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Full Length Research Paper

Evaluation and comparison of antifungal activities of *Terminalia catappa* and *Terminalia mantaly* (Combretaceae) on the *in vitro* growth of *Aspergillus fumigatus*

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Many surveys performed around the world have mentioned that among the plant species belonging to Combretaceae family, *Terminalia catappa* is the most requested medicinal plant. In recent decades, traditional healers in southern region of Côte d'Ivoire prefer to use the bark of *Terminalia mantaly* instead of those of *T. catappa*. The purpose of this study is to compare the pharmacological activities of these plants. The results of the anti-fungal activities of aqueous, hydroalcoholic and residual extracts on the growth of *Aspergillus fumigates*, a fungal pathogen, show that *Terminalia mantaly* water extract is 64 times more active than *T. catappa* water extract; hydroalcoholic extract of *T. mantaly* is 2 times more active than hydroalcoholic extract of *T. catappa* and residual extract of *T. mantaly* is 128 times more active than residual extract of *T. catappa.* Analysis of these results shows clearly that *T. mantaly* extracts are more active than extracts of *T. catappa.* The choice of *T. mantaly* and abandon of *T. catappa* by traditional healers in making medicines against skin infections is due to abundance of this plant in all areas and settlements and its excellent activities on many pathogens.

Key words: Côte d'Ivoire, *Terminalia catappa*, *Terminalia mantaly,* antifungal activity.

INTRODUCTION

Ethnopharmacological surveys performed around the world have mentioned that among the plant species belonging to Combretaceae family, *Terminalia catappa* is the most requested medicinal plant (N'guessan, 2008). In Côte d'Ivoire, the roots bark decoction is used as anti-pyretic (Zirihi, 1991; N'guessan, 1995). In Nigeria, the decoction of leaves is used as medicine against malaria and abdominal pains (Amenoudji, 1990). In Togo and Benin root barks decoction is used in the treatment of various dermatosis (Amenoudji, 1990; Batawila et al., 2005; Baba-moussa, 1999; Baba-moussa et al., 1999). In Phillipines, leaves extract is used against leprosies (Burkill, 1997). In recent decades, traditional healers in southern region of Côte d'Ivoire prefer to use the bark of *Terminalia mantaly* instead of those of *T. catappa* (Zirihi, 1991). According to Coulibaly (Coulibaly, 2006), roots of *T. catappa* and leaves of *Terminalia mantaly* are used against the loss of voice. Indeed, unlike *T. catappa*, *T. mantaly* abounds in this part of Côte d'Ivoire, this plant is found along roads side, streets and even in the settlements (Aké-Assi, 1984). The purpose of this study is to compare the pharmacological activities of *T. catappa* a medicinal plant well known and well studied to those of

