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RESEARCH ARTICLE

COMPARATIVE STUDY CHROMATOGRAPHIC FRACTIONS ACTIVITIES FROM *TERMINALIA IVORENSIS* AND KETOCONAZOLE AS STANDARD ANTIFUNGAL ON *IN VITRO* GROWTH OF *TRICHOPHYTON MENTAGROPHYTES VAR. INTERDIGITALE*

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ABSTRACT

The present study was undertaken to evaluate *in-vitro* antifungal activity of Ketoconazole and extracts from trunk's barks of *Terminalia ivorensis* A. Chev (Combretaceae). *In vitro* antifungal activity of all the extracts was done by agar slant. Extracts were incorporated in Sabouraud medium culture and extract has been inserted to this medium according to the Agar slant method and Ketoconazole were used as standards for antifungal assay. Antifungal activity was determined by diminution of mushroom in the assay tubes. For each extract five tests were done. Antifungal activity was more pronounced against *Trichophyton mentagrophytes var. interdigitale.* The fraction F_8 showed best antifungal activity. *T. ivorensis* barks extracts showed better antifungal activity than a Ketoconazole. Demonstration of antifungal activity of *T. ivorensis* provided the scientific basis for the use of this plant in the traditional treatment of diseases and may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious and cutaneous diseases. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the treatment of various infections caused by opportunists' mushrooms.

Keywords: *Terminalia ivorensis*, Antifungal Activity, chromatographic fractionation, delipidation.

INTRODUCTION

The infectious diseases became extensive. Among them, candidosis, cryptococcosis, aspergillosis are mycosis in progression (Chabasse, 1994; Dromer and Dupont, 1996; Kra, 2001; Rosenhein and Itoua-N, 1989). This situation is related to several factors of which most projecting are:

- lack of adequate medical structures;
- qualified staff shortage;
- lack of tools for diagnosis;
- self medication;
- bad using of the drugs;

Even advent of HIV in human viral pathology in the years 1980 which had the most negative impact on medical evolution in under African western area. Indeed, problem of aids which carry away propagation of a great number of opportunist infections. In spite of medical drugs against mycosis, therapeutic rate of failure is high (Belhadj *and al*., 1994; Dupont *and al*., 1990).

The inefficiency of current treatments led populations stripped to direct itself towards pharmacopeia plants for their cure (Adjanohoun and Aké-Assi, 1979; Ebrahim, 2003; Lorougnon, 1995; Pousset, 1989; Zirihi, 1991).

In fact, medicinal plants use by populations exists since old times. More than 80% of populations use plants for their primary health care (Karou *and al*., 2006**)**. However the badly using of medicinal plant could have health accidents (renal insufficiency, cardiopathies, and intoxications).

To help populations from medicinal plants use, our team had work to extract active principles from medicinal plants by checking their therapeutic virtues and in order to give them scientific basis. Among many plants requested by faith healers, *T. ivorensis* (Combretaceae) is used against diarrheal, diabetes, hypertension, parasites and coughs. This plant is also used in treatment of cutaneous infections, buccal and teeth infections. To check these anti-infectious virtues, antifungal activities of hydroalcoholic extract of *T. ivorensis* is improved on the *in vitro* growth of *Trichophyton mentagrophytes var. interdigitale*.

MATERIAL AND METHODS

Plant Material

The Plant is a powder obtained from trunk's barks from *T. ivorensis* (Combretaceae) codified TEKAM 2. These barks were collected in the Nangui Abrogua University area (Abidjan-Côte d'Ivoire).

Extraction

The barks were collected, washed, dried with sun's shelter at a temperature between 25 and 27°C and were returned out of powder fine with an electric crusher of IKA-MAG type. Hundred (100) grams of this powder were extracted in a mixture from solvent with 70% from ethanol and 30% from water by homogenization in Blender. After six (6) cycles of homogenization, the homogenate obtained was dried in a white fabric and was filtered successively twice on cotton and once on paper whatman 3mm. The filtrate was concentrated with a rotary evaporator of BÜCHI at

60°C (Zirihi *and al*., 2003). This gave hydroalcoholic extract called X_0. Then, a portion of 10g from X_0 had been delipided with the soxhlet with hexane. A vegetable oil codified X_{41} is obtained and also a non hexano-soluble residue called X_{42}. 3,21 g of X_{42} extract was then chromatographied on freezing Sephadex G_{25} column (50 cm height with a diameter of cm). Distilled water was the phase's mobile and the rate of the flow is 0,125 mL/min. Nine (9) fractions of 10 mL each one (1 to 9) were collected which had been concentrated separately using a rotary evaporator of BÜCHI type. All extracts obtained from X_0 were tested separately on the *in vitro* growth of *T. mentagrophytes var. interdigitale*.

