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Exploration of the anticandidosic activity of *Piptadeniastrum africanum* Hook (Fabaceae)

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Abstract

Aim: Natural substances which derive from plants, have multiple interests, both in the cosmetic and pharmaceutical domain. In this context, we investigated the anticandidosic activities of *Piptadeniastrum africanum*, a medicinal plant used in the Haut-Sassandra Region to treat bacterial and fungal infections.
Methods: In order to be able to exploit a possible therapeutic activity, the aqueous and ethanolic extracts 70% of *Piptadeniastrum africanum* were tested on *Candida albicans*, *Candida tropicalis* and *Candida Glabrata* using the agar diffusion technique.
Results: The 70% ethanolic extract exhibited the best antifungal activity on *Candida tropicalis* and *Candida albicans* with respective CMFs of 12.5 mg /mL and 25 mg /mL.
Conclusions: The results obtained in this study, shows the richness of *Piptadeniastrum africanum* in chemical substances and which could represent a new potential source of bioactive molecules in therapeutics.
Keywords: Antifungal activity, anticandidosic activity of *Piptadeniastrum africanum*, *Candida*, plant extract.

1. Introduction

Medicinal plants have been used as an important source of medicines for thousands of years of human history, and even today they form the basis of systematic practices of traditional medicine around the world [1]. The failure of conventional pharmaceutical treatments related to the multidrug resistance of microbial strains, the high incidence of adverse effects associated with them, the high price of drugs and the lack of health infrastructure in developing countries make The majority of the population depends on natural or complementary medicine to heal themselves [2]. Today, the discovery of drugs from plants is based primarily on the isolation of bioactive molecules [3-4]. Unfortunately, despite all the efforts made by medicine, we notice in these years that infectious diseases have experienced a strong upsurge. In fungal infections, Candidiasis is very common infections in people living with the human immunodeficiency virus (HIV) [5]. In addition, sensitivity to antifungal is found to be lower in some strains of Candida due to pressure; Medications, mutation phenomena and the strong progression of opportunistic infections [5]. In order to help people to take a real advantage of natural substances, an ethnobotanical survey was conducted with the aim of finding new bioactive molecules with antimicrobial activities from Ivorian medicinal products. As a result of this ethnobotanical survey, *Piptadeniastrum africanum* was selected because of the frequency of its use in the treatment of microbial infections in the Haut-Sassandra Region. The experimental work performed in this study consisted of extractions from

Piptadeniastrum africanum stem bark and evaluation of the antifungal activities of crude extracts of *Candida albicans*, *Candida tropicalis* and *Candida glabrata*.

2. Material and methods

2.1 Material

2.1.1 Plant material

The used plant material is made of the trunk bark of *Piptadeniastrum africanum* collected in the Department of Issia (Côte d'Ivoire), whose identification was made by the National Center of Floristry of Côte d'Ivoire.

2.1.2 Biological material

The biological material includes three *Candida* strains *Candida albicans*, *Candida tropicalis* and *Candida glabrata* (opportunistic fungi largely responsible for candidiasis). The three strains of *Candida* were provided by the mycology department of the UFR of Medical Sciences of Felix HOUPHOUËT-BOIGNY University (Abidjan, Ivory Coast)

2.2 Methods

2.2.1 Preparation of extracts

The stem bark of *Piptadeniastrum africanum* was harvested, cut and dried out of the sun. After drying, these barks were crushed and made into powder. To obtain the total aqueous and ethanolic extracts, the powder of the bark of *Piptadeniastrum africanum* was extracted as follows: 100 g of powder of the bark are introduced into a beaker of 200 mL. We added 1 liter of distilled water. The resulting mixture is homogenized in a blender for 10 minutes [6]. The homogenate obtained is filtered on hydrophilic cotton and then on Wattman paper (3 mm). The obtained filtrate is concentrated using a rotary evaporator Bücher type at a temperature of 60°C, which allowed us to have the total aqueous extract (TAE). Five grams (5 g) of the total aqueous extract are constituted and separately submitted by partition in 100 ml of 70% ethanol. After decantation, the different phases were separated and concentrated using a rotary evaporator. We obtain respectively the following extracts: the ethanolic phase, called the 70% ethanolic extract and the residual phase resulting from the ethanol-water partition, called the residual extract. The 70% ethanolic and aqueous extracts were tested separately on the *in vitro* growth of the three fungal strains.

