = 12.5 mg/mL

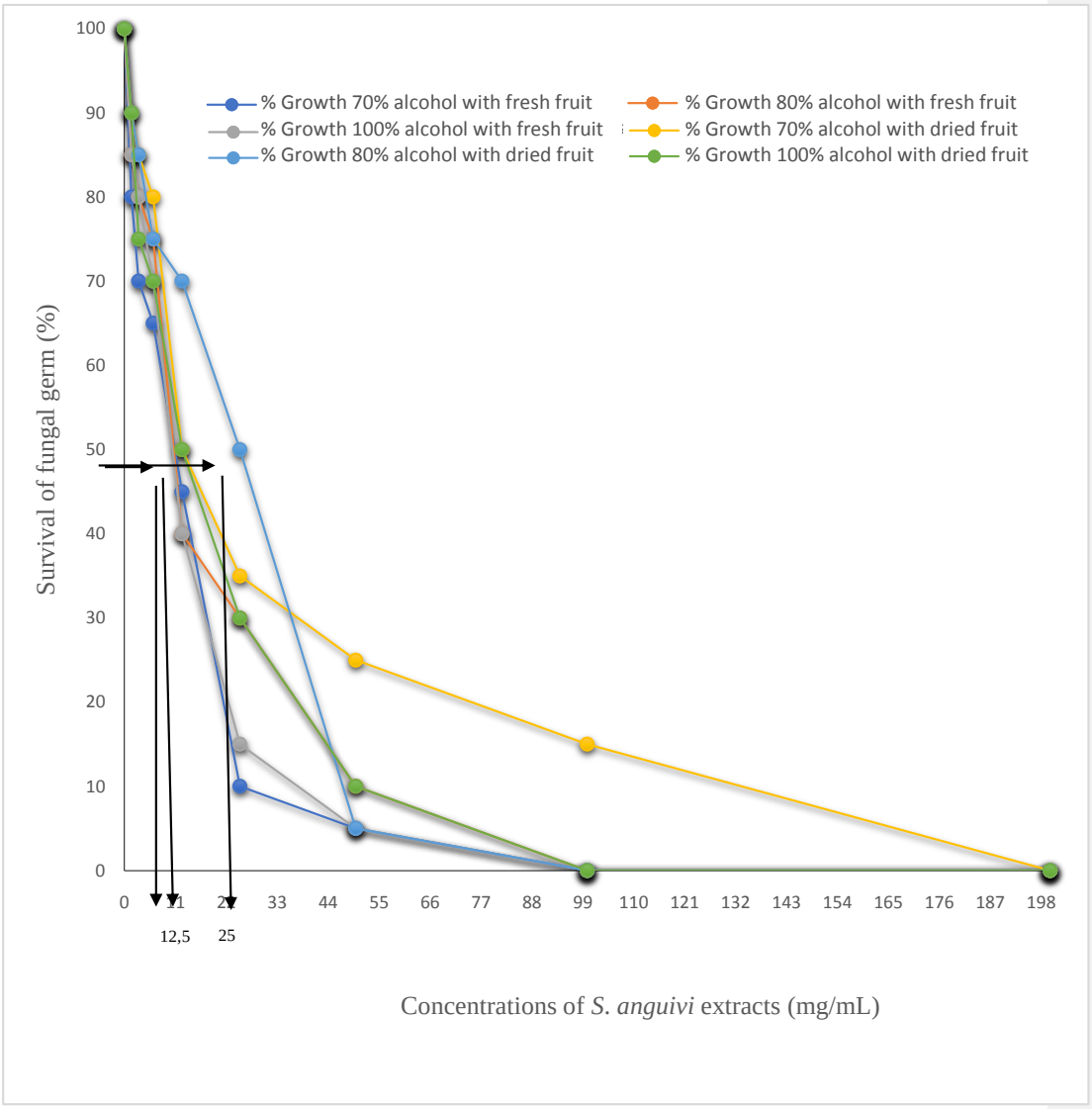

Figure 3: Activity curves for fresh and dried fruit extracts on Candida albicans

Antifungal activity of various extracts on Trichophyton rubrum

The antifungal activity of S. anguivi was carried out on a fungal species, Trichophyton rubrum. After 5 days of incubation, compared with the growth control tube, there was a progressive decrease in the number of colonies in the experimental tubes, inversely proportional to the concentration of extract in the tubes.

