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Optimization of the in vitro antifungal activity of hydroalcoholic extract of *Terminalia ivorensis A. Chev.*

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ABSTRACT

In an effort to help people get real benefit of the effectiveness of medicinal plants which are less expensive and affordable, an hydroalcoholic extract of Terminalia ivorensis coded TEKAM2 has been improved and the extracts obtained were tested on the in vitro growth of Candida albicans and Aspergillus fumigatus. The antifungal tests were performed on Sabouraud medium in which the plant extract were incorporated according to the method of inclined tube double dilution. Concentrations ranging from 390 to 1.52 µg /mL. The results showed that all extracts tested have led to a significant inhibition of in vitro growth of C. albicans and A. fumigatus. Among these extracts, the fraction F_8 has the best antifungal activity with the lowest antifungal parameter values on C. albicans (MFC = 6.09 µg /mL and IC_{50} = 1.8 µg /mL) and A. fumigatus (MFC = 3.04µ g / mL and IC_{50} = 0.36µ g /mL). Fraction F_8 is more active on the latter strain.Therefore Fraction F_8 concentrates the active molecules of TEKAM 2 better.

Keywords: Anti*fungal*, TEKAM 2, *Terminalia ivorensis*

INTRODUCTION

In recent year's infectious diseases showed strong upsurge in the West African sub region. Among these infections, candidiasis, cryptococcosis, aspergillosis are fungi with sharp progression **[6, 9, 17]**. Several factors are responsible for that situation. Among them, state poverty causing lack of adequate health facilities, lack of qualified medical personnel, lack of diagnostic tools, self-medication and drug abuse can be mentionned. The advent of HIV /AIDS can also be added to these factors

Indeed, HIV / AIDS poses therapeutic problems and carried with it a large number of opportunistic infections associated with immunosuppression. Despite the existence of drugs against fungal infections, therapeutic failure rate is high **[5, 7, 8]**.

All these factors have led the people majority are financially poor to turn towards the utilization of plants in the pharmacopoeia to treat their ailments **[3, 11, 16]**. Indeed, the use of medicinal plants by the people existed since ancient traditions and over 80% of people use these plants for their primary care **[7]**. However abusive use of these

medicinal plants exposed them to various accidents (renal failure, heart disease, various intoxications). In order to help these people to get real benefit without any risk from the use of medicinal plants, our team began for over a decade research work to extract the active ingredients of many medicinal plants in order to identify the healing properties granted to these plants and to establish a scientific basis for the use of these plants in the pharmacopoeia. Among the most used plants by traditional healers include *Terminalia ivorensis* which is used for its antidiarrhea, antidiabetics, antihypertensives, antiparasitic and anticough virtues. It is also used to treat oral and skin infections **[4, 10, 18, 15]**.

The aim of this work is to verify the validity of the anti-infective virtues granted to *Terminalia ivorensis* and improve the antifungal activity of a defattened hydroalcoholic extract of this plant against *C. albicans* and *A. fumigatus*.

MATERIALS AND METHODS

Microbial isolates used

Strains of *Candida albicans* (No. 896/AB of 10.01.2000) and *Aspergillus fumigatus* (No. 896/AB of 10.01.2000) have been provided by the Department of Mycology of the Faculty of Medical Sciences, University of Félix Houphouët Boigny-Abidjan. These strains were isolated from people living with HIV (PLHIV) in the Department of infectious diseases at University Teaching Hospital Treichville in Côte d'Ivoire.

Preparation of plant extracts

The plant material used is a powder coded TEKAM2, obtained from the bark of the trunk of *Terminalia ivorensis*. These barks were collected from the campus of the University of Nangui-Abrogoua, Abidjan. These barks were collected, washed, dried under shade at a temperature between 25 and 27 ° C and made into a fine powder using an electric grinder type IKA-MAG. Hundred grams of powder were macerated in ethanol (70%) by homogenization in a blender. The homogenate obtained was filtered twice on cotton wool, then once on Whatman 3MM paper. The filtrate is evaporated to dryness with a rotary evaporator Büchi type at 60°C, giving 70% ethanolic extract of mass 30grm noted X_0 **[19]**. X_0 was defatted with 1 L of hexane soxhlet and it gave a residue of 27 grams in the cartridge coded X_{42}. Then 3.21 grams of X_{42} was chromatographed on Sephadex G25 gel column with a height of 50 cm and diameter of 1 cm. The mobile phase was distilled water and the flow velocity was 0.125 mL / min. Nine fractions of 10 mL each numbered 1 to 9 are collected separately and concentrated using a rotary evaporator Büchi type. The antifungal activity of nine fractions evaluated against C. *albicans* and *A. fumigatus*.