2.2.2 Preparation of culture medium

The cultures of *Candida albicans*, *Candida tropicalis* and *Candida glabrata* were made on medium Sabouraud Chloramphenicol Agar (HIMEDIA / Ref: M 1067-500G Lot 0000215703). The incorporation of the different plant extracts to Sabouraud agar was made according to the double dilution method, in bent tubes [6-7]. All extracts (aqueous, ethanol) were tested separately. Each series contains for each plant extract 10 test tubes containing plant extracts and 2 control tubes including a test without plant extract, used as growth control of the germs, the other without germs and without extract serving as a control of the sterility of the medium culture. For the 10 test tubes, the concentrations vary from 50 mg/mL to 0.097 mg/mL according to a ½ reason geometric connection. After incorporation of the extracts, all 12 tubes of each series are sterilized by autoclaving at 121 °C for 15 minutes and then slanted at room temperature to allow their cooling and solidification of the agar [8].

2.2.3 Performing antifungal tests

The inoculum was prepared from the young cultures of the three *Candida* strains (48 hours old). The initial suspension (called 100) concentrated at 106 cells/mL was first prepared, by homogenization of a colony of *Candida albicans* in 10 ml of sterile distilled water. From suspension 100, a second suspension (10^{-1}) was prepared by dilution to 1 /10^{th} of the first. The latter was concentrated at 105 cells/mL. For each of the test tubes of each series of the three extracts (aqueous, ethanolic), the culture of the seeds were made on media previously prepared by inoculation into transverse streaks (until exhaustion) of 10 µl of the suspension. This corresponds to 1000 seeded cells.

The cultures thus produced were incubated at 300°C. After 48 hours and 72 hours of incubation at 30°C, the *Candida albicans* colonies were counted by direct counting using a colony counter pen. Growth in the experimental tubes of each series was evaluated as percent survival, compared to 100% survival in the growth control control tube [6,9].

The processing of the experimental data made it possible to determine the antifungal parameters (MIC, CMF, IC_{50}). To check the fungicidy of the extracts, all the tubes from the MIC were transplanted into new test tubes containing Sabouraud Chloramphenicol Agar medium and incubated under the same conditions as previously for 72 to 120 hours. At the end of the incubation time, each extract is evaluated as fungicidal if no colony is present on the surface of the new agar, otherwise the plant extract is declared fungistatic[10-11].

3. Results

3.1 Anticandidosic activity of the total aqueous extract of *Piptadeniastrum africanum*

The results of the evaluation of the antifungal activity of the total aqueous extract of *Piptadeniastrum africanum* on *Candida albicans*, *Candida tropicalis* and *Candida glabrata* were translated as a survival curve in Figure 1. The survival curves of *Candida albicans*, *Candida tropicalis* and *candida glabrata* are decreasing in appearance and far from the y-axis. The IC_{50}, MIC and CMF parameters determined are greater than 50 mg/mL (Table I).

3.2 Anticandidosic activity of the ethanolic extract 70% of *Piptadeniastrum africanum*

For these three germs, the survival curves are decreasing with average slopes (*Candida albicans* and *Candida tropicalis*) except for the survival curve of *Candida glabrata*, which is very far from the ordinate axis. The survival curve of *Candida tropicalis* is a little closer to the y-axis than that of *Candida glabrata* (Figure 2). *Candida tropicalis* is therefore more sensitive to ethanolic extract than *Candida glabrata*. The parameters IC_{50}, MIC and CMF are shown in Table I.

