

ACKAH JACQUES AUGUSTE ALFRED BOGNAN¹, YAYE YAPI GUILLAUME^{1*}, YAPI HOUPHOUET FELIX¹, KRA ADOU KOFFI MATHIEU¹, DJAMAN ALLICO JOSEPH^{1,2}

¹ Laboratoire de Pharmacodynamie-Biochimique, UFR Biosciences
Université de Cocody-Abidjan, 22 BP 582 Abidjan 22 (Côte d'Ivoire).

² Département de Biochimie Médicale et Fondamentale, Institut Pasteur de Côte d'Ivoire, 01 BP 490 Abidjan 01(Côte d'Ivoire).

*Corresponding Author, E-mail: yaveyapi@yahoo.fr

Abstract

Aims: To evaluate antifungal activities of *Terminalia mantaly*.

Study design: Agar double dilution method in slope tubes.

Place of study: Department of Biochemistry (Laboratory of Biochemistry-Pharmacodynamic).

Methodology: Agar double dilution method in slope tubes was adopted to determine anticryptococcal activity. The fungal isolate was used in this study: *Cryptococcus neoformans*. This germ was isolated from patients in the infectious diseases department of the University Teaching hospital of Treichville-Abidjan (Côte d'Ivoire).

Results: All extracts of *Terminalia mantaly* exhibited marked antifungal activity in dose-effect relationship. The lowest MFC (24.37µg/mL) and IC₅₀ (5.87µg/mL) values were recorded for extract T₄₋₂ obtained after degreasing the concentrated hydro-alcoholic extract T₀ of *Terminalia mantaly*.

Conclusion: The results suggest that *Terminalia mantaly* possess antifungal property. It exhibited on the *in vitro* growth of *Cryptococcus neoformans*.

Keywords: Antifungal activity, cryptococcosis, opportunists, *Terminalia mantaly*

1- INTRODUCTION

These last years, infectious knew a strong outbreak connected to several factors among which one of the most marking is the advent of the HIV/AIDS in the human viral pathology (Neofytos *et al.*, 2009; Michel, 2011).

This pandemic difficult to contain and to eradicate has not only more negative impacts on the sanitary evolution in most of the African countries but also it has involved and amplified the distribution of certain number of pathologies among which mycoses (McNeil *et al.*, 2001). Among these opportunist mycoses, cryptococcosis takes up a disturbing place. With the global emergence of AIDS, the incidence of cryptococcosis is increasing and now represents a major life-threatening fungal infection in these patients. The most serious infections usually develop in patients with defective cell-mediated immunity. Indeed, *Cryptococcus* may cause pulmonary diseases and central nervous system (CNS) fungal infection (life-threatening meningitis or meningoencephalitis) potentially mortal to the patients of the HIV/AIDS who has developed from 80 to 90% this infection (Bicanic *et Harrison*, 2005; Gaudry *et al.*, 2008; Park *et al.*, 2009), to the patients undergoing organ transplantation, to the patients undergoing corticosteroid treatment and to the patients with sarcoidosis. Today and in spite of the existence of numerous antifungal and the therapeutic combinations, cryptococcosis are part of the three

most dangerous fungal infections in hospitable environment (Schwarz *et al.*, 2006; Seddon *et al.*, 2009).

Furthermore, in the face of lack of equipment, the insufficiency of medical tool and especially the lack of efficient medicines and the increasing of the poverty, the population mainly the African ones turned to traditional medicines to relieve their ills (Babayi *et al.*, 2004; Gurib-Fakim, 2006). But the excessive use of these healing plants can have serious consequences even dramatic on this population. Then, it turns out to be necessary to secure this traditional medicine. So, an ethnobotanic study made in Côte d'Ivoire allowed to list several healing plants with turned out to be reliable biological activities. Among the selected plants represent plants with anti-infective activities of which *Terminalia mantaly* H. Perrier (combretacea) used in the traditional environment to satisfy several troubles among which the fungal infections (Rivière *et al.*, 2005).

