

Antifungal Activity of *Solanum Anguivi* Lam (Solanaceae) on *Candida Albicans*

Ouattara Sitapha^{1,2*}, Kporou Kouassi Elisée³, Kra Adou Koffi Mathieu¹, Appia Franck Hermann Desire¹, YAPO Adou Francis¹, Djaman Allico Joseph^{1,4}

¹Biology and Health Laboratory, Felix Houphouët-Boigny University, Cocody, Abidjan, Ivory Coast, 22 BP 582 Abidjan 22

²National Pedagogical Institute of Technical and Vocational Education (NPITVE), Abidjan, BP 08 2098 Abidjan 08 Ivory Coast

³Department of Biochemistry and Microbiology, Research Unit of Bioactive Natural Substances, Jean Lorougnon Guede University, Daloa, Ivory Coast, BP 150 Daloa

⁴Medical and fundamental Biochemistry, Pasteur Institute / Company, Cocody, Abidjan, Ivory Coast, 01 BP 490 Abidjan 01

DOI: [10.36347/sajb.2023.v11i10.009](https://doi.org/10.36347/sajb.2023.v11i10.009)

| Received: 17.09.2023 | Accepted: 23.10.2023 | Published: 27.10.2023

*Corresponding author: Ouattara Sitapha

Biology and Health Laboratory, Felix Houphouët-Boigny University, Cocody, Abidjan, Ivory Coast, 22 BP 582 Abidjan 22

Abstract

Original Research Article

In Ivory Coast, as elsewhere in Africa, *Solanum anguivi* Lam (or *Solanum distichum*) is generally used in traditional medicine to treat bacterial and fungal infections. Given the importance of this plant, the 70%, 80% and 100% extracts of fresh and dried fruits were evaluated on the *in vitro* growth of *Candida albicans*. 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. Extracts from fresh fruit showed good activity on *C. albicans*.

Keywords: *Solanum anguivi* Lam, antifungal.

Copyright © 2023 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

INTRODUCTION

Throughout history, man has been able to rely on nature to provide for his basic needs (food, shelter, clothing) and also for his medical needs. Indeed, the use of plants for therapeutic purposes is an ancestral practice throughout the world. For thousands of years, mankind has used plants found in nature to treat and cure diseases (Sanago, 2006).

Thus, the therapeutic use of plants in the treatment of human illnesses is not only of public interest, but has also evolved with the history of mankind, according to the World Health Organization (WHO).

Some 65% to 80% of the world's population rely on traditional medicine to meet their primary healthcare needs. This practice is due to the accessibility and availability of traditional medicine, poverty, the high cost of modern medicines and the harmful side effects caused by synthetic drugs (Biyiti *et al.*, 2012).

Adjanohoun and Aké-Assi (1979) have inventoried 5,000 species of medicinal plants from the Ivorian flora in order to preserve this knowledge, but also

to enable the development of new medicines effective in the treatment of illnesses, by working on plants reputed to be active and whose properties and uses it is up to modern research to understand.

Such is the case of *Solanum anguivi* Lam (Solanaceae), a plant used in food with hepatoprotective and antimalarial virtues, according to a survey of traditional practitioners.

The aim of the present study was also to evaluate the antifungal properties of the dried and fresh fruits of the *Solanum anguivi* Lam (Solanaceae) plant on *Candida albicans*, which is involved in many candidiasis diseases.

I. MATERIALS AND METHODS

I.1 MATERIALS

Plant material

The plant material used is a powder obtained from the dried and fresh fruits of *Solanum anguivi* Lam (Figure 1). The plant was identified at the National Center of Floristic (NCF) Université Félix HOUPHOUËT BOIGNY.



Figure 1: *Solanum anguivi* Lam (Solanaceae)

Fungal strains

The fungal material was *Candida albicans* from the laboratory of the National Center of Floristic (NCF), Université Félix HOUPHOUËT-BOIGNY (Côte d'Ivoire).

Technical study material

Germs were cultured on Sabouraud agar (BIO-RAD/ Ref: 2019-07; Lot: 64406698) at acid pH 5.7. This medium (in g/L distilled water) is composed of 10 g peptone, 40 g glucose and 15 g agar.

Harvesting and drying plant fruits

Fresh fruits were obtained from vendors in Abidjan and shade-dried in a dry, well-ventilated area for around 7 days. After drying, we crushed the fruit using a blender. Fresh fruit was also crushed.

Preparation of the various hydroalcoholic extracts

Hydroalcoholic extracts (70%, 80% and 100%) of both dried and fresh fruit were prepared according to the method described by Zirihi *et al.*, 2003.

