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ANTIFUNGAL ACTIVITIES OF SOME EXTRACTS OF STREPTOGYNA CRINITA ON IN VITRO GROWTH OF CANDIDA ALBICANS, CANDIDA TROPICALIS, CRYPTOCOCCUS NEOFORMANS AND ASPERGILLUS NIGER

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ABSTRACT

Mycoses establish one of the infectious pathologies furthermore in pus emergent especially because of its eukaryotic cellstructure.Thus he turns out to be necessary to solve this health problem by other medicinal alternatives especially that the current molecules present more and more limits. Thus the species *Streptogyna crinita*, a medicinal plant was selected to estimate its antifungal activity. Thus, by bioguided extractions, 3 extracts of which the aqueous extract (S_{Aq}), hydro-ethanol extract (S_0) and the aqueous phase of a partition hexane-water extract (SH-E_1) were tested *in vitro* growth of 4 fungal germs (Candida albicans, Candida tropicalis, Cryptococcus neoformans and Aspergillus niger). After incubation (48 hours) at 30 °C, it appears that the SH-E_1 extract has the best values of antifungal parameters. The values of MFC are 580 µg/mL (*Candida albicans and Candida tropicalis*) and 1170 µg/mL (*Cryptococcus neoformans and Aspergillus niger*). The species *Streptogyna critina* is a medicinal plant with antifungal activity. SH-E_1 extract is the most active extract.

KEYWORDS: Antifungal activity, *Streptogyna critina*, phytotherapy.

1. INTRODUCTION

Infectious diseases have become increasingly emerging in recent years. The resistance to modern drugs is also increasing.[1,2] This is the case of fungal infections that are opportunistic or primitive infections generally encountered in immunocompromised patients.[3,4,5] Of these fungal infections, *candidiasis*, *cryptococcosis* and *aspergillosis* constitute the trio that endangers the health of many individuals, especially in the developing countries.[6,7,8] Moreover, existing antifungal molecules are rare and their toxicity is often very high. It is therefore a matter of a public health problem for the clinical practitioners and governments whose main objective is the development of new drugs.[9,10,11,12]

Then it is necessary to find other alternative treatments or molecules capable of curbing this scourge. Thus medicinal plants, an inexhaustible source of therapeutic molecules, constitute a major asset in the treatment of numerous pathologies.[13,14,15] Even today, despite the evolution of science, two thirds of the pharmacopoeias have recourse to their healing properties. Thus, the new pathogens and the emergence of resistance underline the lack of means of struggle. Currently, the search for new molecules from plants is a substantial step in the development of new drugs.[16,17]

An ethnobotanical survey in the Daloa region (Ivory Coast) resulted in the selection of several medicinal plants including *Streptogyna crinita*. This species, a Poaceae, are used in the treatment of many infections and some cutaneous affection in a traditional environment, especially in the region of Daloa (Côte d'Ivoire).

The purpose of this study is to evaluate the spectrum of antifungal activity of *Streptogyna crinita* on the *in vitro* growth of 4 fungal species, they are: Candica albicans, Candida tropicalis, Cryptoccocus neoformans and Aspergillus niger.

2. MATERIAL AND METHODS

The plant material used was the powder obtained from the crushing of the leaves of *Streptogyna crinita*. It is well known for its anti-infectious properties.

Four (4) fungal isolates, including *Candida albicans*, *Candida tropicalis*, *Cryptococcus neoformans* and *Aspergillus niger*. These fungal germs were provided by the mycology laboratory of the Pasteur Institute (Abidjan, Côte d'Ivoire) taken from patients suffering from

deep mycoses. In addition, the current equipment of a microbiology laboratory has been used for biological tests.

Leaves of *Streptogyna crinita* were collected and dried at room temperature. Then, the dried leaves were crushed and the powder obtained was used for the preparation of the extracts. Then the total aqueous and hydro-ethanolic extracts were obtained according to the method described by Zirihi *et al*., in 2003[18] by homogenizing 100g of powder in a blender separately in one litre of distilled water or in hydro-ethanolic mixture. The obtained homogenate was filtered in a square of clean fabric, then filtered three times on hydrophilic cotton and once on Whatman 3 mm filter paper. The filtrates were concentrated in an oven at a temperature of 60°C. The aqueous extract is coded S_{Aq} and the hydro-ethanol extract S_0**.**

Subsequently, a hexane-water partition was made from the hydro-ethanolic extract by homogenizing for 48 hours, 20 g in a solvent mixture (hexane-water, 50/50, v/v). After decantation, separation and drying in an oven at 30°C only the aqueous phase from the hexane-water partition (SH-E_1) was obtained.

These three extracts (S_{Aq}, S_0, SH-E_1) were tested separately on the *in vitro* growth of the four (4) fungal isolates.

-Preparation and incorporation of plant extract into agar Sabouraud agar was prepared according to the manufacturer's instructions and distributed in test tubes numbered from 1 to 10 as follows: 20mL in tube N°1and 10 mL in the others.