Hence, in line with the current trend of finding naturally occurring anti-cryptococcosis activity, this study was designed to evaluate *in vitro* anti-cryptococcosis activity of the extracts of *Terminalia mantaly* on the growth of *Cryptococcus neoformans*, the major germ of *Cryptococcus* which causes illness in human.

2- MATERIAL AND METHODS

2-1- Fungus used

For these tests, the isolate *Cryptococcus neoformans* was used. It was supplied by the laboratory of mycology of Medical Sciences department of the University of Cocody-Abidjan (Côte d'Ivoire). This germ was isolated from patients in the infectious diseases department of the University teaching hospital of Treichville-Abidjan (Côte d'Ivoire).

2-2-Preparation of extracts

The tem barks harvested, cut and dried at shade were crushed. The powder obtained codified TEKAM1. The crude extracts (aqueous and hydro-alcoholic) were prepared as follows:

Hundred grams of TEKAM1 was extracted in blender with one liter (1L) of distilled water or with one liter (1L) of solvent mixture (70 % of pure ethanol and 30 % of distilled water) respectively. After grinding, the homogenate obtained in each case was centrifuged in a square of fabric and then filtered on absorbent cotton and on wathman paper 3mm. Afterward, aqueous filtrate were concentrated under a vacuum at 60°C and the extract obtained constitutes the crude aqueous extract T_{Aq}. The hydro-alcoholic filtrate was evaporated to dryness and allowed to obtain the crude extract T₀.

Besides from the T₀ extract, 8 other extracts were prepared. Indeed, 3 portions of 10g are subjected to a liquid / liquid partition in 400 mL of 3 different mixtures of solvents (hexane-water, ethyl acetate-water and dichloromethane-water; v/v: 50/50). The various phases obtained were separated and were dried under a vacuum. The following extracts were obtained: the hexane phase (plant oil) (T1-1), the aqueous phase of the partition hexane-water (T1-2), the acetate phase (T2-1), the aqueous phase of the partition ethyl acetate-water (T2-2), the dichloromethane phase (T3-1) and the aqueous phase of the partition dichloromethane-water (T3-2). Moreover, another portion of 10g of the extract T₀ was degreased at soxhlet in 350 mL of hexane. We obtain T4-1 (the hexane phase of the degreased extracts, plant oil) and T4-2 (the degreased extract).

After, all the obtained extracts were tested for biological assays.

2-3- Culture media

For the Culture media preparation, we used the Sabouraud agar (Bio-Rad / ref: 64449; Batch: 8B2212). This medium was prepared according to the instructions of the manufacturer's protocol.

The inclusion of the plant extracts in the agar was made according to the method of the double dilution (Holt, 1975). Ten test tubes were used per series among which 8 containing the plant extract. The concentrations of these tubes ranged from 780 to 6.09 $\mu\text{g/mL}$ binding by a geometrical reason of $\frac{1}{2}$. The 2 other tubes are used as control tubes in which one was without plant extract without germ was used as sterility control to the culture medium and the other one without plant extract as used to control the growth of the germ.

Then, all tubes were removed by the use of forceps sterilized by flaming at 121°C for 15 minutes and were inclined to room temperature of the laboratory to cooling and solidification of the agar.

2-4- Antifungal assays

A fresh colony of *Cryptococcus neoformans* (48 hours of incubation) was homogenized in 10 mL of sterilized distilled water. The suspension 10^0 obtained was concentrated to 10^6 cells/mL. Afterward, a second suspension (10^{-1}) has been prepared by dilution ($1/10^{\text{th}}$) from the suspension 10^0 . It's charged to 10^5 cells /mL.

Besides for each tests tube except the sterility control tube, the culture of the germ was made by sowing of 10 μL of the suspension 10^{-1} . It corresponds to 1000 cells sowed.

After 48 hours incubation at 30°C , the colonies were counted with a pen colony counter (serial number 23382 Scinceware de Bel-Art). Furthermore, the growth in the experimental tubes was estimated as a percentage of survival calculated compared to 100 % survival in the control tube growth (Holt, 1975).

