ANTIFUNGAL ACTIVITY OF Terminalia superba (COMBRETACEAE)

Kra Adou Koffi Mathieu1,*, SIAKA Sohro2, Ahon Gnamien Marcel1, KASSI Amian Brise Benjamin3, OUATTARA Sitapha1, AW Sadat2, COULIBALY Adama1,4, SORO Yaya2 and DJAMAN Allico Joseph1,5

1Laboratory of Pharmacodynamic Biochemistry, UFR of Biosciences, Université Félix Houphouët Boigny/ 22 B.P. 582 Abidjan 22 Côte d’Ivoire
2Laboratory of Industrial Process Synthesis, Environment and Energy News, Institut National Polytechnique Félix H. B
3Laboratory Structural Organic Chemistry of UFR SSMT University Félix Houphouët Boigny/22 B.P 582 Abidjan 22 Côte d’Ivoire
4Université Péléphor Gon Coulibaly, Korhogo
5Department of Clinical and Fundamental Biochemistry, Pasteur Institute, Abidjan, Côte d’Ivoire

Received – January 22, 2015; Revision – February 15, 2015; Accepted – April 05, 2015
Available Online – April 25, 2015

DOI: http://dx.doi.org/10.18006/2015.3(2).162.173

KEYWORDS

Terminalia superb
Anticandidosic
Ellagic acid derivative

ABSTRACT

The aim of the present study was to optimize the anticandidosic activities of Terminalia superb (TEKAM4) and the identification of major compounds present in the most active chromatographic fraction. The hydroethanolic extract TEKAM4-X0 was prepared by homogenization employing a blender. Two derivatives extracts of TEKAM4-X0 (X1-1 and X1-2) were obtained by a liquid/liquid partition of TEKAM4-X0 in a mixture of hexane and water (v/v). Three chromatographic fractions (F1, F2 and F3) from X1-2 were separated by means of Sephadex-LH20 gel filtration chromatography. All the extracts were incorporated to Sabouraud according to the agar slanted double dilution method. Ketoconazole was used as standards for antifungal assay. The entire fractions were tested on the previously prepared medium culture containing 1000 cells of C. albicans. Antifungal activity was determined by evaluating antifungal parameters values (MFC and IC50). Lastly, the structures of 2 isolated compounds were elucidated by combination of Flash chromatography and spectroscopic methods, including MS, and multiple stage RMN experiments.

All the article published by Journal of Experimental Biology and Agricultural Sciences is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License Based on a work at www.jebas.org.

* Corresponding author
E-mail: mattykra@yahoo.fr (KraAdouKoffi Mathieu)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.
1 Introduction

Terminalia superba Engl. & Diels (Combretaceae) used by various traditional healers for the treatment of bacterial, fungal and viral infections in Côte d’Ivoire (Ahon et al., 2012). Furthermore, Kra et al. (2014) reported the antifungal activities and established the antimicrobial virtues of this plant. Results of various investigations have proved that hydro-ethanolic crude extract of T. superba stem bark shows strong in vitro antifungal activity against C. albicans. The present study was led to optimize the antifungal activities of the isolated and identified active ingredients of T. superba against C. albicans.

2 Materials and Methods

2.1 Plant Material

The plant material was a powder of the stem bark of T. superba. The pieces of barks were collected from the Mopri forest (Côte d’Ivoire) during ethnobotanical investigations of 2014. In the first step of the study some samples of T. superba were identified and taxonomically authenticated at the Herbarium of the floristic national center of the Felix Houphouët Boigny University (Abidjan, Côte d’Ivoire) by comparison with available specimens (Harvested from Tonkoui mountain on 26th February 1969, L. Aké Assi Number 10477). Furthermore large amount of stem bark were collected and carefully checked to single out all undesired specimen parts. The barks were then cut to small fragments and carefully shade dried during two weeks at ambient temperature under laboratory condition with continuous ventilation away from sunlight and dust. After drying all the vegetal pieces were crushed to fine powder with an electrical grinder. At last, powders were hermetically sealed in polyethylene bags and stored away from light and moisture until used for extraction (Yaye et al., 2012; Ouattara et al., 2013a; Kra et al., 2014).

2.2 Extraction

The hydro-ethanolic crude extract was prepared from the stem bark powder of T. superba. For this hundred grams (100g) of bark powder were extracted in one liter solvent with a mixture of ethanol (70%) and water (30%) by homogenization in a blender. The homogenates obtained were first wrung out in a fabric square and then filtered twice successively with absorbent cotton and once with Whatman 3mm filter paper. The resulting filtrate was concentrated under vacuum using a Büchi rotary evaporator at 60°C (Zirihi et al., 2003). Obtained dark powder is the hydro-ethanolic crude extract (35 g) which was codified TEKAM<sub>1</sub>-X<sub>0</sub>.