Microorganism Studied

T. mentagrophytes var. interdigitale weas provided by mycology department from Medical Sciences of University Félix Houphouët Boigny-Abidjan. Germ was taking on aids patients from infectious diseases service of Treichville's hospital (Côte d'Ivoire).

Antimicrobial Assay

Fungal germs culture on medium previously prepared was made by sowing of 1.000 cells of *T. mentagrophytes var. interdigitale*. The cultures were incubated at 30°C for 7 days. After that, germs were counted with germs pen meter. The growth in the 10 experimental tubes was evaluated on percentage survival, compared to 100% of survival in the pilot tube of growth control. Data processing permit to see fungicidal concentration minimal (FMC) values and determine 50% of inhibition concentration (IC_{50}) values graphically (on extracts activity curves).

RESULTS AND DISCUSSION

Results

The tests of the nine (9) fractions resulting from the X_{42} TEKAM 2 extract gave the antifungal values of parameters consigned in **table I**.

Table I: Antifungal values of the parameters of the extracts resulting from extract hydroalcoholic X_0 from TEKAM 2 and from Ketoconazole at 7 days of incubation at 30° C.

	Trichophyton mentagrophytes var. interdigitale										
	X_{42}	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F8	F_9	Ketoconazole
FMC (µg/mL)	48,75	97,5	48,75	48,75	48,75	48,75	24,37	24, 37	6,09	24,37	97,5
IC_{50} (µg/mL)	10	3,78	0,96	2,5	2,5	2,5	0,96	1,08	1,08	19,8	9,13

Among them, by taking account of the values of FMC, three groups of fractions were distinguished:

- Those whose values of the FMC are identical to X_{42} extract from TEKAM 2 having been used for the chromatography, these are the fractions F_2, F_3, F_4 and F_5;

- Those whose values of the FMC are higher than that of the X_{42} extract, it is about the F_1 fraction;

- And finally those whose values of the FMC are lower than that of the X_{42} extract, these are the fractions F_6, F_7, F_8 and F_9. In this last group only the F_8 fraction has the lowest values of antifungal parameters with a value of FMC = 6,09 µg/mL and IC_{50} = 1,08 µg/mL. In comparison with the performances of the F_8 fraction it was retained for a comparative study with as standards for antifungal assay (Ketoconazole) which has given on *T. mentagrophytes var. interdigitale* the following values of antifungal parameters FMC = 97,5 µg/mL and of IC_{50}= 9,13 µg/mL. In a general way, after 7 days of incubation to 30°C, compared to the pilot tube of control of growth a progressive reduction in the number of colonies was observed as the concentrations of the fractions and Ketoconazole increased in the experimental tubes. The data relating to the F_8 fraction and Ketoconazole translated in the form of curves (**figure 1**) took a decreasing form. However, the slope of the curve of the F_8 fraction is stronger than Ketoconazole.

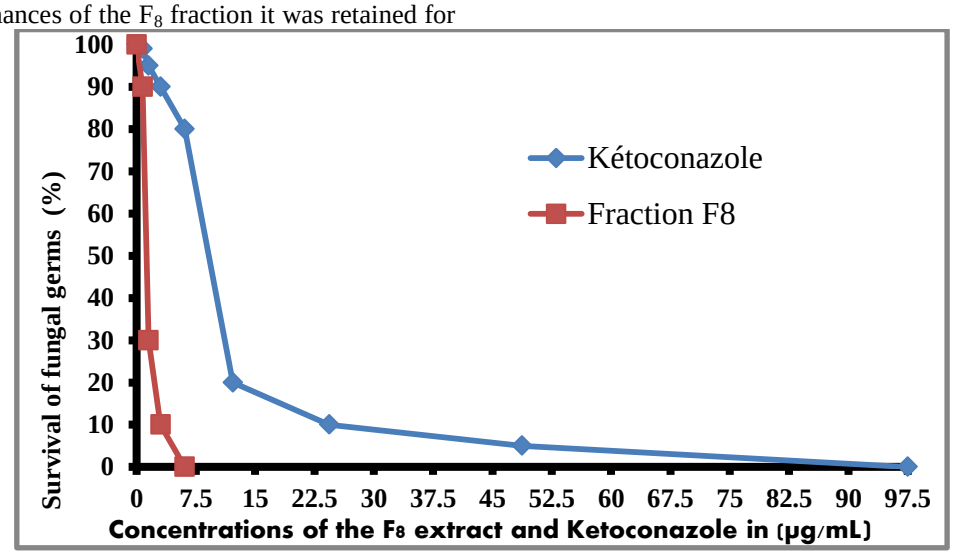

Figure 1: Compared curves of activity of the F_8 extract and Ketoconazole

DISCUSSION

The F_1 fraction whose value of CMF is higher than X_{42} extract translates is less active than the basic extract X_{42}.