The absence of germs in the sterility control tube reflects the sterility of the culture medium used and shows that the manipulations were carried out under ideal aseptic conditions.

The results showed that the extract inhibited the in vitro growth of the fungal strain, according to a dose-response relationship enabling minimum fungicidal concentrations (FMC) and minimum inhibitory concentrations (MIC) to be determined.

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Also at 5 days, it was observed that in tubes with concentrations below the MIC value, the number of colonies increased, but that of the MIC tube remained stable. In other words, the extract acts according to a dose-response relationship.

Antifungal parameter values for extracts from fresh fruit

MIC at 70% = 50 mg/mL FMC is greater than 100 mg/mL, therefore the extract is fungistatic

MIC at 80% = 25 mg/mL FMC is greater than 100 mg/mL, so the extract is fungistatic

MIC at 100% = 12.5 mg/mL FMC is greater than 100 mg/mL, so the extract is fungistatic

Antifungal parameter values for dried fruit extracts

MIC at 70% = 50 mg/mL FMC = 50 mg/mL

MIC at 80% = 50 mg/mL FMC = 50 mg/mL

MIC at 100% = 100 mg/mL FMC = 100 mg/mL

With fresh fruit, the MIC varies from 50 to 12.5 mg/mL and is fungistatic, while with dry extract the MIC and FMC remain unchanged (50 mg/mL) at 70% and 80%, while at 100% the MIC is equal to the FMC = 100 mg/mL, giving fungicidal activity on Candida albicans. It is therefore clear that for all these extracts, the type of extract influences the various antifungal parameters.

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Figure 3 shows the six activity curves obtained from colony count data in experimental tubes for which fungal germ growth was evaluated as percentage survival calculated against 100% survival in the growth control tube. This allows us to compare extract activity at different extraction percentages.

The steeper the slope of a sensitivity curve, the more active the extract and the more sensitive the strain. In this figure, we can see that all the curves have a decreasing trend with slopes of varying steepness. The activity curves for 70%, 80% and 100% extracts with fresh fruit, and for 80% dried fruit, are steeply sloping. In contrast, the other curves illustrating extract activity on C. albicans have a shallow slope. These six activity curves were used to determine inhibitory concentrations for 50% germ survival (IC50).

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With regard to the performance of the extracts on the germ tested, FMC values varied according to extract type and extraction percentage. However, the difference in activity was atIC50 level. Furthermore, extracts were ranked from most active to least active in the following order: 100%, 70% and 80% of fresh fruit extracts were considered the most active, compared with 100% extracts, followed by 70% and 80% of dried fruit extracts, which were the least active.

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IC50 values for extracts from fresh fruit