After this step, 10g of TEKAM<sub>1</sub>-X<sub>0</sub> were submitted to liquid/liquid partition in 400 mL of a hexane/water mixture (v/v, 50/50). After decantation, these two phases were separated and concentrated under vacuum using a Büchi rotary evaporator at 60°C which gave two extracts: X<sub>1</sub> (hexane phase) and X<sub>1,2</sub> (aqueous phase) (Achak et al., 2008; Yaye et al., 2012; Ouattara et al., 2013c).

On the basis of the antimicrobial investigations of the above extracts, 2 g of X<sub>1,2</sub> were subjected to column chromatography on Sephadex-LH<sub>20</sub> gel and eluted with a MeOH gradient system. The flow velocity was 0.125 mL/min. Forty sub-fractions (SF<sub>1</sub> to SF<sub>40</sub>) of 20 mL each were collected separately. All these fractions were then pooled following analytic TLC investigations in three new major fractions: F<sub>1</sub> (sub-fractions 1 to 6: 120 mL), F<sub>2</sub> (sub-fractions 7 to 18: 240 mL) and F<sub>3</sub> (sub-fractions 19 to 40: 440 mL). TLC plates (Mcherey-Nagel, ALUGRAM<sup>®</sup> SILG/UV<sub>254</sub> 0.2mm silica gel 60 Å) were visualized under UV light at 254-365 nm and/or spraying them with appropriated reagents (Meyer, Dragendorff, Liebermann Burchard, NEU, Vanilline sulfuric). The antifungal activity of the 3 fractions was evaluated against C. albicans.

2.3 Fungal germs tested

The tested fungus was the clinical and identified strain of C. albicans (n° 896/AB of 10.01.2000). This strain was provided by the Laboratory of Mycology Medical Sciences Faculty Felix Houphouët Boigny University (Abidjan, Côte d’Ivoire). C. albicans is an opportunistic fungus (Abi-Said et al., 1997; Agoumi, 2000). It is a normal human commensal but it becomes pathogenic when the immune system fails (Chabasse, 1994) and caused about 83% of infections (Agoumi, 2000; Develoux and Bretegane, 2005).

The results of the antifungal assays showed that TEKAM<sub>1</sub>-X<sub>0</sub>, X<sub>1,2</sub> and fractions were capable to inhibit the in vitro growth of C. albicans. The results of the MFC determination indicated that all extracts shows an inhibition ranging from 97.5 μg/mL to 12.1875 μg/mL on C. albicans. Furthermore, all extracts as well as fractions F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> exhibited a very high level of antifungal and antiviral activity. Among these extracts, fraction F<sub>3</sub> was the most active and it was reported 32 times more active than Ketoconazole. The physicochemical investigations (Flash chromatography, MS, UV, MNR) of F<sub>3</sub> led to isolate 2 compounds namely 4,4′-di-O-methylellagic acid and 4-O-methylellagic acid that are the antifungal active principles of this fraction.
2.4 Antimicrobial Test

The antifungal activities were assessed by determining antifungal parameters values which are MFC (minimal fungicidal concentration; concentration that inhibit 99.99% of growth in the experimental tube compared to the witness tube of growth control) and IC50 (Concentration for 50% of inhibition; graphically determined) around each assay. For each extract six replicate trials were conducted against C. albicans and Ketoconazole was used as standards for antifungal assay. The antifungal tests were carried out on culture medium Sabouraud (Biomerieux, 51078 Ref: 777666501).

The incorporation of plant extracts in to the agar was made using the agar slanted double dilution method (Ajello et al., 1963; Holt, 1975; Zirihi et al., 2003). After that, all ten tubes of each series were sterilized in an autoclave at 121°C for 15 minutes and then inclined with small base at room temperature to allow their cooling and so permitted to calculate the minimal fungicidal concentration. It also made it possible to plot the curves of colony counter pen (Cienceware number 23382). The processing of these data was made by half.

Fungal germs culture on previously prepared slants agar was made by inoculating 1000 cells of C. albicans (Holt, 1975). These cultures were incubated at 30°C for 48 hours. At the end of the incubation period, colonies were counted with the help of colony counter pen (Cienceware number 23382). The growth in the 10 experimental tubes was expressed as survival percentage, calculated compared to 100% of growth in the witness tube of growth control. The formula of this calculation has been mentioned below. The processing of these data permitted to calculate the minimal fungicidal concentrations (MFC) values. It also made it possible to plot the curves of activity of the extracts and the graphically determination of the concentrations for 50% of Inhibition (IC50) values. Formula to calculate the survival percentage -

\[ S = \frac{n}{N} \times 100 \]