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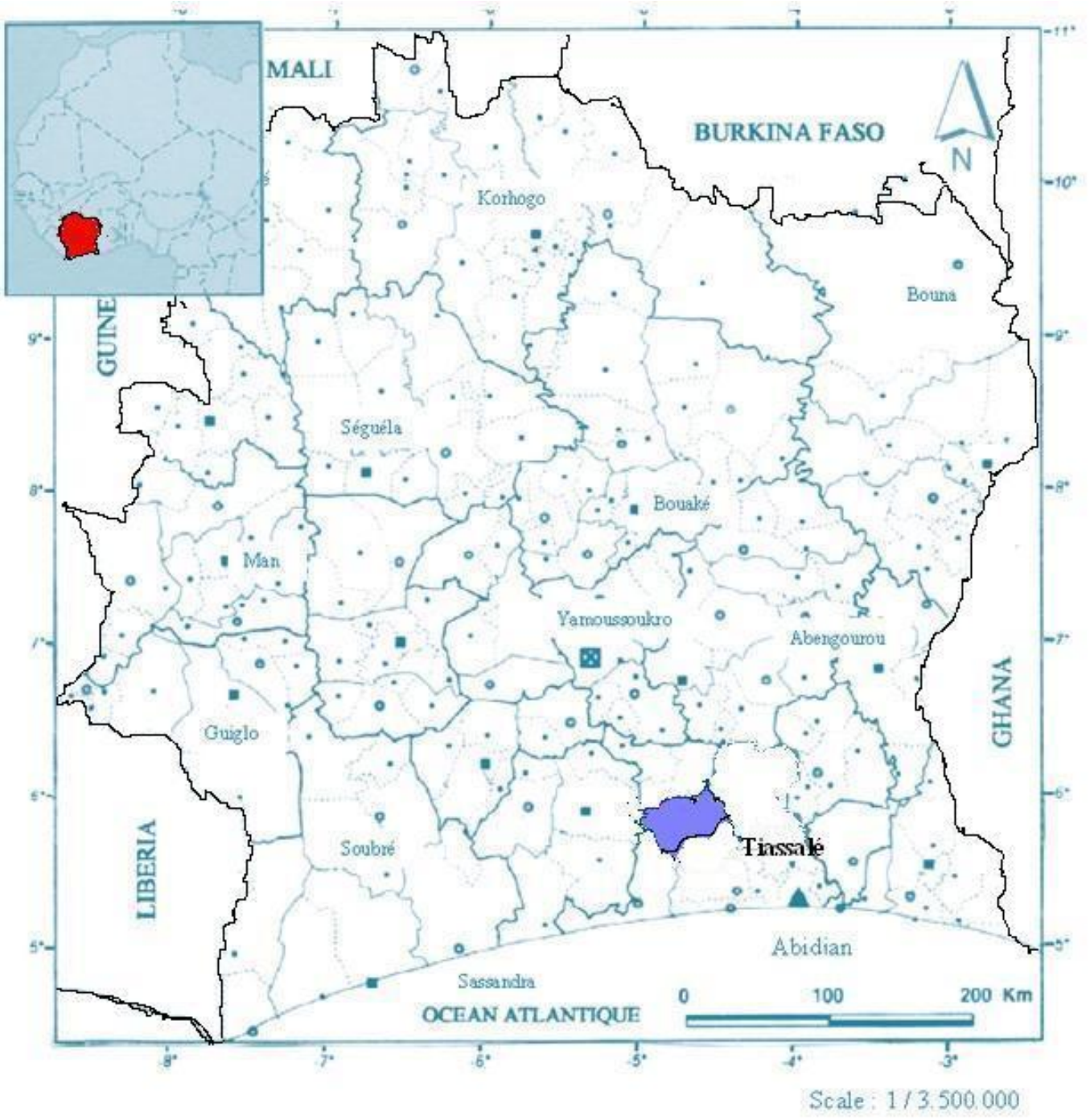

Figure 1. Situation of the study site; Geographical situation of Côte-d'Ivoire in West Africa; Geo graphical situation of the department of Tiassalé, in Côte-d'Ivoire; (CEDA, 2001, modified by Coulibaly).

T. mantaly introduced in 1972 in Côte d'Ivoire. We present here the results of the anti-fungal activities of aqueous, ethanolic and residual extracts in the in vitro growth of *Aspergillus fumigatus* a fungal pathogen that causes very severe and irreversible lung infections in Human immunodeficiency virus (HIV) positive patients.

MATERIALS AND METHODS

Study site

Our investigations took place in Tiassalé Department (Figure 1), Located at 130 km of Abidjan. Tiassalé is part of the Southern forest of Côte-d'Ivoire (West Africa), in the guinea field of the

Figure 2. *Terminalia mantaly* H. Perrier (Combretaceae): Portion of stem bark. Picture by Coulibaly K., Tiassalé, 06/12/08.

mesophilic sector, characterized by dense moist semi-deciduous forest. Currently, the original vegetation has been degraded by human activities (Chevalier, 1948). Annual average pluviometry is about 1708.73 mm of water. Its climate, warm and humid, is characterized by two seasons: a dry season from December to February with two dry month, January and February, and a long rainy season from March to November, with the largest recorded in June, the other in October; between the two month, there is a period of less rainfall during August.