The treatment of experimental data was used to determine:

- The Minimum Inhibitory Concentration (MIC): it is the smallest extract concentration in the tube where there is no growth visible at the eye.

- The Minimum Fungicidal Concentration (MFC): it is the smallest extract concentration in the tube which gave 99.99 % inhibition compared to control tube growth or conversely it is the tube which leaves 0.01 % survival. It's obtained after transplanting the agar of the tube corresponds to the MIC.

- The Concentration for 50 % Inhibition (IC_{50}): it is the concentration which gave 50 % inhibition. This parameter was determined graphically.

These parameters allow comparing extracts between them.

3- RESULTS AND DISCUSSION

After incubation at 30°C for 48 hours, we was observed compared with control tube a progressive decrease in the number of colonies as the concentrations of the plant extracts increased in the experimental tubes. This was observed in all series of the 10 extracts. These results are average statistics of 6 experiments by extract.

Effective inhibitions were observed at different concentrations level according to extracts (Table I). The experimental data translated of sensitivity curves are presented in figure 1 for different partitions extracts.

Table I: Antifungal parameters of the different extracts of *Terminalia mantaly*

Extracts		Antifungal parameters	
		MFC (µg/mL)	IC ₅₀ (µg/mL)
Crude extracts	T _{Aq}	195	35
	T ₀	97.5	16.5
T ₀ Partitions	T ₁₋₁	390	150
	T ₁₋₂	48.75	9.5
	T ₂₋₁	195	62.5
	T ₂₋₂	24.37	10
	T ₃₋₁	780	345
	T ₃₋₂	48.75	10
	T ₄₋₁	780	165
	T ₄₋₂	24.37	5.87

In general, all sensitivity curves present a decreasing pace with slopes more or less strong slopes according to the extracts. They intersect x-axis at different levels according to the extracts. Concerning the crude extracts, the sensitivity curve of the T₀ extract slope is stronger than of the T_{Aq} extract. However, the extracts T₁₋₂, T₂₋₂, T₃₋₂ and T₄₋₂ presented curves whose slopes are stronger particular the T₄₋₂ extract. The extract T₂₋₁ has curve slope as average. Whereas, the weakest slopes are observed with T₁₋₁, T₃₋₁ and T₄₋₁ (figure 1). The values of the MFC and the IC₅₀ for all the extracts are confine to Table I.

