Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 700-705
Received: 16-06-2020
Accepted: 05-08-2020

Yayé Yapi Guillaume
1) Biology-Health sector, Biochemistry-Microbiology Department, UFR Agroforestry, Jean Lorougnon Guédé University, Bp 150 Daloa
2) Laboratory of Biology and Health, UFR Biosciences, Université Félix Houphouet-Boigny, 22 BP 582 Abidjan 22

Obouayéba Abba Pacôme
1) Biology-Health sector, Biochemistry-Microbiology Department, UFR Agroforestry, Jean Lorougnon Guédé University, Bp 150 Daloa
2) Laboratory of Biology and Health, UFR Biosciences, Université Félix Houphouet-Boigny, 22 BP 582 Abidjan 22

Ackah Jacques Alfred Auguste Bognan
1) Biology-Health sector, Biochemistry-Microbiology Department, UFR Agroforestry, Jean Lorougnon Guédé University, Bp 150 Daloa
2) Laboratory of Biology and Health, UFR Biosciences, Université Félix Houphouet-Boigny, 22 BP 582 Abidjan 22

Djaman Allico Joseph
1) Laboratory of Biology and Health, UFR Biosciences, Université Félix Houphouet-Boigny, 22 BP 582 Abidjan 22

Corresponding Author:
Yayé Yapi Guillaume
1) Biology-Health sector, Biochemistry-Microbiology Department, UFR Agroforestry, Jean Lorougnon Guédé University, Bp 150 Daloa
2) Laboratory of Biology and Health, UFR Biosciences, Université Félix Houphouet-Boigny, 22 BP 582 Abidjan 22

Optimization of antifungal activity and chemical study of *Terminalia mantaly* extracts

Yayé Yapi Guillaume, Obouayéba Abba Pacôme, Ackah Jacques Alfred Auguste Bognan and Djaman Allico Joseph

Abstract
Context: We are witnessing an increasing number of mycosis due to a weakened immune system, resistance and the limited number of antifungal agents. Hence the need to develop other antifungal agents with pronounced activity.
Methodology: The soxhlet extract obtained from hydroalcoolic extract of *Terminalia mantaly* bark was chromatographed on Sephadex gel G25. The extracts obtained and a ketoconazole, were tested on the *in vitro* growth of fungal isolates. A chemical study (phytochemical screening, TLC and HPLC) was carried out.
Results: Of the 19 extracts obtained, the F_8, F_9, F_{10} and P extracts showed the best activities (MFC = 24.37 µg/mL and 12.18 µg/mL respectively on *A. fumigatus* and *C. albicans*, *C. neoformans*). The chemical study revealed the phenols and quinones that could be responsible for this activity.
Conclusion: This extraction resultd in more pronounced antifungal activity which would be due to phenols and/or quinones.

Keywords: *Terminalia mantaly*, column chromatography, antifungal, chemical

Introduction
Advances in medicine have made it possible to better control the evolution of many diseases that were once fatal. However, the list of new fungi emerging in medicine is constantly growing and are involved in pathologicals process (Bioforma, 2002) [1]. They are less visible and are responsible for more or less serious conditions called mycoses.
These mycoses have increased considerably and their etiological agents have diversified in recent years, largely due to the growing number of patients with impaired defences caused by pathologies such as cancer, HIV infection and by treatments such as organ transplantation, corticosteroid treatment or immunosuppressants (Marchetti *et al.*, 2004 ; Lehrnbecher *et al.*, 2010) [2, 3]. It should also be remembered that, despite medical progress, existing antifungal molecules present many limitations. Indeed, most of these antifungals have certains toxicities or contraindications and are even very limited in number without omitting the increasingly frequent resistances (Bretagne, 2009; Resplandy, 2017) [4, 5].
Thus, in order to find new therapeutic pathways, research in medical mycology requires new approaches (Piens *et al*., 2003) [6], hence that of pharmacopoeia to find new series of molecules that would be less toxic given of their natural synthesis and pronounced activity. Thus among these species of plants, work carried out on Terminalia mantaly has already revealed satisfactory antimicrobial results on both bacterial and fungal germs (Yayé *et al*., 2011 & 2012; Ackah *et al*., 2013 & 2014) [7, 8, 9, 10].
The objective of this study is to optimize the activity of Terminalia mantaly extract obtained from a series of bioguided extractions (Yayé *et al*., 2011 & 2012; Ackah *et al*., 2013) [7, 8, 9] and to evaluate this activity on *in vitro* growth of 3 fungal isolates (*Candida albicans, Aspergillus fumigatus and Cryptococcus neoformans*) which are germs of medical interest (Grillot, 2007; Schmiedel & Zimmerli, 2016; Institut pasteur, 2017) [11, 12, 13].