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 one 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 (Figure 2) (Zirihi *et al.*, 2003).

Preparation of fungal strain inocula

Inocula were prepared separately from 48 hours old young cultures of *Candida albicans*. At least one or two well-isolated colonies were picked with a 2 mm loop and homogenized in 10 mL sterilized distilled water. This suspension gave a stock suspension rated 10^0 , with a load of 10^6 cells/mL. From suspension 10^0 , suspension 10^{-1} was prepared by 1/10th dilution, transferring 1 mL of suspension 10^0 into 9 mL of sterilized distilled water to give a final volume of 10 mL, containing 10^5 cells per mL (Guéde-Guina *et al.*, 1997; Zirihi *et al.*, 2003).

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.

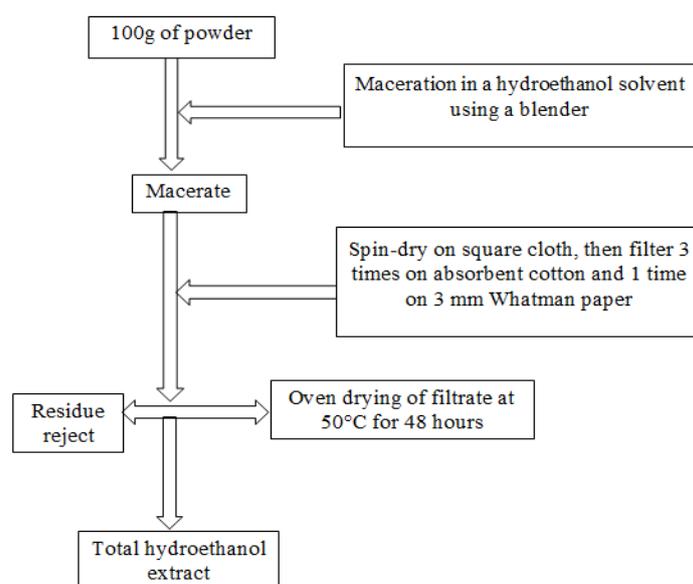


Figure 2: Hydroethanol extraction diagram (Zirihi *et al.*, 2003)

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 no. 1 and 10 mL in the other tubes. Thus, 4g were retained for all series of all extracts. This quantity was homogenized in tube n°1 containing 20 mL of 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 according to a geometric relationship of reason $\frac{1}{2}$. Test tubes 9 and 10 containing 10 mL agar are used as control tubes. Tube 9, without plant extract, is used as a control for germ growth, and tube 10, without plant extract but without germs, is used as a control for sterility of the culture medium and working conditions. 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 (Kra, 1997; Kra, 2001; Kouakou *et al.*, 2007).

Antifungal tests

For each series, with the exception of the sterility control tube, 10 μ L of suspension 10^{-1} (concentration 10^5 cells/mL) was seeded in transverse streaks (until exhaustion), giving 1000 seeded cells. The resulting cultures were incubated at 37°C for 48 hours (Guédé-Guina *et al.*, 1997; Zirihi *et al.*, 2003; Dewanjee *et al.*, 2007).

At the end of the incubation period, colonies were counted by estimation against the TC (Guédé-Guina *et al.*, 1997; Zirihi *et al.*, 2003).

II. RESULTS AND DISCUSSION

RESULTS

Extraction yields

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

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 *in vitro* growth of the fungal strain, according to a dose-response relationship enabling minimum fungicidal concentrations (MFC) and minimum inhibitory concentrations (MIC) to be determined.

In fact, after subculturing the experimental tubes corresponding to the MICs on new agar, no growth was observed after 48 h of incubation for the extracts obtained from dried fruit (70% and 80%), hence the notion of fungicide. The four remaining extracts obtained from fresh and dried fruit, for which growth was observed after transplanting, underline the notion of fungistatic. Indeed, after 5 days, tubes with concentrations below (Table I) the MIC showed an increase in colony number, while those above the MIC remained unchanged, hence the dose-response relationship.

Table I: Antifungal Parameter Values

Percentage of ethanol	MIC (mg/mL)	MFC (mg/mL)	IC ₅₀ (mg/mL)	Characteristics
DRY FRUITS				
70%	200	200	12,5	Fungicide
80%	100	200	25	Fungicide
100%	100	>200	12,5	Fungistatic
FRESH FRUIT				
70%	100	>200	11	Fungistatic
80%	100	>200	10,60	Fungistatic
100%	100	>200	10,3	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.