Vegetal material

T. mantaly

T. mantaly grows 10 to 20 m with an erect stem and layered branches. Bark is pale grey and smooth. Leaves are smooth and bright green when young and in terminal rosettes; they are 5 to 7 cm long with short stem, apex broadly rounded and base tapred (Figures 2 to 5). Flowers small, greenish in erect spikes. Fruit small oval, about 1.5 cm long

T. catappa

T. catappa is a mean tree, a Mesophanerophyte from 25 to 30 m of height and of 1.60 m of diameter. The trunk of *T. catappa*, without footing, comprises a rhytidom split lengthwise (Figures 1 and 3); the branches are staged. The simple leaves, bound without peduncles, are in subvertilled tufts with. Flowers without petals, gathered in terminal tufts are bisexual.

Preparation of extracts

The drugs (stems barks) of *T. catappa* coded « CTA » and *Terminalia mantaly* coded « MTA», used in this study, were collected in the region of Tiassalé, South of Côte d'Ivoire. The collected drugs, were washed up, cut in small dices and dried at the laboratory at the ambient temperature (25 to 27°C) during three weeks and crushed out fine powder. Powders were extracted separately with a blinder, according to the method of Zirihi and Kra (2003), as follows: 100 g of powder of barks from each plant were extracted hot (decoction) with one liter of distilled water using a Mixer (Blinder). After three cycles of extraction, the solution obtained was filtered, then the solvent of extraction (water) was eliminated using a rotary evaporator; the paste obtained was freeze-dried; it constituted the total water extracts codified (CTA_{wat} and MTA_{wat}). 30 g of each total water extract were treated by 300 ml of hydroalcoholic solution (70% Ethanol and 30% distilled water) using a separating funnel. Two phases were obtained (the alcoholic upper phase (CTA_0, MTA_0) and a residue named (CTA_1, MTA_1). CTA_0, MTA_0, CTA_1 and MTA_1 were freeze-dried. All extracts obtained were kept in glass sterilized bowls at -20°C. The six extracts of CTA_{wat}, MTA_{wat}, CTA_0, MTA_0, CTA_1 and MTA_1 were tested on the *in vitro* growth of *A. fumigatus*.

Microbial stock and realization of *in vitro* tests

The stock of *A. fumigatus* on which we worked was provided to us by the Laboratory of Mycology of the U.F.R of Medical Sciences of the University of Cocody-Abidjan (Côte d'Ivoire). The incorporation of different extracts of CTA and MTA at the Sabouraud agar was

Figure 3. *Terminalia catappa* Linn. (Combretaceae): Portion of stem bark. Picture by Coulibaly K., Tiassalé, 10/01/09.

Figure 4. *Terminalia mantaly* H. Perrier (Combretaceae): Young leaves and fruits. Picture by Coulibaly K. Tiassalé, 06/12/08.

Figure 5. *T. catappa* Linn. (Combretaceae): Leaves and fruits. Picture by Coulibaly K., Tiassalé, 10/01/09.

done according to the double dilution method, in angle sloping tubes. The extracts were tested separately. For the *T. mantaly* extract, each set comprises of 10 test tubes for the water extract and 12 test tubes for the ethanolic and residual extract in which we have, respectively eight and ten test tubes containing vegetal extracts, and two testimony tubes including one without vegetal extract serving to control the growth of germs, the other one without germs and without the extract, serving to control the sterility of the field of cultivation. The concentrations of the extracts vary from 3.125 mg/ml to 24 µg/ml for the eight test tubes and from 12.5 mg/ml to 24 µg/ml for the ten test tubes (according to a geometric line of ½ reasons). For the *T. catappa* extract the set representing the water extract and the residual extract comprises of 15 test tubes, including 13 test tubes, and 02 testimony tubes.

The concentrations of the extracts from the 13 test tubes vary from 100 mg/ml to 24 µg/ml. Concerning the ethanolic extract we have 14 test tubes, including 12 test tubes and 02 testimony tubes. The concentrations of the extracts vary from 50 mg/ml to 24 µg/ml. After the incorporation of the extracts, all the tubes of each set are sterilized with an autoclave at 121°C, during 15 min and then inclined at room temperature, to allow the agar-agar to quench itself and to solidify itself (Ajello et al., 1963; Holt, 1975; Guede-Guina et al., 1995). For each set of the different extracts, the antifungal tests were carried out by the cultivation of 1000 cells of *A. fumigatus* on the fields previously prepared. All the cultivations were incubated at 30°C during 72 h. The colony of *A. fumigatus* was numbered and the growth in experimental tubes of each set was assessed in percentage of survival, calculated in proportion to 100% of survival in the testimony tube of control of the growth (Ajello et al., 1963; Holt, 1975; Guede et al., 1995).