Material and Methods
Material
The plant material is made up of extracts from the bark of Terminalia mantaly. For the antifungal evaluation, *Candida albicans, Aspergillus fumigatus et Cryptococcus neoformans* are used where are provided by the laboratory of Mycology of UFR of Medical Sciences of the University of Cocody-Abidjan (Côte d'Ivoire). This germ was isolated from patients in the infectious diseases.

A usual antifungal agent ketokonazole was also used in this study. And Sabouraud Agar buffered to pH 5.7 was used to determine the activity of the plant extracts.

Methods

Preparation of extracts

Ten (10) g of the extract T_0 was degreased in 350 mL of hexane. Thus that made it possible to obtain T_{4-2} (degreased extract) (Yayé *et al*., 2011 & 2012; Ackah *et al*., 2013) [7, 8, 9]. Subsequently 5 mg of this extract diluted in distilled water were chromatographed on a Sephadex G25 gel filtration column whose characteristics are 1 cm in diameter, 53 cm in height of the gel with a flow rate of 1.48 cm^3/min. The fractions obtained were dried in an oven at 60 °C.

In addition, some of these fractionated extracts (F_8, F_9 and F_{10}) were merged in the same proportion to form a new fraction P. The efficacy of this extract was assessed on the *in vitro* growth of the 3 fungal isolatesalong with that of usual antifungal, ketoconazole (powder).

Culture medium

The culture medium was prepared according to the instructions of the manufacturer's protocol. The inclusion of the various plant extracts in the agar was made using the method of the double dilution agar slopes (Zirihi *et al*., 2003) [14]. Ten test tubes were used per series of which 8 containing the plant extract. The concentrations of these tubes ranged from 780 to 6.09 µg/mL. The 2 other tubes were regarded as control tubes in which one was without plant extract for the control of the growth of fungal germs and the other without plant extract without germs was used as sterility control.

Then, the 10 tubes were removed by the use of forceps sterilized by flaming for 15 min at 121 °C. The tubes were inclined to room temperature of the laboratory to cooling and solidification of the agar.

Antifungal Assessment

A 48-hour colony of each germ was used for the preparation of the inoculum. The inoculum has been prepared by dilution of 1/10 and each test tubes except the sterility control tube, the culture of the germ was made by sowing of 10 µL of the fungal suspension (10^{-1}) corresponded 1000 cells.

After incubation at 30 °C for 48 hours, the colonies were numbered with a pen colony counter. Moreover, the growth in the experimental tubes was assessed as a percentage of survival calculated compared to 100% survival in the control tube growth. The experimental data were used to determine the antifungal parameters as minimun inhibition concentration (MIC) and minimum fungicidal concentration (MFC). In effect, the value of MFC were determined after dilution of 10^{-1}, 10^{-2}, 10^{-3} et 10^{-4} of content of the tube of MIC.

Phytochemical screening

The chemicals components were screened only in the extracts T_{4-2} and F_8, F_9, F_{10} and P following the results of the antifungal assessment. To describe the availability of chemical compounds in these extracts, scores ranging from 0-3 (absence or presence) have been assigned. Thus, the absence was symbolized by a score of 0, the presence in small quantities by a score of 1, the presence in average quantity by a score of 2 and finally in large quantity by a score of 3.

In addition, the methods used for revealing the presence of chemical components are those used classically in the phytochemical screening of medicinal plants. Thus, the phenols were highlighted by the reaction to ferric chloride at 2%, cathechic tannins by stiasny reagent. As for gallic tannins, sodium acetate added to ferric chloride at 2% was used. The alkaloids were detected by Dragendorff, valser mayer and Bouchardat reagents. Saponins were highlighted by measuring the height shaking. The reaction to cyanidrin made it possible to highlight the presence of flavonoids. Quinones were revealed by bortraegen reagent. About sterols and terpens, the Lieberman-Buchard reagent was used. Lastly, ammonia hydroxide at 10% was used for Coumarins.