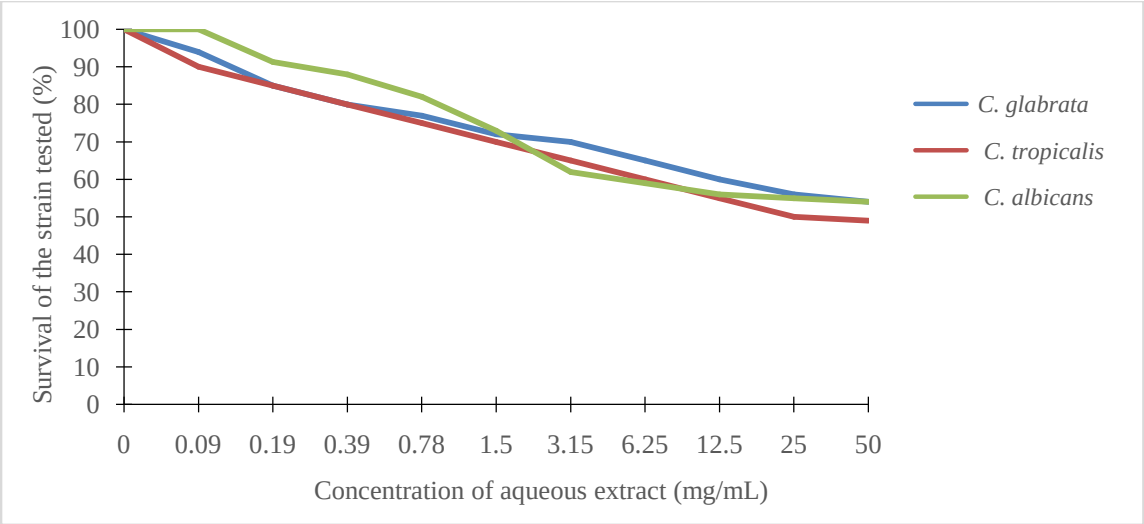

Figure 1: Susceptibility of *Candida albicans*, *Candida tropicalis* and *Candida glabrata* to the total aqueous extract of *Piptadeniastrum africanum*

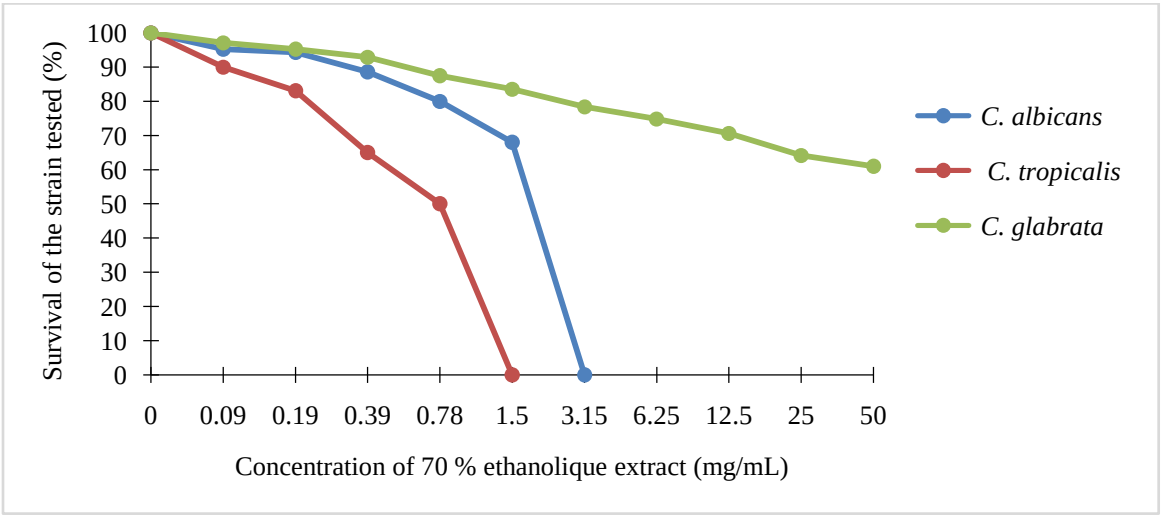

Figure 2: Susceptibility of *Candida albicans*, *Candida tropicalis* and *Candida glabrata* to ethanol extracts 70% *Piptadeniastrum africanum*