RESULTS

Antimicrobic tests

After 48 h of incubation at 30°C, we observe comparatively to the testimony bowls, a progressive reduction of colonies number with an enhancement of plant extract concentrations in experimental tubes. This is observed for all the sets (Figures 6 to 11). The experimental data expressed in the form of curves of sensibilities are summarized in Figure 12. The values of the antifungal parameters, MFC (Minimal Fungicid Concentration) and CI_{50} (Concentration for 50% Inhibition) of the 06 extracts, are recorded in the Table 1. All the six curves representing the evolution of the activity of each extract, presenting a deceasing speed, with slopes of more or less strong.

DISCUSSION

The botanical study of these two plants shows great differences; stems, barks, leaves and fruits are different. The analysis of the results of the microbiological tests shows that *A. fumigatus* is sensitive to all the extracts (CTA_{wat}, MTA_{wat}, CTA_0, MTA_0, CTA_1 and MTA_1), according

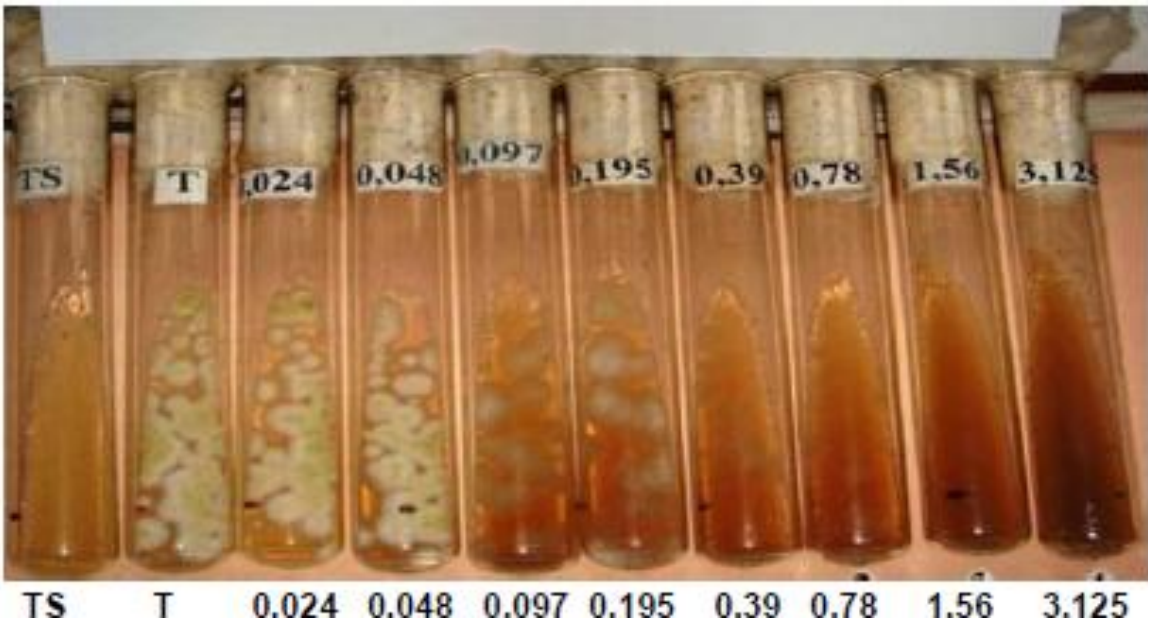

Figure 6. *A. fumigatus:* Aspect of culture in presence of water extracts of *T. mantaly,* at different concentrations.

Figure 7. *A. fumigatus:* Aspect of culture in presence of water extracts of *T. catappa* at different concentrations.

to a relation dosis-dependent. In all experiments we noticed effective inhibition of *A. fumigatus* growth after 48 h of incubation and at 30°C. The comparison of all antifungal parameters shows that the six plants extracts are active on *A. fumigatus* growth. *T. mantaly* extracts activities comparisons shows the following results:

MFC_{MTAwat} / MFC_{MTA1} = 1.56 / 0.78 = 2; MFC_{MTAwat} /MFC_{MTA0}= 1.56 / 1.56 = 1; MFC_{MTA0} / MFC_{MTA1} = 1.56 / 0.78 = 2.