The Figure 3 shows the six activity curves obtained from colony count data in the experimental tubes, for which fungal germ growth was evaluated as a percentage of 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 have a steep slope, while the other curves have a shallow slope. These six activity curves were used to determine inhibitory concentrations for 50% germ survival (IC₅₀).

With regard to the performance of the extracts on the germ tested, MFC values varied according to the condition of the fruit and the type of extract. However, the difference in activity was at IC₅₀ level. Thus, the ranking of extracts from most active to least active gives the following order: 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.

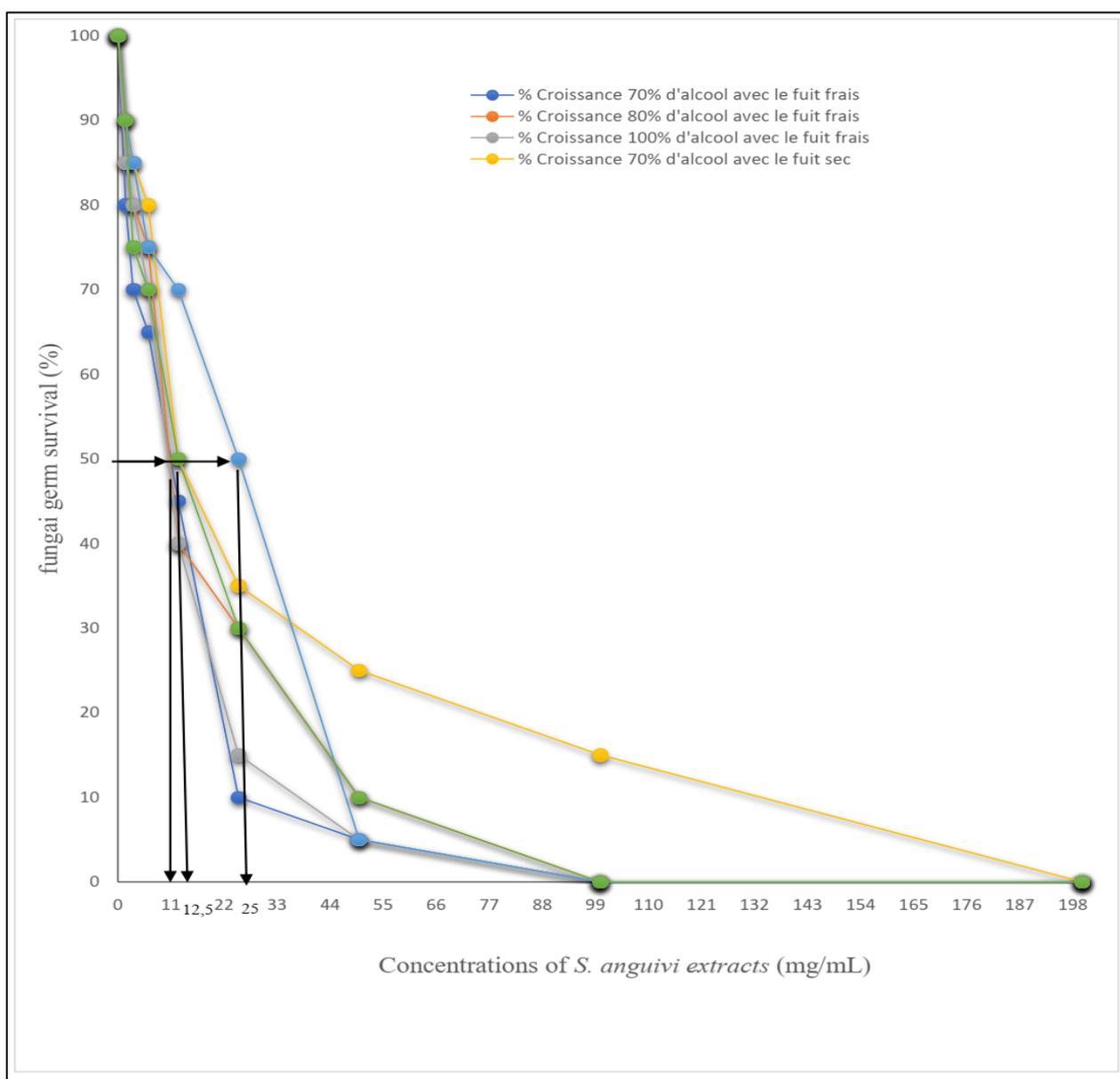


Figure 3: Activity curves for fresh and dried fruit extracts on *Candida albicans*

DISCUSSION

Preparation of the 70%, 80% and 100% hydroethanol extracts with dried *S. anguivi* fruit gave high yields of 14.55%, 21.54% and 14.18%, compared with 8.22%, 13.36% and 10.25% with fresh fruit. 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 Djahra (2014), 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 (Ribéreau-Gayon, 1968). 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 (Seidel, 2005). Moreover, according to Djeridane *et al.*, (2005), 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.