Table I: Values of the antifungal parameters of the total aqueous and ethanolic extract 70%

Plant species	Fungal germ	Different extracts	Value of the anticandidosic parameters in mg /mL			Fungicidal
			MIC	IC_{50}	CMF	
Piptadeniastrum africanum	*Candida albicans*	TAE	> 50	> 50	> 50	Fungistatic
		70 %EE	3,15	2	25	Fungicide
	Candida tropicalis	TAE	> 50	> 50	> 50	Fungistatic
		70 %EE	1,5	0,78	12,5	Fungicide
	Candida glabrata	TAE	> 50	> 50	> 50	fungistatic
		70 %EE	> 50	> 50	> 50	Fungistatic

4. Discussion

The different extracts used in this work were prepared from water. The choice was motivated by the fact that water is the main solvent used for preparations in traditional medicine. In the second part of our study, due to the lack of activity with the aqueous extract, we improved the activity of the aqueous extract by using another solvent for extraction. Considering the work of Zirihi *et al* [6], we chose 70% ethanol as extraction solvent. The analysis of the results of the antifungal tests with the extracts of *Piptadeniastrum africanum* shows that *Candida tropicalis* and *Candida albicans* are only sensitive to the tested ethanol extract (*Candida tropicalis* CMF_{EE}70% = 12.5 mg / mL and *Candida albicans* CMF_{EE} 70% = 25 mg / mL). When *Candida glabrata* is resistant to both extracts tested, For the ethanolic extract, our results show that there is a gradual decrease in the number of colonies as a function of the increase of the concentration of the extract in the tubes. Analysis of the results with the antifungal parameters (CMF and IC_{50}) of the 70% ethanolic extract of *Piptadeniastrum africanum* confirms that *Candida albicans* and *Candida tropicalis* are only the most sensitive strains to the ethanolic extract tested. Moreover, the efficacy ratio established on the basis of the CMF and IC_{50} values, shows that the 70% ethanolic extract is more active than the aqueous extract of *Piptadeniastrum africanum*. A difference in composition between the two extracts, related to the extraction method used according to Thagara *et al* [12] could explain these results. This observation is supported by several works including those of other researchers [13-14] which showed that ethanol allows a better concentration of the principles assets compared to ETA. According to these authors, when the total aqueous extract is changed to the ethanolic extract, certain chemical groups are eliminated and others are concentrated. This is the case of less polar derivatives such as terpenes, the presence of which in sufficiently high proportion is reported after a phytochemical study of the different extracts studied [15]. A comparative analysis of our results on *Candida albicans* with those of previous work carried out on the same fungal strain showed that 70% ethanolic extract of *Piptadeniastrum africanum* has a better anticandidosic activity than extracts of *Ceibapentadra* (CMF = 50 mg /mL), *Entandrophragm acylindricum* (CMF = 50 mg /mL) and *Kaya ivoirensis*(CMF = 100 mg /mL). Moreover the comparison of our results with those of Zirihi *et al* [6] reveals that the 70% ethanolic extract of *Piptadeniastrum africanum* is 2 times more active than the hydroalcoholic extract of *Microglos sapyrifolia* (CMF = 50 mg /mL). But less active than extracts of *Terminalia superba* (CMF = 0.39 mg /mL) and *Terminalia catappa* (CMF = 0.78 mg /mL) respectively obtained by Ackah *et al* [7] and Coulibaly *et al* [10].

Then a comparative analysis of our results on *Candida tropicalis* with those of previous work carried out on the same fungal strain showed that the 70% ethanolic extract of *Piptadeniastrum africanum* has the same anticandidic activity as the Bersamine ointment (CMF = 12.5 mg /mL).

5. Conclusion

The 70% ethanolic extract of *Piptadeniastrum africanum* exhibited relatively high anticandidosic activity on strains of *Candida tropicalis* and *Candida albicans*. It is of practical interest in the fight against mycosis, which is strongly resurging.

Conflict of Interest:

We do not have any conflict of interest.

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