Figure 8. *A. fumigatus*: Aspect of culture in presence of 70 % ethanolic extracts of *T. mantaly*, at different concentrations.

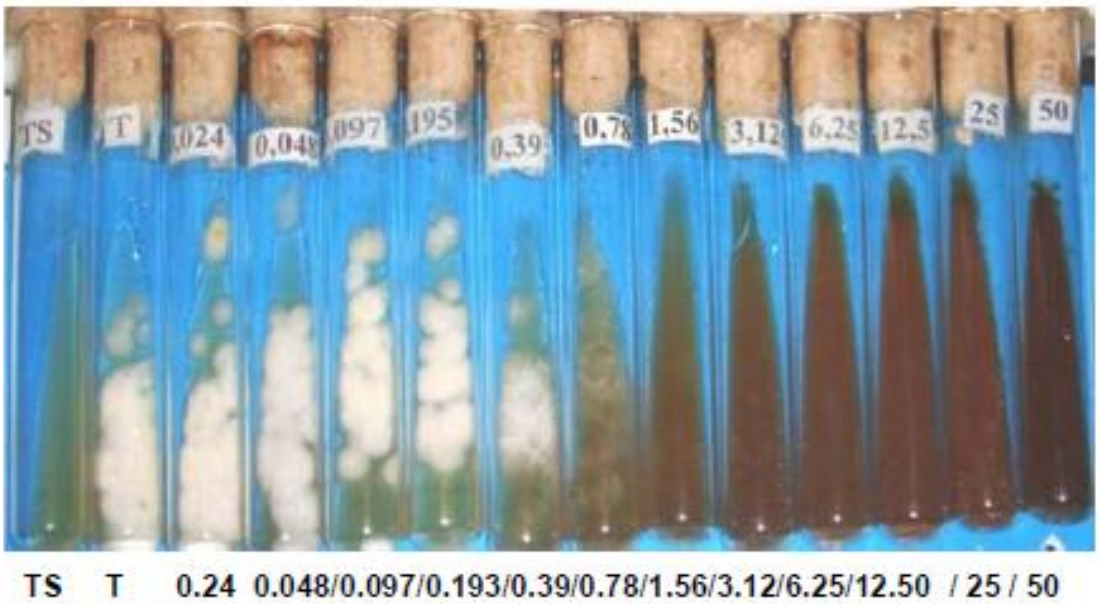

Figure 9. *A. fumigatus*: Aspect of culture in presence of 70% ethanolic extracts of *T. catappa*, at different concentrations.

According to these results residual extract (MTA_1) is twice more active than the water extract of *T. mantaly* (MTA_{wat}). Activities of water extract (MTA_{wat}) and hydroalcoholic extract (MTA_0) are the same. *T. catappa* extracts activities comparisons shows the following results:

MFC_{CTAwat} / MFC_{CTA1} = 100 /100 =1 MFC_{CTA1} / MFC_{CTA0}= 100 / 3.125 = 32; MFC_{CTAwat} / MFC_{CTA0} = 100 /3.125 = 32.