Candida albicans showed sensitivity to the various hydroalcoholic extracts. MFC values ranged from 50 mg/mL to 200 mg/mL, depending on the extract. The results of the antifungal tests showed that the extracts were active on *C. albicans*, 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 *C. albicans* growth at concentrations of 50, 100 and 200 mg/mL, corresponding to the MIF.

With *C. albicans*, fresh fruit is also very active, 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. Comparison of these results with those of other studies reveals that certain extracts are more active than the hydroethanol extract of *Morinda morindoides* (MFC=250000 µg/mL) on the *in vitro* growth of *Candida albicans* (Bagré, 2004). Also, with the hydroethanolic extract of *Spermacace verticillata* (CMF = 100000 µg/mL) on the *in vitro* growth of *Candida albicans* (Zihiri *et al.*, 2007).

In view 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 *C. albicans*. We conclude from this analysis that the extracts have good antifungal activities. In fact, dry extracts better concentrate the active principles of *Solanum anguivi* Lam.

CONCLUSION

Medicinal plants are still the most reliable source of active ingredients known for their therapeutic properties.

We can say that these results are very encouraging and demonstrate the richness of this plant in antifungal molecules. In addition, *Solanum distichum* may constitute a natural resource of new substances with biological activity.

ACKNOWLEDGMENTS

At the end this study, financial support to research and pedagogy unity of biochemical of pharmacodynamics, the national center of floristics from department of University Felix HOUPOUET BOIGNY in Cocody-Abidjan (Ivory Coast) and unit of fundamental Medical Biochemistry of Pasteur institute (in Cocody-Abidjan; Ivory Coast) are gratefully acknowledged.

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

REFERENCES

- Sanago, R (2006). The role of medicinal plants in traditional medicine. University of Bamako (Mali), 53p.
- Biyiti, L. F., Tamze, V., Nnanga, N., Agbor, A. G., & Ganguou-Pieboji, J. (2012). Formulation of an antibacterial ointment based on an ethanolic extract of *Tabernaemontana crassa* Benth trunk barks. *European Scientific Journal*, 10, 87-95.
- Adjanohoun, E. J., & Aké-Assi, L. (1979). Contribution to the census of medicinal plants in Ivory Coast. National Floristic Center of the National University of Ivory Coast. Tome 1: pp 23-30.
- Zirih, 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.
- Kra, A. K. M. (1997). Evaluation of the effects of a new natural-source anti aspergillus agent. DEA, Université de Cocody-Abidjan, Côte d'Ivoire 30 p
- Kra, A. K. M. (2001). Evaluation and improvement by chromatographic sequencing of the antifungal action of MISCA against *Aspergillus fumigatus*. Doctorat 3e cycle thesis. UFR Biosciences. Université de Cocody Abidjan, Côte d'Ivoire 126 p.
- Kouakou, A. V., N'guessan, J. D., Kra, A. K. M., & Guédé-Guina, F. (2007). Antifungal activity and phytochemical screening of THOS (aqueous extract of *Thonningia sanguinea*). *Rev Med Pharm Afr*, 20, 45-52.
- Guédé-Guina, F., Kra, K. A. M., Vangah-Manda, M., Bonga, G. M., & De Souza, C. (1997). Inhibition by MISCA-F2 of the growth of *Aspergillus fumigatus*; *Candida albicans* and *Cryptococcus neoformans*, 3 opportunistic fungal germs of AIDS, *Afrique Biomédicale*, 2, 11-16.
- Dewanjee, S., Kundu, M., Mait, 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*. *Tropical Journal of Pharmaceutical Research*, 3, 773-778.

- Djahra, A. B. (2014). Phytochemical study and antimicrobial, antioxidant, antihepatotoxic activity of Marrube blanc or Marrubium vulgare L. Doctoral thesis, University of Annaba (2014).
- Riberau-Gayon, P. (1968). Phenolic compounds in plants. Ed. Dinod. p173-200.
- Seide, 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.
- 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 Chemistry*, (97), 654–660.
- Bagré, I. (2004). Anti-infective spectrum of BGG, an antidiarrheal substance from a natural source. Memory of D.E.A de Biotechnology, Option: Pharmacology of natural substances, University of Cocody, Ivory Coast, 29p.