Preparation of culture media

The antifungal tests were performed on Sabouraud culture medium (Bio-RAD/Réf: 64494, Lot: 7A2211). The incorporation of plant extract was made according to the method of double dilution in inclined tube. The series consisted of 11 test tubes with 9 test tubes containing the plant extract and 2 control tubes. Of these two tubes, one without plant extract served as control for the growth of the germs , while the other germ-free and without extract served as a control for controlling the sterility of the culture medium. The range of concentrations of the extract in the tubes ranged from 390 to 1.52 µg / mL with a geometrical connection in order of ½. All tubes were autoclaved (121°C for 15 min.), Then inclined to small base at room temperature to allow cooling and solidification of the medium.

Antimicrobial test

Culture of germs on previously prepared medium was made by seeding 1000 cells of each strain of *C. albicans and A. fumigatus*. The cultures thus produced were incubated at 30 ° C for 48 hours. After this incubation time, colonies were counted by direct counting with a colony counting pen (CEINCEWARE serial number 23382) and the growth in the 10 experimental tubes was assessed by percentage of survival, calculated with reference to 100% survival in the control tube growth control. The processing of these data allowed not only determining minimum fungicidal concentrations (MFC), but also to draw the extracts activity curves and graphically determines the concentrations for 50% inhibition (IC_{50}).

RESULTS

The nine fractions have certainly antifungal activity with MFC on *C. albicans and A. fumigatus* vary from 390 µg / mL to 6.09 µg / mL and 48.75 µg / mL to 3.04 µg / mL, respectively (Table 1). Also, fractions F_3, F_5, F_7 and F_8 each

have MFC less than or equal to X_{42} (MFC = 12 µg / mL) on *C. Albicans*, but on *A. fumigatus*, all MFC obtained (F1 to F9) are lower than that of X_{42} (48.75µ g / mL) (Table 1). However, only the fraction F_8 have the best antifungal activity with lowest antifungal parameter values on *C. albicans* (MFC = 6.09 µg / mL and IC_{50} = 1.8 µg / mL) and *A. fumigatus* (MFC = 3.04 µg / mL and IC_{50} = 0.36 µg / mL).

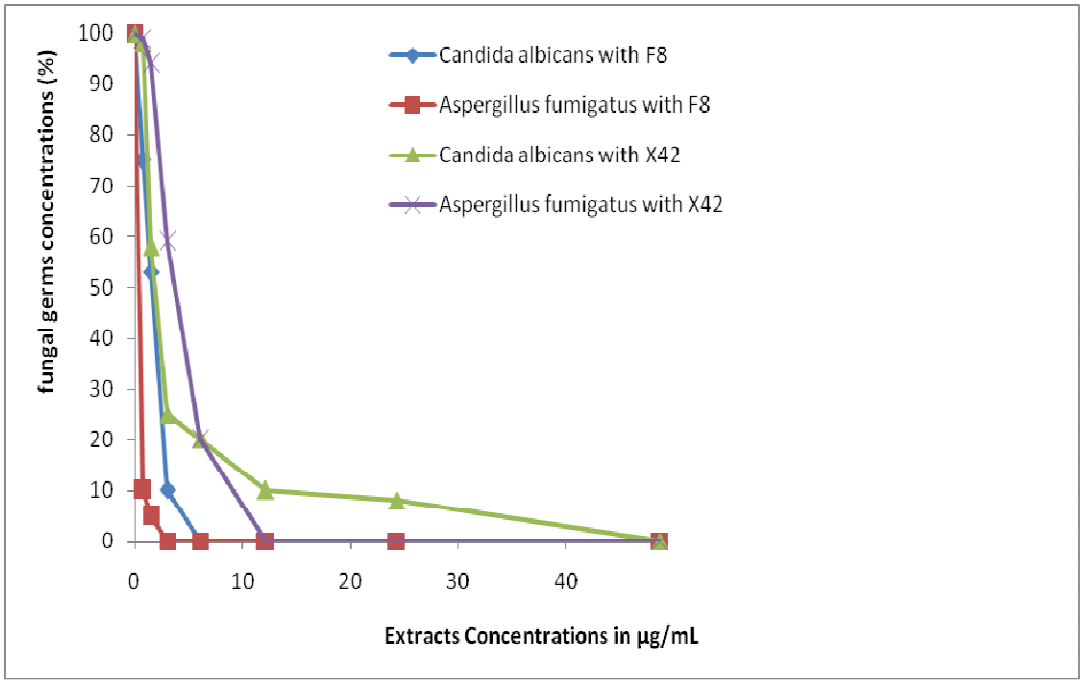

Figure 1: Antifungal activity of the extract X_{42} and fraction F_8 of *Terminalia ivoriensis* against *Candida albicans* and *Aspergillus fumigatus*