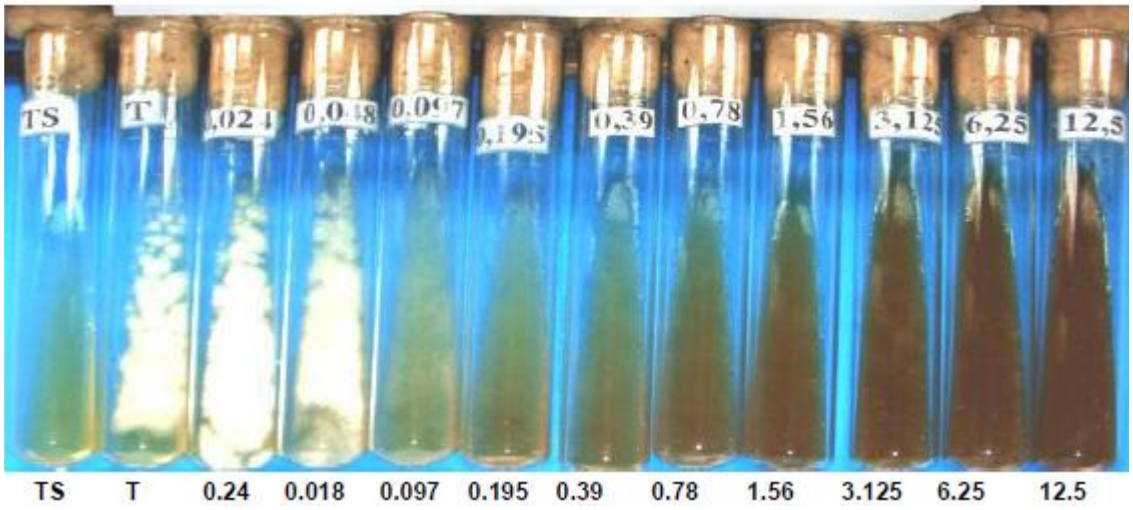

Figure 10. *A. fumigatus*: Aspect of culture in presence of residual extracts of *T. mantaly,* at different concentrations.

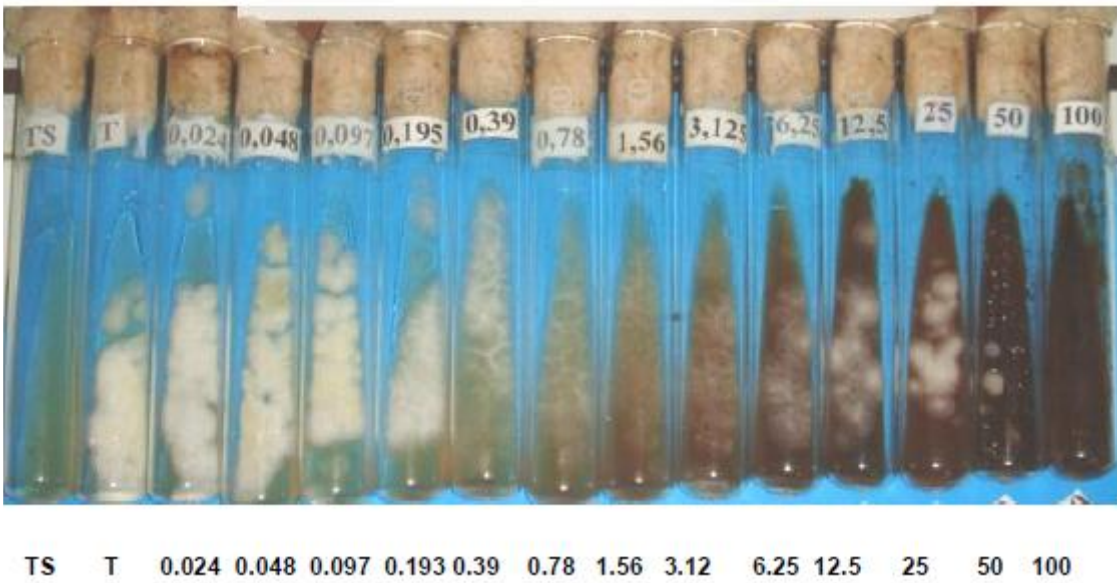

Figure 11. *A. fumigatus*: Aspect of culture in presence of residual extracts of *T. catappa,* at different concentrations.

The comparisons of these results show that hydroalcoholic extract (CTA_0) of *T. catappa* is 32 times more active than water (CTA_{wat}) and residual extract (CTA_1).

In order to know the plant which is more active, water extracts, hydroalcoholic extracts and residual extracts are compared separately. -water extracts: MFC_{CTAwat} /MFC_{MTAwat} =100/ 1.56 = 64; this ratio show that *T. mantaly* water extract is 64 times more active than *T. catappa* water extract. Hydroalcoholic extracts: MFC_{CTA0}/ MFC_{MTA0} = 3.125/1.56 =2; According to this proportion *T. mantaly* hydroalcoholic extract is 2 times more active than hydroalcoholic extract of *T. catappa*. -residual extracts: MFC_{CTA1} / MFC_{MTA1} = 100/ 0.78 = 128; this ratio proves that *T*. *mantaly* residual extract is 128 times more active than residual extract of *T. catappa*. Analysis of these results shows clearly that *T. mantaly* extracts are more active than extracts of *T. catappa.* Ackah (2004) and Ouattara (2005), tested, respectively the 96% alcoholic residue (MISCA-F3) of *Mitracarpus scaber* (MFC = 18.750 mg/ml) and the 70% alcoholic residue (MISCA-F2) of *Mitracarpus scaber* (MFC = 50.000 mg/ml)