Thin Layer Chromatography (TLC) of fractions F_8, F_9, F_{10} and P

A TLC with fractions F_8, F_9 and F_{10} was been requiered. Thus, on an aluminum plate covered with a layer of silica gel (8 cm x 5 cm), a horizontal line was drawn 1.5 cm from the base on which was deposited a 15 µL drop of each fraction 3 cm apart. The drops are first dried at room temperature, then the plate was immersed from the bottom edge into a glass vat containing 100 mL of the eluent consisting of ethyl acetate, methanol and distilled water in the respective proportions of 81-11-8 then the vat was hermetically closed.

After about 2 hours the experiment was stopped and the plate was removed from the vat and dried. The revelation was made with an ethanolic solution of 10 mL at 10% potassium hydroxide for the detection of quinones and a 5% solution of iron chloride in 0.5 M hydrochloric acid for the phenols. In addition, a visualization was made at 365 nm.

As for the P fraction before the TLC, this fraction was solubilized in methanol: G_1 (deposit) and G_2 (methanol soluble part). This TLC was carried under the same conditions as before but this time the aluminium plate is 8 cm x 3 cm and the horizontal line was drawn 1 cm from the base and on which was deposited 2 drops of 10 µL of the 2 sub-fractions. The ethyl acetate/methanol mixture in the proportions 70-30 was used as eluent. The detection was also done at 365 nm.

High Performance Liquid Chromatography (HPLC)

For the performance of this chromatography, the sampling characteristics were programmed using a computer (Table I) and the column used is a C18 RP (Reverse Phase). The elution was constituted from a binary mixing system (mobile phase) in gradient mode, with variable proportions of 0.1% TFA (Tri Fluoro Acetic Acidified Water) and 100% acetonitrile. The flow rate was set at 1 ml/min and the volume injected at 10 µL. The elution time was set at 60 min. At the exit of the column, the compounds are detected at UV 365 nm.

Table 1: Programming for HPLC Chromatography

Flow rate (ml/min)	Time (min)	% A	% B	% C
1	0	95	0	5
	10.0	85	0	15
	45.0	70	0	30
	50.0	0	0	100
	55.0	0	0	100

A: H_2O at 0.1% TFA; B: Methanol ; C : Acetonitrile

Results

Antifungals results

We collected 18 fractions of 10 mL which constitute 18 extracts after drying. These 18 extracts and the usual antifungal agent, ketoconazole, have been used to determine the MIC and MFC values (Table II).

These MFC values coincide with those of MIC on the *in vitro* growth of the 3 fungal germs tested. In addition, analysis of

the results of the antifungal activity of these extracts on the *in vitro* growth of the 3 fungal germs tested shows that these different extracts as follows:

- The extracts with MFC values >780 µg/mL: F_1, F_2, F_3, F_4, F_{17} and F_{18}.
- The extracts which inhibit the growth of fungal germs with MFC values higher than that of their base extract ($T_{4\text{-}2}$): F_5, F_6, F_7, F_{11}, F_{12}, F_{13}, F_{14}, F_{15} and F_{16}.
- and the extracts whose inhibition is effective at MFC values lower than those of the base extract : F_8, F_9, F_{10} (MFC = 24.37 µg/mL and 12.18 µg/mL respectively for *Aspergillus fumigatus* and yeasts (*Candida albicans* and *Cryptococcus neoformans*).

Further more, extract P shows that the MFC values obtained are identical to those of extracts F_8, F_9 and F_{10} taken separately.