IC50 at 70% = 8.9 mg/mL

IC50 at 80% = 12.5 mg/mL

IC50 at 100% = 8.6 mg/mL

IC50 values for extracts from dried fruit

CI50 at 70% = 4.9 mg/mL

CI50 at 80% = 9.3 mg/mL

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CI50 at 100% = 3.22 mg/mL

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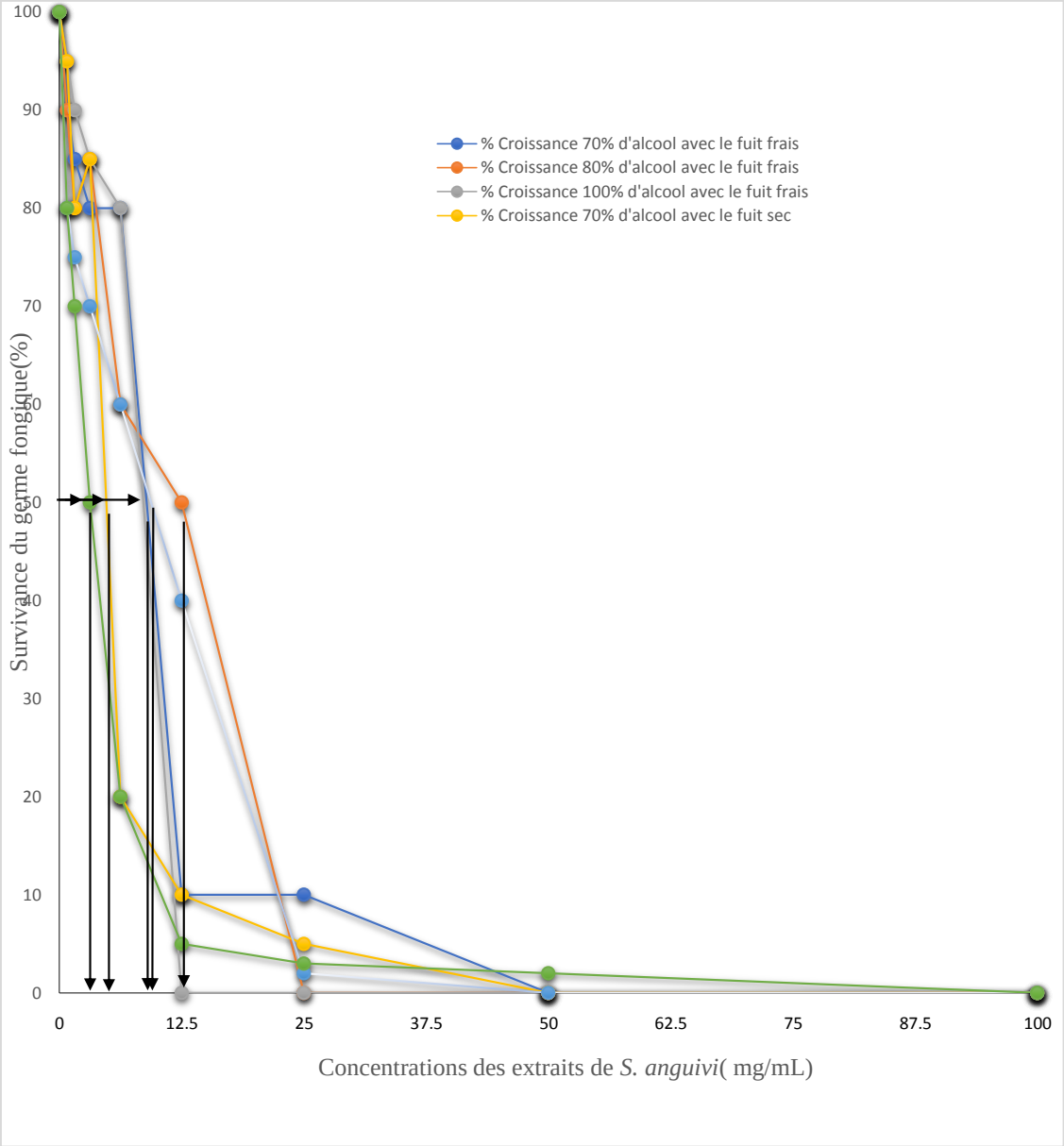
% Croissance 70% d'alcool avec le fuit frais
% Croissance 80% d'alcool avec le fuit frais
% Croissance 100% d'alcool avec le fuit frais
% Croissance 70% d'alcool avec le fuit sec
Survivance du germe fongique(%)
Concentrations des extraits de S. anguivi(mg/mL)
0
10
20
30
40
50
60
70
80
90
100
0
12.5
25
37.5
50
62.5
75
87.5
100

Figure 4: Activity curves for fresh and dried fruit extracts on Trichophyton rubrum

All the fungal species studied showed sensitivity to the various hydroalcoholic extracts. FMC values varied between 50 mg/mL and 200 mg/mL, depending on the extract. The results of the antifungal tests showed that the extracts were active on the fungal strains tested, and that this

activity was dose-dependent. The minimum inhibitory concentration values obtained show that the extracts have varying degrees of antifungal activity. Some extracts effectively inhibited the growth of certain germs at concentrations of 50, 100 and 200 mg/mL, corresponding to the FMC. On the basis of the average of the various IC50 values, dried fruit is more active on T. rubrum than fresh fruit.

On the other hand, with C. albicans, fresh fruit is also very active on these germs, taking into account the respective IC50 values, which are very close. From these IC50 values, we deduce that extracts from fresh fruit are 10.63 times more active on C. albicans than dried fruit, while dried fruit is 5.80 times more active on T. rubrum than fresh fruit. Comparison of these results with those of other studies reveals that certain extracts are more active than the hydroethanol extract of Morinda morindoides (FMC of 250000 µg/mL) on the in vitro growth of Candida albicans [16]. Also with the hydroethanolic extract of Spermacace verticillata (FMC = 100000 µg/mL) on the in vitro growth of Candida albicans [4] .

In the light of the above, we deduce that when we increase the percentage of alcohol in the extractions, we improve the antifungal activity of the extracts on both C. albicans and T. rubrum. In fact, dry extracts better concentrate the active principles of Solanum anguivi Lam.