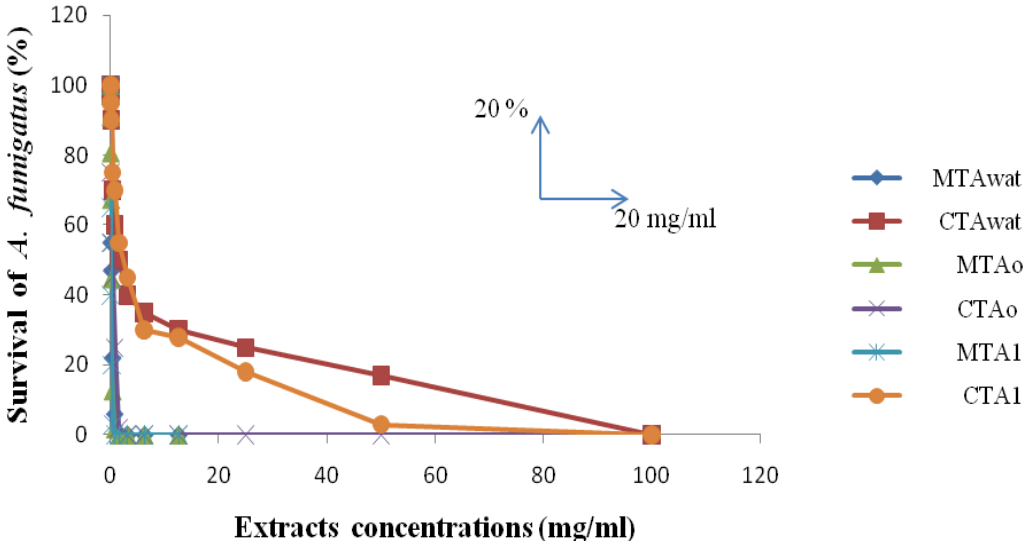

Figure 12. Sensitivity of *A. fumigatus* to the extracts of *T. mantaly* and T. *catappa* (MTA_{wat}, CTA_{wat}, MTA_0, CTA_0, MTA_1 and CTA_1).

Table 1. Antifungal parameters Values of the six extracts of the MTA and the CTA at 48 h of incubation and at 30°C.

Type of extract		Antifungal parameters	
		CI_{50} (mg/ml)	CMF (mg/ml)
Water extracts	MTA_{wat}	0.098	1.56
	CTA_{wat}	0.84	100
Hydroalcoholics extracts	MTA_0	0.10	1.56
	CTA_0	0.197	3.125
Residual extracts	MTA_1	0.043	0.78
	CTA_1	1.58	100

on the same germ and in the same experimental conditions, we can say that *Terminalia mantaly*, MFC = 0.78 mg/ml (MFC_{MTA1}); present a better activity than *M. scaber*.

CONCLUSION AND PERSPECTIVES

According to all these results we can make the following observations: water, hydroalcoholic and residual extracts of *T mantaly* and *T catappa* inhibits the *in vitro* growth of *A. fumigatus*, hydroalcoholic extract of *T. mantaly* is 2 times more active than hydroalcoholic extract of *T. catappa*, residual extract of *T. mantaly* is 128 times more active than residual extract of *T. catappa.* Analysis of these results shows clearly that *T. mantaly* extracts are more active than extracts of *T. catappa. T. mantaly* extracts are more active than those of *M. scaber* on the *in vitro* growth of *A. fumigatus.* The choice of *T. mantaly* and renunciation of *T. catappa* by traditional healers in making medicine against skin infections is due to abundance of this plant in all areas and settlements and its excellent activities on many pathogens. Phytochemical analysis associated to antifungal tests is needed to isolate the active compound.

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