The incorporation of the various plant extracts into this agar and for each series of tubes was made according to the double dilution method in inclined tubes[18] by homogenizing 3g of each extract in the agar of the tube N°1 Half of the volume of this homogeneous mixture was transferred into tube N°2 using a vortex and then homogenized. This operation was repeated for tube N°3, and so on to tube N°8 where half of the volume of the homogeneous mixture was rejected.

Agar plates N°9 and N°10 served as controls. Thus, the contents of the tube N°9 served as fungal germ growth control and that of tube N°10 served as control of the sterility of the culture medium (without germs and without extract).

For the 8 test tubes, the concentrations vary from 150000 to 150 µg/mL biding by a geometric ratio of 2.

After incorporation of the extracts, all 10 tubes of each series were sterilized by autoclaving at 121°C for 15 minutes, and then sloped with a small pellet at room temperature to allow their cooling and solidification of the agar.

-Preparation, seeding and counting

For each fungal organism, a colony of 48 hours was removed with the aid of a loop and then homogenized in 10 mL of sterilized distilled water and the solution obtained was concentrated to 10^6 cells/mL. This suspension was diluted to $1/10^{th}$, by transferring 1 mL in 9 mL of sterilized distilled water, thus giving a concentration of 10^5 cells/mL (suspension 10^{-1}). Subsequently, the culture of different fungal germs was done on previously prepared medium, except in the sterility control tube, by transverse striations seeding of 5 µL of the suspension 10^{-1} with the aid of a micropipette.

The cultures thus produced were incubated in an oven at 30°C for 48 hours and the numbering of the colonies of the various fungal germs was done by direct counting and then their growth in the experimental tubes was evaluated in % of survival.

The following antifungal parameters, namely the Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) were determined by transplanting the culture of MIC after 48 hours, confirms whether the MIC is equal to or different from the MFC and the Concentration for 50% inhibition (IC_{50}) determined graphically made it possible to evaluate the activity of the extracts.

The comparison of the activity of different extracts was made on the basis of the MFC values, if necessary on the basis of the IC_{50} values.

3. RESULTS AND DISCUSSION

We observed after 48hours of incubation the action of S_{Aq} on the *in vitro* growth of *Candida albicans, Cryptococcus neoformans, Candida tropicalis* and *Aspergillus niger* compared to the control tube, a progressive decrease in the number of colonies in the experimental tubes as concentration increases in the tubes.

Inhibitions were observed with the naked eye at different concentrations depending on the extracts, it is the MIC. In addition, in the various manipulations, it was verified that the MIC values are equal to those of the MFC and that the species *Streptogyna crinita* has a clear and effective fungicidal activity on the *in vitro* growth of the fungal seeds tested (Table 1).

In view of these results, the optimization of the antifungal activity was made using organic solvents according to the work of Zirihi *et al.*, 2003[18]

The action of the S_0 (hydro-ethanolic) extract on the *in vitro* growth of *Candida tropicalis*, *Aspergillus niger*, *Candida albicans* and *Cryptococcus neoformans* is summarized in Table 1.Among the total extracts, the S_0 extract concentrates better the active ingredients. Therefore, it has been selected for tests to improve antifungal activity. Its partition ($SH\text{-}E_1$) gave as the value of MFC:

- 580 µg/mL for *Candida albicans* and *Candida tropicalis*.
- 1170 µg/mL for *Cryptococcus neoformans* and *Aspergillus niger*.

Table 1: Compared values of antifungal parameters of extract (S_{Aq}, S_0, $SH\text{-}E_1$).

Extracts	Fungal germs	Antifungal parameters (µg/mL)	
		MFC	IC_{50}
S_{Aq}	*Candida albicans*	2340	828.36
	Candida tropicalis	1170	372
	Cryptococcus neoformans	1170	168.48
	Aspergillus niger	> 1170	Indetermined
S_0	*Candida albicans*	580	369.72
	Candida tropicalis	1170	378
	Cryptococcus neoformans	1170	444.6
	Aspergillus niger	1170	325
$SH\text{-}E_1$	*Candida albicans*	580	255.06
	Candida tropicalis	580	290
	Cryptococcus neoformans	1170	409.5
	Aspergillus niger	580	482.4

The dynamism of infectious vectors in the face of therapeutic agents makes all practitioners and researchers unanimously come to the conclusion that the development of new therapeutic agents is necessary. In the diversity of approaches used, the plant world remains a universe with great potential for the discovery of new substances.[13,14,15]

Among these selected plants, *Streptogyna crinita P. Beauv* is a species used mostly by traditional healers especially in the region of the upper Sassandra.

This study was conducted not only to verify the anti-infective properties possessed by this plant, and to improve this activity by bio-guided partitioning.

Initially, the aqueous extract was prepared, due to the fact that water is the solvent mostly used in the traditional recipes. The aqueous extract (S_{Aq}) presented an interesting antifungal activity (Table 1).