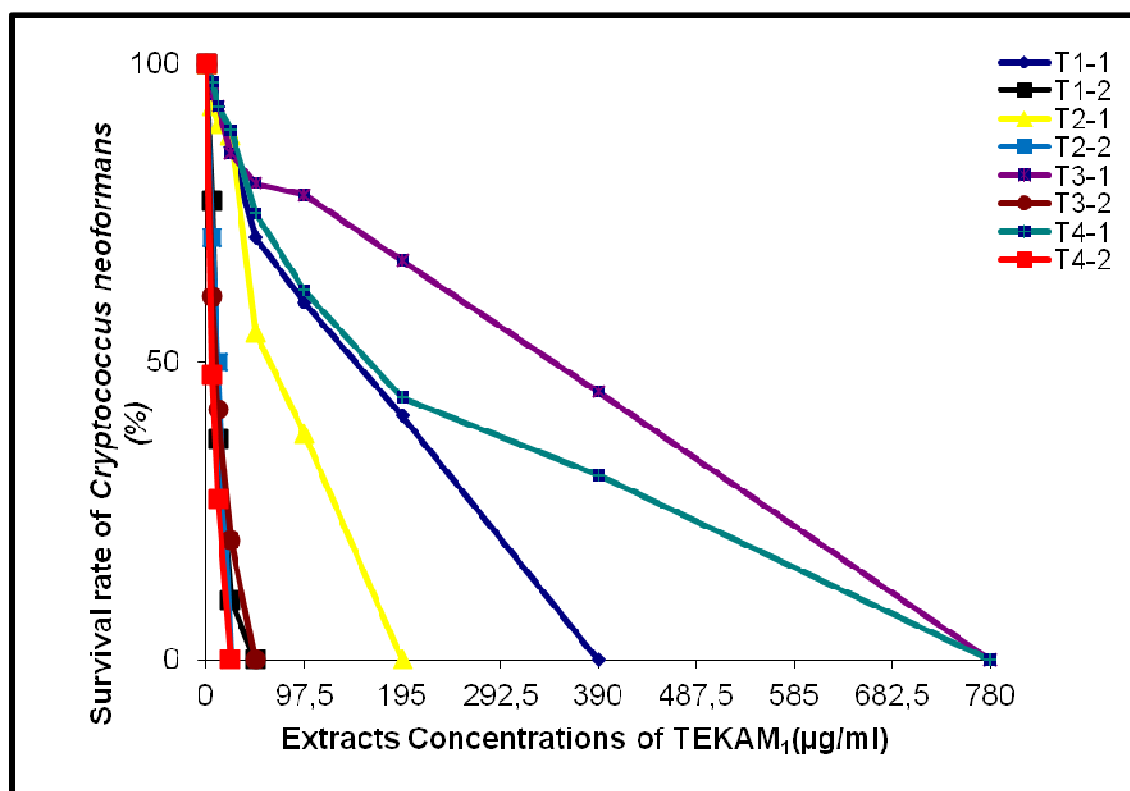


Figure 1: Activity of T_0 partitions extracts of *Terminalia mantaly* on the *in vitro* growth of *Cryptococcus neoformans*

Taking for granted that for the traditional medicine treatment, a great part of the population use water as solvent, we have checked the anti-infected properties granted to the specie of plant by testing the aqueous extract first. The very interesting results obtained, bring us to improve the performance of this extract. From then on, we used other solvents among which the ethanol, the ethyl acetate, the dichloromethane and the hexane in different methods.

The analysis of all these results reveals that the tested isolate is sensitive to drugs according to a measure and effect relationship and extracts. The curves of sensitivity obtained with each extract allow comparing their performance. Indeed, more the slope of a curve is strong more the extract is active.

It rereleases from the analysis of the crude extracts (T_{Aq} and T_0) that the T_0 extract is twice times more active than the extract T_{Aq} on the *in vitro* growth of *Cryptococcus neoformans* (CMF = 97.5 µg / ml against 195µg / ml for T_{Aq}) (Table I).

Besides the aqueous extract results are better than those obtained by **Fyhrquist, 2007** on the *in vitro* growth of *Cryptococcus neoformans*. In fact, the aqueous extracts of *Combretum molle*, *Combretum padoïdes* and *Terminalia kaiserana* gave as values for MFC 12500µg/ mL, 6250µg/mL and 1560 µg/mL respectively. The T_{Aq} extract is still 64 times, 32 times and 8 times respectively more active than the aqueous extracts of these different species. It's the same in comparison to the results of **Ouattara et al., 2007** which found a value for MFC=6250µg/mL with the aqueous extract of *Thonningia sanguinea* on the *in vitro* growth of *Cryptococcus neoformans*. As matter of fact, T_{Aq} is still 32 times more active. Moreover the hydro-alcoholic extract T_0 is also 32 times more active than the hydro-alcoholic extract of *Thonningia sanguinea* which value for MFC is 3125µg/mL (**Ouattara et al., 2007**).

Besides, the results of 8 other extracts reveal that from all these extracts the most active is the extract T_{4-2} . Its sensitivity curve presents the strongest slope (Figure 1) and the values of its antifungal parameters are the best (Table I). The MFC analysis values of these extracts reveal that the T_{4-2} extract is still 32 times more active than extracts T_{3-1} and T_{4-1} , 16 times more active than T_{1-1} . It is also 8 times more active than the T_{2-1} extract and 2 times that T_{1-2} and T_{3-2} extracts. As for the T_{2-2} extract, it presents the same value for CMF but the comparison made based on the value of IC_{50} reveal that the T_{4-2} extract is the most active ($IC_{50} = 5.87\mu\text{g/mL}$ against $10\mu\text{g/mL}$ for T_{2-2}). Furthermore when we compare the values of MFC of T_{4-2} extract to those of the crude extracts, it emerges that this extract is still 8 times more active than T_{Aq} and 4 times more active than its basic extract (T_0).