Table 2: Table of MFC values obtained on the *in vitro* growth of the 3 fungal isolates

Extracts and Antifungal	*Aspergillus fumigatus*	*Candida albicans*	*Cryptoccocus neoformans*	Groupe
$T_{4\text{-}2}$ (base extract)	**48.75**	**24.37**	**24.37**	
F_1	>780	>780	>780	
F_2	>780	>780	>780	
F_3	>780	>780	>780	G_1
F_4	>780	>780	>780	
F_5	780	390	390	
F_6	390	195	195	G_2
F_7	97.5	48.75	48.75	
F_8	24.37	12.18	12.18	
F_9	24.37	12.18	12.18	**G_3**
F_{10}	24.37	12.18	12.18	
F_{11}	48.75	24.37	24.37	
F_{12}	97.5	48.75	24.37	
F_{13}	195	48.75	48.75	
F_{14}	195	97.5	48.75	
F_{15}	390	195	195	G_2
F_{16}	780	195	390	
F_{17}	>780	>780	>780	
F_{18}	>780	>780	>780	G_1
Ketoconazole	97.5	390	48.75	

Phytochemical screening results

The extract $T_{4\text{-}2}$ is rich (score 3) in phenols and quinones. However, alkaloids are present in small quantities (score 1) and the other chemical compounds are absent (score 0) (Yayé *et al*., 2012)[8]. We notice the same results with the extracts F_8, F_9, F_{10} and P witch also contain phenols and quinones with a score of 3. One can also note traces of alkaloids (score 1) (Figure 1).

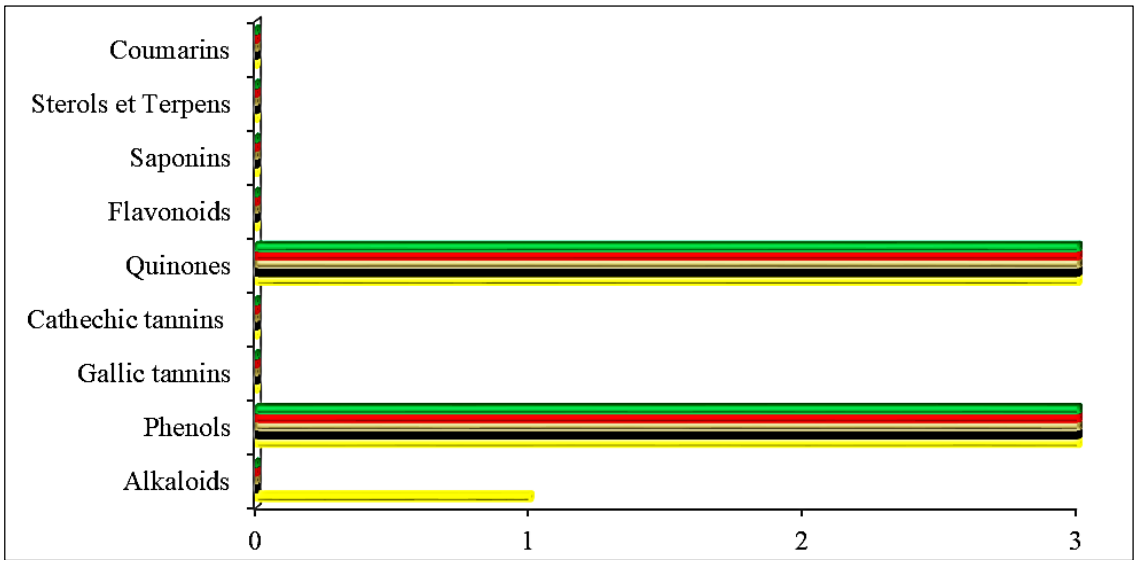

Fig 1: Chemical compound present in $T_{4\text{-}2}$, F_8, F_9, F_{10} and P extracts

Chromatogram results

Two hours after the development of the TLC chromatogram of the extracts F_8, F_9 and F_{10}, the 3 corresponding tasks have been revealed and show the same frontal ratio at UV 365 nm (Rf = 0.77).

As for the development of that of the extract P, 2 tasks are observed for sub-fraction G_1, and 1 task for the sub-fraction G_2. One of the tasks in the sub-fraction G_1 has the same profile as that of the sub-fraction G_2 (Rf = 0.88), the other task having a value of 0.76. (Figure 2).

Fig 2: Photograph of fraction P chromatogram at UV 365 nm

The HPLC chromatogram confirms the presence of 2 chemical compound. Thus, at UV 365 nm two (2) distinct intense peaks are observed at Retention Times of 3.15 min with an absorbance of 220 mAU and 12.64 min with an absorbance of 90 mAU. These peaks correspond to the major compounds contained in the extract P.