CONCLUSION

The results obtained in this study confirm the properties.

The two fungal species studied are sensitive to S. anguivi extracts according to a dose-response relationship;

All the extracts tested have effective antifungal activity on the in vitro growth of the species studied;

T. rubrum appears to be the fungal species most sensitive to S. anguivi extracts, unlike C. albicans, which remains more or less sensitive to extracts;

REFERENCES

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The author(s) should follow the journal reference format.

[1]. **WHO, 2003** Traditional Medicine Strategy. Publication WHO, 56th World Health Assembly, 5p, www. apps.who.int/gb/archive/pdf_files/WHA56/fa5618.pdf. Consulted on 05/23/2010.

[2]. **Biyiti, L. F., Tamze V., Nnanga, N., Agbor, A.G., Gangoue-Pieboji, J., 2012**. Formulation of an antibacterial ointment based on an ethanolic extract from the bark of the trunk of the *Tabernaemontana crassa Benth*. *European Scientific Journal* 10 : 87-95.

[3]. **Maghrani, M., Zeeggwagh, N., Michel, J., et Eddoules, M., 2005.**Antihypertensive effect of *Lepidium sativum L*. in spontaeneously hypertension rats, *Journal of Ethnopharmacology*, 100(102), p.193 - 197.

[4]. **Zirihi, G. N., Kra, A. K. M., Guédé-Guina, F., 2003.**Evaluation of the antifungal activity of Microglossa pyrifolia (Lamarck) O. Kantze (Asteraceae) "PYMI" on the in vitro growth of Candida albicans, *Journal of Medicine and Pharmacy Africa*, 17, p. 11 - 18.

[5]. Ajello, L., Geord, L. K., Kaplan, W. et Kaufman, L., 1963. Laboratory manual for Medical Mycology. 2nd Ed. John Wiley and sons, New York, USA.158 p.

[6]. **Holt, R. J., 1975.** Laboratory test of antifungal drugs. *J Clin Path*, 18 : 767 – 774.

[7]. **Maury, M., 1987.** Laboratory media and reagents PASTEUR. Microbiology-immunology.3rd Ed. onaphi, Paris, France. 115 p.

[8]. **Guéde-Guina, F., Kra, K. A. M., Vangah-Manda, M., Bonga, G. M. et De Souza, C., 1997.**Inhibition by MISCA-F2 on *Aspergillus fumigatus*; *Candida albicans* and Cryptococcus *neoformans*,growth 3 opportunists fungal germs ffrom AIDS, *Afrique Biomedical*, 2, 1997, 11-16.

[9]. **Kra, A. K. M., 2001.** Evaluation and improvement by chromatographic sequencing of the antifungal action of MISCA against *Aspergillus fumigatus*. Doctoral Thesis 3rd cycle. UFR Biosciences. University of Cocody Abidjan, Ivory Coast 126 p.

[10]. **Kouakou, A. V., N'guessan, J. D., Kra A. K. M., et Guéde-Guina, F., 2007.**Antifungal activity and phytochemical screening of THOS (aqueous extract of *Thonningia sanguinea*). *Rev Med Pharm Afr*, 20 : 45 – 52.

[11]. **Dewanjee1, S., Kundu1, M., Maiti1, A., Majumdar, R., Majumdar, A., Mandal, S.C., 2007.***In vitro* evaluation of antimicrobial activity of crude extract from plants *Diospyros peregrine*, *Coccinia grandis* and *Swietenia macrophylla*. Trop J Pharm Research. 3 : 773-778.

[12]. **Djahra, A. B., 2014.** Phytochemical study and antimicrobial activity, antioxidant, antihepatotoxic of white *Marrube* or *Marrubium vulgare L*. Doctoral thesis, University of Annaba. (2014).

[13]. **Riberau-Gayon, P., 1968.** phenolic compounds in plants. *Ed.Dinod*. p173-200.

[14]. **Seidel, V., 2005.** Initial and Bulk Extraction. In : Sarker S D, Latif Z and Gray A I. Natural products isolation. *Humana Press (Totowa)*, pp: 27-37.

[15]. **Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, A., Stocker, C., Vidal, N., 2005.** Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem*. Vol. (97) : 654–660.

[16]. **Bagré, I., 2004.** Anti-infective spectrum of BGG, an antidiarrheal substance from a natural source.. Mémory of D.E.A Biotechnology, pharmacology of natural substances option, Cocody University, Ivory Coast, 29p.

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