In addition, comparing the results of the most active T_{4-2} extract to those of **Fabri et al., 2008** on the *in vitro* growth of *Cryptococcus neoformans* with the hexanic and acetatic extracts of *Mitracarpus frigidus*, reveal that the T_{4-2} extract is still 13 times and 3 times respectively more active than these extracts whose values of MFC are $313\mu\text{g/mL}$ (hexanic extract) and $78\mu\text{g/mL}$ (acetatic extract).

Moreover, a previous study has revealed that the most active extract T_{4-2} is very rich in Polyphenols and in quinones. These compounds would be at the origin of the antifungal activity of this extract and even the plant (**Yayé et al., 2012**). The antifungal activity of phenols and the quinones were proved by **Branthner et al., 1996**; **Cowan, 1999** and **Harbonne et al., 1999**.

4- CONCLUSION

This study confirms the use of this species of plant as an anti-infectious in the traditional medicine. It reveals its antifungal potential. From this study, we retain what follows:

- The tested isolate *Cryptococcus neoformans* is sensitive to the drugs according to a measure and effect relationship.
- Crude extracts, the most active is the hydro-alcoholic extract (twice more active than the aqueous extract).
- Among partition extracts, the most active is the T_{4-2} extract. It is still 4 times more active than its basic extract T_0 .
- The obtaining method of T_{4-2} constitutes the best way of the principle active selection and concentration.
- The best performances are always observed with the aqueous phases of the different partitions.
- The most active extract T_{4-2} is rich in phenols and in quinones. They would be at the origin of the antifungal activity.

It would be thus desirable to pursue these works by committing a series of chromatography which could certainly allow to optimize the antifungal activity and to determine the chemical compounds of this antifungal activity.

ACKNOWLEDGMENTS

The authors are grateful to:

- The Laboratory of parasitology and mycology of the faculty of Medical Sciences of the University of Cocody-Abidjan for having provided the germ used in this study.
- The National Floristic Center for identifying this specie.
- Members of the laboratory of Biochemistry-Pharmacodynamic for their valuable contribution.

206 - Laboratory technicians for their collaboration

207 **AUTHORS' CONTRIBUTIONS**

208 This work was carried out in collaboration between all authors.

209 ACKAH designed the study, wrote the first and final draft of the manuscript, and wrote the protocols.

210 YAYE executed a scientific study, and checked the results.

211 YAPI, KRA and DJAMAN checked the results and managed literature and scientific searches of the
212 study.

213 All authors read and approved the final manuscript.

214 **REFERENCES**

215 1- Babayi H., Kolo, I., Okogun J. I., Ijah U. J. J., (2004). The antimicrobial activities of methanolic
216 extracts of *Eucalyptus camaldunensis* and *Terminalia catappa* against some pathogenic
217 microorganisms. *Biokemistri*, 16: 106-111.

218 2- Bicanic T. and Harrison T., (2005). Cryptococcal meningitis. *British Medical Bulletin*, 72: 99-118.

219 3- Brantner A. Z., Males S., Pepeljnak S. and Antolic A., (1996). Antimicrobial activity of *Paliurus spina-*
220 *christi* Mill. *Journal of Ethnopharmacology*, 52: 119-122.

221 4- Cowan M. M., (1999). Plant products as antimicrobial agents. *Clinical Microbiology Revue*, 12: 564-
222 582.

223 5- Fabri R. L., M.S. Nogueira, F.G. Braga, E.S. Coimbra, E. Scio, (2008) : *Mitracarpus frigidus* aerial
224 parts exhibited potent antimicrobial, antileishmanial and antioxidant effects. *Bioresource Technology*,
225 100: 428- 433.