Other peaks of much smaller sizes appearing respectively at 4.37 min (A ≈ 20 mAU), 9.63 min (A ≈ 28 mAU) and 34.00 min (A ≈ 20 mAU) correspond to traces of chemical compounds (very minority). (Figure 3).

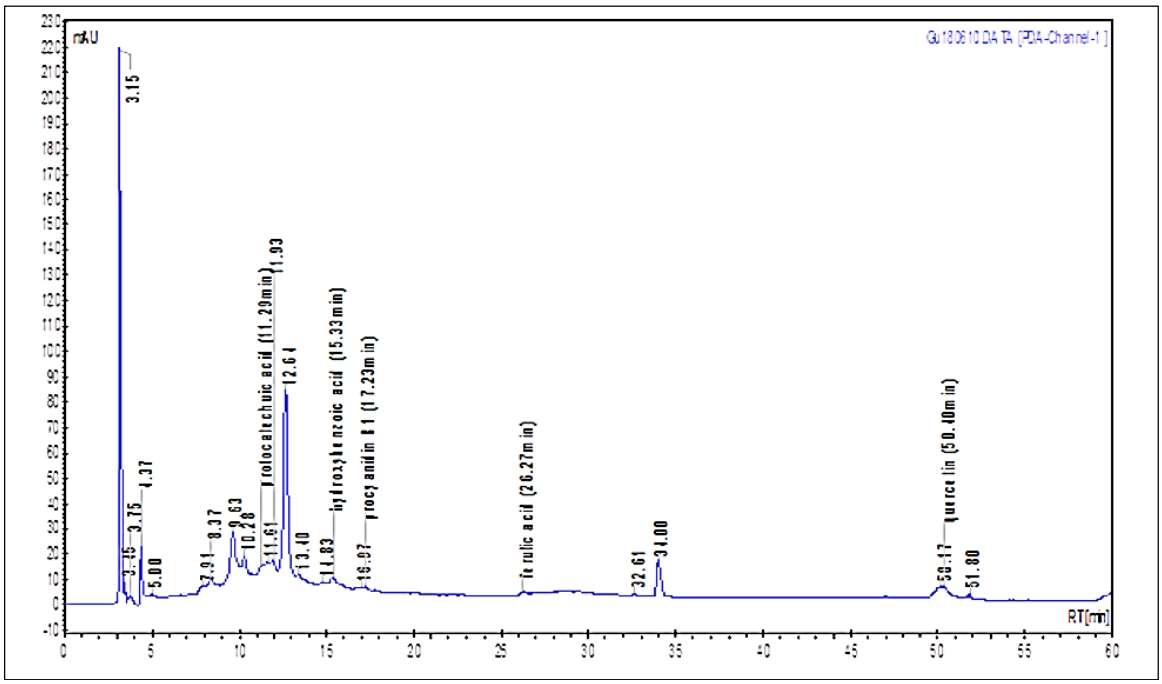

Fig 3: HPLC chromatogram at 365 nm

Discussion

The antifungal activity of the T_{4-2} extract has been proven by the work of Yayé *et al*., 2011 [7] and 2012 [8] and those of Ackah *et al*, 2013 [9]. This activity is fungicidal. Thus in view of these performances, this extract has been optimized by Soxhlet extraction since the aqueous phases are the most active according to these same authors.

For the fractional extracts, the results obtained make it possible to classify them into 3 groups. Thus group G_1 is the group whose extacts have MFC values > 780 µg/mL (F_1, F_2, F_3, F_4, F_{17} and F_{18}), group G_2 constitues extacts of values of MFC > CMF T_{4-2} (F_5, F_6, F_7, F_{11}, F_{12}, F_{13}, F_{14}, F_{15} and F_{16}) and group G_3 is the group whose extracts have CMF values < CMF of the T_{4-2} extract (F_8, F_9 and F_{10}).

On the other hand, the most active extract in the group G_1 is the extract F_4. This activity evolves in the direction of obtaining the extacts. Indeed, from extract F_1 to extract F_4, the activity changes from the least active to the most active. It is the same for extracts F_{17} to F_{18} which evolves from the most active to the least active. This same remark is made with those of group G_2. In fact, the activity is increasing from F_5 to F_7 and decreasing from F_{11} to F_{16}.