In addition, the fractions F_2, F_3, F_4, F5 and X_{42} would have a same activity. However, considering the values of IC_{50} it appeared that all these fractions had IC_{50} values lower than X42 extract. Thus, They are more active than the basic extract X_{42}.

On the other hand the fractions F_6, F_7, F_8 and F_9 whose FMC values are lower than that of the X_{42} extract show than they are more active than the basic extract X_{42}. Among them, the fractions F_6, F_7, F_9 of FMC value is 24,37 µg/mL improved 2 times the activity of the basic extract X_{42}. Considering IC_{50} value, the F_6 fraction which had low value is the best. Moreover the F_8 fraction which had the lowest FMC value (6,09 µg/mL), had the best antitrichophytic activity. Indeed on the basis of report/ratio $FMC_{F\ (6,7or\ 9)}$ / FMC_{F8} = 4, it is shown that the F_8 fraction was 4 times more active than F_6, F_7 and F_9 fractions. Antitrichophytic activity of the F_8 fraction is very interesting; it was desirable to compare with antifungal standard basis. The results are better than those of other authors who had tested ethanol extracts from medicinal plants on *Trichophyton mentagrophytes var interdigital*. For example, *Pteleosis suberosa* (IMC = 250 µg/mL), *Combretum glutinosum* (IMC = 1000 µg/mL), *C. hispidum* (IMC = 4000 µg/mL), *C. molle* (IMC = 250 µg/mL), *C. nigricans* (IMC = 250 µg/mL), *Terminalia avicennidoides* (IMC = 250 µg/mL), *T. moellis* (IMC = 250 µg/mL) (Baba-Moussa *and al*., 1999).

Ketoconazole was selected because it is an antifungal molecule usually prescribed to patients in the treatment of the mycosis. The results obtained with Ketoconazole showed that molecule which FMC value was 97,5 µg/mL is less active on *T mentagrophytes var*. *interdigitale* than vegetable F_8 fraction which FMC value was 6,09 µg/mL. The antifungal activity of F_8 fraction is 16 times better than Ketoconazole. In a general way F_8 fraction significantly improved not only the activity of the basic extract X_{42} but as it shown a definitely better activity at Ketoconazole on the basis which FMC value is 8 times more active than X_{42} extract and 16 times more active than Ketoconazole.

Moreover of the clear inhibiting activities observed for each fraction and Ketoconazole indicated that they are fungicidal. The progressive reduction in of colonies in experimental tubes coupled with the decreasing shape of the curves shown that extracts act according to relation amount-effect.

CONCLUSIONS

This study confirms a real antifungal activity of *T. ivorensis*. The X_{42} extract starting was active on *T. mentagrophytes var*. *interdigitale*. The various fractions from TEKAM 2 and Ketoconazole inhibited the *in vitro* growth. F_8 fraction was the best with lowest parameters values (FMC = 6,09 µg/mL and IC_{50} = 1,08 µg/mL). The method of extraction as described by ZIRIHI *and al*.in 2003 combined of a delipidation with hexane followed by chromatographic fractionation made it possible to obtain a fraction of *T. ivorensis* which was real interesting antifungal activity.

It arises this method of extraction is a way which concentrated better active ingredients of TEKAM 2. From this analysis, it comes out the following aspects:

T. mentagrophytes var *interdigitale* was sensitive to various TEKAM 2 extracts and Ketoconazole according to relation amount-effect;

- improvement of the antifungal activity of hydroalcoholic extract from TEKAM 2 by chromatography with water distilled like eluant.

- the method of extraction which combines solvents ethanol-water (70:30) followed by a hot delipidation with the soxhlet then of chromatographic fractionation concentrated better active ingredients of TEKAM 2.

The present study justifies the use in traditional medicine of TEKAM 2 to cure skin diseases.

In prospect, studies of screening phytochimic of the F_8 fraction from TEKAM 2 followed by thin layer chromatography and NMR could help to identify nature and active molecule to improve the activity of F_8 fraction from TEKAM2.

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