226 6- Fyhrquist P., (2007). Traditional medicinal uses and biological activities of some plant extracts of
227 African *Combretum* Loeffl., *Terminalia* L. and *Pteleopsis* Engl. Species (Combretaceae). Thesis,
228 University Main Building Helsinki, 183p. www.ethesis.helsinki.fr .

229 7- Gaudry S., D. Roux, J.-D. Ricard, (2008) : Les méningo-encéphalites. *Les essentiels*, Elsevier
230 Masson SAS, Paris, p.481-494.

231 8- Gurib-Fakim A., (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular*
232 *Aspects of Medicine*, 27: 1-93.

233 9- Harborne J.B., Baxter H. and Moss G. P., (1999). Phytochemical dictionary. A handbook of
234 bioactive compounds from plants, 2nd Ed. London, UK. Taylor & Francis.

235 10- Holt R. J., (1975). Laboratory test of antifungal drugs. *Journal of Clinical Pathology*, 28: 767-774.

236 11- McNeil M. M., S. L. Nash, R. A. Hajjeh, M. A. Phelan, L. A. Conn, B. D. Plikaytis, and D. W.
237 Warnock, (2001). Trends in Mortality Due to Invasive Mycotic Diseases in the United States, 1980-
238 1997. *Clinical Infectious Diseases*, 33: 641-647.

239 12- Michel G., (2011). Mycoses invasives au cours du traitement des maladies malignes de l'enfant.
240 Elsevier Masson SAS, Paris. *Archives de Pédiatrie*, 18 : 3-7.

241 13- Neofytos D., Horn D., Anaissie E., Steinbach W., Olyaei A., Fishman J., Pfaller M., Chang C.,
242 Webster K. and Marr K., (2009). Epidemiology and Outcome of Invasive Fungal Infection in Adult
243 Hematopoietic Stem Cell Transplant Recipients: Analysis. *Clinical Infectious Diseases*, 48: 265-73.

- 14- Ouattara B., Kra A. M., Coulibaly A. et Guédé-Guina F., (2007). Efficacité de l'extrait éthanolique de *Thonningia sanguinea* sur *Cryptococcus neoformans*. *Cahier d'études et de recherches francophones/ santé*, 17 : 219-22.
- 15- Parc B.J., Marston B.J., Govender N. and Pappas P.G., (2009). Estimation Of The Current Global Burden Of Cryptococcal Meningitis Among Persons Living With HIV/AIDS. *AIDS*, 23: 525-530.
- 16- Rivière C., Nicolas J.-P., Caradec M.-L., Désiré O. et Schmitt A., (2005). Les plantes de la région Nord de Madagascar : une approche ethno pharmacologique. *Bulletin de la Société Française d'Ethnopharmacologie et de la Société Européenne d'Ethnopharmacologie*, 36: 36-50.
- 17- Schwarz P., F. Dromer, O. Lortholary and E. Dannaoui, (2006). Efficacy of Amphotericin B in Combination with Flucytosine against Flucytosine-Susceptible or Flucytosine-Resistant Isolates of *Cryptococcus neoformans* during Disseminated Murine Cryptococcosis. *Antimicrobial Agents Chemother.* 50: 113-120.
- 18- Seddon J., N Mangeya , R. F. Miller , E. L. Corbett et R. A. Ferrand, (2009). La récurrence de la méningite cryptococcique dans le VIH chez les patients infectés à la suite de reconstitution immunitaire. *International Journal of STD & AIDS*, 20 : 274-275.
- 19- Yayé Y. G., Ackah J. A., Kra A. K. M., Djaman J. A., (2012). Anti-Fungal Activity of Different Extracts of *Terminalia Mantaly* H. Perrier on the *in Vitro* Growth of *Aspergillus Fumigatus*. *European Journal of Scientific Research*, 82 : 132-138.

281

282

283

284

285

286

287

288

UNDER PEER REVIEW