As for extracts of group G_3 (F_8, F_9 and F_{10}), analysis of the MFC values reveals that these extracts have the same MFC value on each fungal isolate. It is the same is true for the extract P. These extracts (F_8, F_9 and F_{10}) in reality constitute the same active fraction, hence the extract P. However, this performance differs according to the fungal germs studied and is twice as active as the T_{4-2} base extract.

Chromatographic fractionation on Sephadex G25 gel has therefore made it possible to better concentrate the active ingredients contained in the non-hexanosoluble extract (T_{4-2} extract). These results of the P extract are better than those obtained by Kporou *et al*., 2010[15] with a chromatographic extract F_8 obtained from a hexanic extract of *Mitracarpus scaber* (CMF=781 µg/mL) on the *in vitro* growth of *C. albicans*. The same applies to the work of Aref *et al*., 2010[16] which obtained inhibition percentages of 100% and 80% respectively on the *in vitro* growth of yeasts (*C. albicans*, *C. neoformans*) and *A. fumigatus* for a concentration of 500 µg/mL of Ethyl acetate/hexane chromatographic extract (50 : 50) obtained from the methanolic latex extract of *Ficus carica*.

Furthermore, comparison of the activity of this extract P with that of the antifungal agent ketoconazole on the various fungal germs tested reveals that the extract P is more active. This activity is more pronounced on the growth of *C. albicans* than on the other 2 species (*A. fumigatus* and *C. neoformans*). Indeed, several studies have proven the resistance of *C. albicans* species to azoles, especially ketoconazole (Cartledge *et al*., 1997, Müller *et al*., 2000; Monroy-Pérez *et al*., 2016) [17, 18, 19]. This would explain the level of difference in this comparison with ketoconazole which is one of the antifungal molecules referred to in Côte d'Ivoire.

In parallel to the study of antifungal activity, the study of phytochemical screening coupled with chromatographic studies have allowed to have an idea of the chemical compound(s) that would be responsible for this activity. Previous work by Yayé *et al*., 2012[8] showed the presence of phenols and quinones in the T_{4-2} extract. These results are confirmed for the fractions F_8, F_9, F_{10} and P and reveal that this antifungal activity of the extract P would be linked to phenols and/or quinones (two aromatic-based chemical compounds) which could act either in isolation or synergistically.

Besides, the TLC of extracts F_8, F_9 and F_{10}, indicate that these 3 extracts contain the same types of molecules because they have the same profile. The TLC of extract P revealing the presence of 2 chemical compounds (Rf = 0.76 and 0.88) also reflects the results of the phytochemical screening. This assume that these extracts with the same biological activity would contain the same 2 chemicals.

In addition, the chromatographic profile of the HPLC indicates that extract P contains 2 major chemicals compounds at the retention times of 3.15 min and 12.64 min. These relatively low retention times indicate that these chemicals compounds would be highly polar compounds. Indeed, with reversed-phase HPLC, the most polar chemicals compounds are released before the least polar (Jandera and Chutaček, 1985; Chromacademy, 2016) [20,21]. Subsequently the presence of these two peaks revealing the presence of two chemical compounds implies that the extract P is relatively pure. The antifungal activity of this fraction can be attributed to one or both compounds.

Thus link chemical studies (phytochemical screening, TLC and HPLC) of the extract P reveals that these chemical compounds at the basis of the antifungal activity could be phenols and/or quinones. These results are in line with those reported by of scientific work. In fact, phenols are known for their antifungal properties by their toxicity and in some cases by their inhibitory action on growth or their interaction with enzymes of fungal germs (Alves *et al*., 2014; Onarian & Bayram, 2017 ; Carvalho *et al*., 2018) [22, 23, 24]. As for quinones, they have also proven their antifungal properties (Meazza *et al*., 2003; Futuro *et al*., 2018) [25, 26]. They act on polypeptides and enzymes of the membrane of microbial cells by forming irreversible complexes with the nucleophilic amino acids of these proteins thus neutralizing their functions or preventing the activity of their substrates (Stern *et al*., 1996; Cowan, 1999) [27, 28]. In addition, other quinones such as anthraquinones inhibit the synthesis of their nucleic acids (Harbonne *et al*., 1999) [29].