The comparison of these results with those of other studies carried out on *Candida tropicalis* revealed that the results on *Streptogyna crinita* are better than those obtained by Fezan *et al.*, 2007[19] with aqueous plant extracts of *Erigeron floribundus, Borreria latifolia and Borreria verticillata, Euphorbia hirta, Turraea heterophylla and Vernonia colorata*. In fact, they obtained MFC values greater than 2000 µg/mL. The same is true of the work of Kporou*et al.*, 2009[20] who found a value of MFC = 100000 µg/mL with the aqueous extract of *Mitracarpus scaber* on the *in vitro* growth of this same species. The *Streptogyna crinita* (MFC = 1170 µg/mL) S_{Aq} extract is 85 times more active than *Mitracarpus scaber*.

The comparison of the performances of this S_{Aq} extract with those of the aqueous extract of *Microglossa pyrifolia* according to the work of Zihiri *et al.*, 2003[18] having a MFC value = 6300 µg/mL with *Candida albicans* showed that *Streptogyna crinita* is 2 times more active.
It can therefore be said that the use of this plant in the traditional environment by infusion, decoction, poultice and frictions as antifungal is well-justified. These results led us to improve the activity, according to the method of Zirihi *et al.*, 2003[18]

With regard to the hydro-ethanol extract S0, the results showed that all the fungal seeds tested are sensitive to this extract (Table 1). The CMF values obtained revealed that S0 has a more or less pronounced antifungal activity compared to the S_{Aq} extract.

When comparing the activity of this S_0 extract with other extracts, the extract S_0 is better. Concerning for example the species *Candida tropicalis*, the S_0 extract (MFC = 1170 µg/mL) is 5 times more active than the hydro-ethanolic extract of *Mitracarpus scaber* (MFC = 6250 µg/mL) according to the work of Kporou *et al.*, 2009[20] Moreover, the results of *Streptogyna crinita* are better than those of Zirihi *et al.*, 2007[21] who worked on the ethanolic extract of *Mitracarpus villosus* (MV_0) and *Spermacoce verticillata* (SV_0.) whose biological tests were carried out on the isolate of *Aspergillus niger*. They obtained a value of MFC=100000 µg/mL for MV_0 and MFC = 50000 µg/mL for SV_0. S_0 hydro-ethanol extract is 85 times more active

than MV_0 and 42 times more active than SV_0.It appears from the comparison of the results of the total extracts that the active ingredients of *Streptogyna crinita* are more concentrated in the hydro-ethanolic extract. The results of the partition of this extract revealed that the SH-E_1 extract is active on the 4 fungal isolates studied.

Furthermore, the comparison of the activity of the aqueous extract from the hexane-water partition (SH-E_1) and the hydro-ethanol extract (S_0) revealed that SH-E_1 has a better activity than the extract. S_0 on *Candida albicans* and *Cryptococcus neoformans* isolates on the basis of IC_{50} values since the MFC values are identical (MFC of 580 µg/mL for *Candida albicans* and 1170 µg/mL for *Cryptococcus neoformans*). Indeed, these IC_{50}values are 255.06 µg/mL against 369.72 µg/mL for *Candida albicans* and 409.5 µg/mL against 444.6 µg/mL for *Cryptococcus neoformans*. Concerning the *Candida tropicalis* and *Aspergillus niger* germs, the comparison was made on the basis of the MFC values and it appears that the SH-E_1 partition has the lowest values of MFC (580 µg/mL). Thus the SH-E_1 extract is the extract with the best activity on the growth of the 4 fungal germs tested.

The activity of this SH-E_1 extract is better than that of the hexane extract of *Mitracarpus scaber* (Zucc) on the *in vitro* growth of other Candida species (*Candida guilliermondii and Candida parapsilosis*). The MFC values recorded by these researchers are 730 µg/mL for *Candida guilliermondii* and 5980 µg/mL for *Candida parapsilosis*[22] These results are also better than those obtained by Raj *et al.* in 2016[23] who tested the antifungal activity of several extracts of plants (*Ulva lactuca Linn. Ulva fasciata Delile and Ulva reticulate Forsk*). Indeed, *in vitro* hexanic, chloroformic, acetalic, acetonic and methanolic extracts of these plants have MFC values of between 500 µg/mL and 1000 µg/mL. The hexanic extract of the species *Streptogyna crinita* has a clearly interesting activity than that obtained with the hexane extract of *Nyctanthes arbor-tristis and Illicium verum* species (MFC = 50 mg/mL) on the growth of a fungal germ *Malassezia furfur*[24] The hexane extract obtained with *Streptogyna crinita* at this stage of our extraction has a very good antifungal activity.

4. CONCLUSION

This study is part of the research program of our laboratory. In this work, the studied plant *Streptogyna crinita* is a species to which anti-infectious properties are known and appreciated in traditional environment. This study justifies this assertion.

Moreover, the results of these investigations made it possible to understand that, with total extracts, the hydro-ethanolic extract S_0 is more active. The extract of the aqueous part of the hexane-water partition SH-E_1 has a better activity than the hydro-ethanol extract S_0.

This method of extraction allowed us to reach our objective which was not only to evaluate, but also to optimize the antifungal activity of the extracts of this species.

However, this research study must continue in order to determine the chemical compounds acting on these fungal germs and their chemical structure and later their mechanism of action in order to help develop new therapeutic molecules.

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