Table 1: antifungal Parameter Values of fractions from X_{42} of *Terminalia ivorensis* on strains of *Candida albicans* and *Aspergillus fumigatus*

Extracts of *T. ivorensis*		*Candida albicans*		*Aspergillus fumigatus*	
		MFC (µg/mL)	IC_{50} (µg/mL)	MFC (µg/mL)	IC_{50} (µg/mL)
chromatographic fractionation from X_{42}	X_{42}	12.18	2.64	48.75	4.56
	F_1	390	114	6.09	1.56
	F_2	195	40	3.04	0.96
	F_3	12.18	0.9	3.04	0.54
	F_4	48.75	8	3.04	1.02
	F_5	12.18	3.75	6.09	1.62
	F_6	48.75	8	3.04	0.96
	F_7	12.18	2	3.04	0.9
	F_8	**6.09**	**1.8**	**3.04**	**0.36**
	F_9	48.75	4.8	3.04	0.96

In all experimental tubes, different fractions gave a clear and effective inhibition of the growth of both strains. Compared to the growth control tube a gradual decrease in the number of colonies was observed as the concentration of fractions increases gradually. However, only the results of fraction F_8 (the most active fraction) are represented as a graph of activity in the two strains (Figure 1). In general, the two curves show a decreasing pace with slopes varying values depending on the strain. The activity curve of fraction F_8 on *A. fumigatus* has a relatively steep slope with respect to that of *C. albicans*.

DISCUSSION

Different antifungal parameter values observed with 9 fractions showed that these fractions have a real antifungal activity. This activity is higher with the fraction F_8 on the basis of MFC values and IC_{50} determined. However, the equation MFC $_{Candida\ albicans}$ / MFC $_{Aspergillus\ fumigatus}$ = 2, shows that F_8 is 2 times more active on *Aspergillus fumigatus* than agaist *Candida albicans*. In addition, based on the values of MFC, F8 is 16 times more active than the defattened hydroalcoholic extract of X_{42} on *Candida albicans* and 32 times more active against *Aspergillus fumigatus*. Ultimately, F_8 highly concentrated the active ingredients of X_{42}. Compared to the work of **[13]** who found a value of MFC = 781 µg / mL on *C. albicans* with fraction of F_8 a Rubiacea coded MISCA, the fraction F_8 of TEKAM 2 of this study is 128 times more active than MISCA. Fraction F_8 of TEKAM 2 is 3 times more active than *Terminalia catappa* (MFC = 20µg / mL) against *Candida albicans***[1]**.

The decrease in the number of colonies as these fractions increased in the experimental tubes shows that the two fractions TEKAM act against *A. fumigatus* and *C. albicans* in a dose-response relationship. The method of direct defatting soxhlet used in this study followed by chromatographic fractionation is a good method to concentrate the active ingredient of TEKAM2.

CONCLUSION

This study confirms antifungal activity of *Terminalia ivorensis* against *A. fumigatus* and *C. albicans*. The hydroalcoholic extract of defattened X_{42} is certainly active on *A. fumigatus* and *C. albicans*; however, 9 fractions from the fractionation of the residue X_{42} has helped to improve the antifungal activity. Also, the fraction F_8 presented the best antifungal parameter values against both fungal strains studied; *Aspergillus fumigatus* is the most sensitive strain. Extraction method as described by **[19]** associated with defatting with hexane followed by chromatographic fractionation on Sephadex G_{25} yielded a fraction (F_8), which concentrated more one or more chemical compound responsible for the antiaspergillar and anticandidosic activity. This study justifies the use of TEKAM 2 to treat skin disorders by many traditional healers.

Perspective, further studies of phytochemical screening of the fraction F_8 followed by thin layer chromatography and NMR will identify the nature and able to isolate the active molecule responsible for the antifungal activity.

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