Conclusion

The extract P obtained from column chromatography of Sephadex G25 Gel is the most active extract and contains quinones and phenols which are believed to be responsible for this antifungal activity. This activity is more pronounced than that of the non-hexanosoluble base extract. The extraction method used has therefore made it possible to achieve the objective which of optimizing the antifungal activity of partitioned extracts of *Terminalia mantaly* H. Perrier.

Furthermore, further purification of the P extract until the isolation of the molecule would allow to obtain an even greater antifungal activity. Nevertheless, this fraction could already constitute a very attractive option for the development of an Improved Traditional Medicine.

This species does indeed possess anti-infectious properties, hence its use in traditional environments.

Acknowledgements

Authors would like to thank,

- The national floristic center of Félix Houphouet-Boigny University (Côte d'Ivoire) for its help in the identification of this plant species,
- The research team of Pharmacodynamics-Biochemistry of the Félix Houphouet-Boigny University (Côte d'Ivoire) for their support in the biological tests.
- Researchers from the Biochemistry-Microbiology Department of Jean Lorougnon Guédé University of Daloa (Côte d'Ivoire).

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

References

1. Bioforma. Les moisissures d'intérêt médical. Cahier de formation Biologie médicale, N°25-Mars 2002, Paris, France. www.bioforma.net 26 April, 2020
2. Marchetti O, Bille J, Fluckiger U, Eggimann P, Ruef C, Garbino J *et al*. Fungal Infection Network of Switzerland. Epidemiology of candidemia in Swiss tertiary care hospitals: secular trends, 1991-2000. Clinical infectious diseases. 2004; 38(3):311-320.
3. Lehrnbecher T, Frank C, Engels K, Kriener S, Groll AH, Schwabe D. Trends in the postmortem epidemiology of invasive fungal infections at a university hospital. The Journal of infection. 2010; 61(3):259-265.
4. Resplandy F. Antifongiques : les points essentiels. Collège National de Pharmacologie Médicale 2017. http//www.doctissimo.fr, 2020
5. Bretagne S. De nouvelles molécules pour les infections fongiques ? Antibiotiques. 2009; 11(3):133-141.
6. Piens MA, De Monbrison F, Picot S. Étude de la sensibilité des levures aux antifongiques en pratique médicale. La Lettre de l'Infectiologue, Tome. 2003; XVIII, n°6:222-226.
7. Yayé YG, Kra AKM, Ackah JAAB ET, Djaman AJ. Evaluation de l'activité antifongique et essai de purification des principes actifs des extraits de *Terminalia mantaly* (H. Perrier), une combretacée, sur la croissance *in vitro* de *Candida albicans*. Bulletin de la Société Royale des Sciences de Liège. 2011; 80(2011):953-964.
8. Yayé YG, Ackah JA, Kra AKM, Djaman AJ. Anti-fungal activity of different extracts of *Terminalia Mantaly* (H. Perrier) on the *in vitro* growth of *Aspergillus Fumigatus.* European Journal of Scientific Research. 2012; 82(1):132-138.
9. Ackah JAAB, Yayé YG, Yapi HF, Kra AKM and Djaman AJ. Antifungal activity of *Terminalia mantaly* on the *in vitro* growth of *Cryptococcus neoformans.* International Journal of Biochemistry Research & Review. 2013; 3(1):63-73.
10. Ackah JAAB, Kokora AP, Yayé YG, Bahi C, Loukou YG, Coulibaly A, Djaman AJ. Action Spectrum of *Terminalia Mantaly* on the *in vitro* growth of *Pseudomonas aeruginosa.* International Journal for Pharmaceutical Research Scholars. 2014; 3(I-1):795-800.

11. Grillot R. Anidulafungine: activité *in vitro* sur les levures et les champignons filamenteux d'intérêt clinique. Elsevier Masson, Réanimation. 2007; 16:S261-S266.
12. Schmiedel Y, Zimmerli S. Common invasive fungal diseases: an overview of invasive candidiasis, aspergillosis, cryptococcosis, and *Pneumocystis pneumonia*. Swiss medical weekly 2016; 146:w14281.
13. Rapport annuel d'activité 2017 (Année d'exercice 2016) du Centre national de référence Mycoses Invasives et Antifongiques. http // www.pasteur.fr 30 April, 2020.
14. Zirihi GN, Kra AM et Guédé-Guina F. Evaluation de l'activité antifongique de *Microglossa pyrifolia* (Lamarck) O. Kunze (Asteraceae) <<PYMI>> sur la croissance *in vitro* de *Candida albicans*. Revue de Médecine et de Pharmacie d'Afrique. 2003; 17:11-18.
15. Kporou KE, Kra MAK, Ouattara S, Guédé-Guina F, Djaman J. Amélioration par fractionnement chromatographique de l'activité anticandidosique d'un extrait hexanique de Mitracarpus scaber Zucc. sur la croissance *in vitro* de *Candida albicans* et *Candida tropicalis*. Phytotherapie 2010; 8:290-294.
16. Aref HL, Salah KBH, Chaumont JP, Fekih A, Aouni M, Said K. *In vitro* antimicrobial activity of four Ficus carica latex fractions against resistant human pathogens (antimicrobial activity of ficus carica latex). Pakistan journal of pharmaceutical sciences 2010; 23(1):53-58.
17. Cartledge JD, Midgley J, Gazzard BG. Clinically significant azole cross-resistance in *Candida* isolates from HIV-positive patients with oral candidosis. AIDS 1997; 11(15):1839-1844.
18. Müller FMC, Weig M, Peter J, Walsh TJ. Azole cross-resistance to ketoconazole, fluconazole, itraconazole and voriconazole in clinical *Candida albicans* isolates from HIV-infected children with oropharyngeal candidosis. Journal of Antimicrobial Chemotherapy. 2000; 46(2):338-341.
19. Monroy-Pérez E, Paniagua-Contreras GL, Rodríguez-Purata P, Vaca-Paniagua F, Vázquez-Villaseñor M, Díaz-Velásquez C, Uribe-García A, Vaca S. High virulence and antifungal resistance in clinical strains of *Candida albicans*. Canadian Journal of Infectious Diseases and Medical Microbiology 2016; 2016:5930489.
20. Jandera P, Chutaček J. Gradient elution in column liquid chromatography: Theory and Pratice. Journal of Chromatography Libary 1985; 31:xvii-xix.
21. Chromacademy. The Theory of HPLC-Reverse Phase chromatography. Crawfordscientific. http// www.chmacademy.com/lms/Sco5, 2020, 93.
22. Alves CT, Ferreira IC, Barros L, Silva S, Azeredo J, Henriques M. Antifungal activity of phenolic compounds identified in flowers from North Eastern Portugal against Candida species. Future Microbiology 2014; 9 (2):139-146.
23. Onaran A, Bayram M. Determination of Antifungal Activity and Phenolic Compounds of Endemic Muscari aucheri (Boiss.) Baker Extract. Journal of Agricultural Faculty of Gaziosmanpasa University 2017; 35(1):60-67.
24. Carvalho RS, Carollo CA, De Magalhães JC, Palumbo JMC, Boaretto AG, Nunes e Sá IC, Ferraz AC, Lima WG, De Siqueira JM, Ferreira JMS. Antibacterial and antifungal activities of phenolic compound-enriched ethyl acetate fraction from *Cochlospermum regium* (mart. Et. Schr.) Pilger roots: Mechanisms of action and synergism with tannin and gallic acid. South African Journal of Botany. 2018; 114:181-187.
25. Meazza G, Franck E, Dayan, David E, Wedge. Activity of Quinones on *Colletotrichum* species. Journal of Agricultural and Food Chemistry. 2003; 51(13):3824-3828.
26. Futuro DO, Ferreira PG, Nicoletti CD, Borba-Santos LP, Da Silva FC, Rozental S, Ferreira VF. The Antifungal Activity of Naphthoquinones: An Integrative Review. Anais da Academia Brasileira de Ciências. 2018; 90(1&2):1187-1214.
27. Stern LJ, Hagerman AE, Steinberg PD & Mason PK. Phlorotannin-protein interactions. Journal of Chemical Ecology 1996; 22:1877-1899.
28. Cowan M. Plant products as Antimicrobial Agents. Clinical Microbiology Reviews 1999; 12 : 564-582.
29. Harborne JB, Baxter H and Moss GP. Phytochemical dictionary. In: A handbook of bioactive compounds from plants. 2nd Eds, Taylor & Francis, London. 1999, 869-877.