Where:
- S - The survival (%)
- N - Number of colony in one experimental tube and
- N - Number of colony in the witness tube of growth control

2.5 Spectral analysis

The F3 fraction was purified by the flash chromatography GRACE Reveleris Flash System device by using MeOH-CH2Cl2 as eluents (RediSep column: Silica 12g; flow rate: 20 mL/min). This procedure resulted in the isolation of 2 aromatic polyphenolic compounds A and B. Finally these A and B were submitted to SM, and NMR spectral analysis. The 1D and 2D NMR spectra (1H and 13C NMR, COSY, HSQC and HMBC) were performed using a Bruker 400MHz spectrometer. Spectra are recorded in DMSO-d6. The carbon type (CH2, CH and CH) was determined by DEPT-135 experiments. ESIIMS (negative-ion mode) was carried out on a SM-QP 2010 of type Shimadzu spectrometer. UV spectra were recorded on a Varian Cary 300 Bio spectrophotometer.

3 Results and Discussion

The results were summarized in the form of curves of sensitivity, tables, MS and NMR spectra. The curves illustrate the evolution of the survival of C. albicans depending on the variation of the concentrations of the extracts (figure 1). Table 1 summarizes all the values of the antifungal parameters (MFC and the IC50). Figures 2 and 3 illustrate the NMR spectra, while table 2 summarizes all the values of the chemical shifts. The structures of compounds A and B are shown at figure 2. The chromatogram of flash chromatography GRACE shows a peak at 36 minutes with a shoulder at 40 minutes indicating the compound A and B.

| Tested Substances | Values of antifungal parameters against Candida albicans | Ratio CMF X_M/X_T3 |
|-------------------|----------------------------------------------------------|-------------------|
|                   | CI50 (µg/mL) | CMF (µg/mL) |                        |
| TEKAM1-10         | 30.08        | 97.5        | 8                     |
| TEKAM2-1,1        | 18           | 195         | 16                    |
| TEKAM4-1,2        | 10.225       | 48.75       | 4                     |
| TEKAM4-11         | 10           | 97.5        | 8                     |
| TEKAM4-12         | 7            | 24.375      | 2                     |
| TEKAM4-13         | 3            | 12.1875     | 1                     |
| Ketoconazole      | 18.455       | 390         | 32                    |
Figure 1 Sensitivity of *C. albicans* against the extracts (X₀, X₁₁, X₁₂, F₁, F₂ and F₃) from *T. superba*.

Table 2 $^1$H and $^{13}$C-NMR data of active ingredient compounds A and B.

| Position | Compound A $^1$H-NMR δH (ppm) | $^{13}$C-NMR δC (ppm) | Compound B $^1$H-NMR δH (ppm) | $^{13}$C-NMR δC (ppm) |
|----------|-------------------------------|------------------------|-------------------------------|------------------------|
| 1/1’     | —                            | 111.62                 | 1                             | 111.60                 |
| 2/2’     | —                            | 141.19                 | 2                             | 141.16                 |
| 3/3’     | —                            | 152.19                 | 3                             | 152.16                 |
| 4/4’     | —                            | 140.19                 | 4                             | 140.17                 |
| 5/5’     | 7.51 s (2H)                  | 111.42                 | 5                             | 111.40                 |
| 6/6’     | —                            | 112.09                 | 6                             | 112.07                 |
| 7/7’     | —                            | 158.46                 | 7                             | 158.44                 |
| 3 OH     | 10.77 s (2H)                 | 152.19                 | 3 OH                          | 10.77 s (1H)           | 152.16 |
| 4/4’ OMe-| 4.04 s (6H)                  | 60.94                  | 4 OMe-                        | 4.09 s (3H)            | 60.93  |
| 1’       | —                            | —                      |                               | 112.27                |
| 2’       | —                            | —                      |                               | 136.32                |
| 3’       | —                            | —                      |                               | 148.07                |
| 4’       | —                            | —                      |                               | 139.59                |
| 5’       | 7.50 s (1H)                  | 110.15                 |                               | 110.15                |
| 6’       | —                            | —                      |                               | 107.53                |
| 7’       | —                            | —                      |                               | 159.09                |
| 3’OH     | 10.76 s (1H)                 | —                      |                               | 148.07                |
4,4'-di-O-methylellagic acid
ESIMS m/z 329 [M-H]: C16H10O8;
yellow crystals; UV λmax 245.93 nm, 369 nm;
1H-NMR and 13C-NMR: see Table 2

4-O-methylellagic acid
ESIMS m/z 315 [M-H]: C15H8O8;
yellow crystals; UV λmax 245.93 nm, 369 nm;
1H-NMR and 13C-NMR: see Table 2.

Figure 2 Structures of compounds A and B.
Figure 3 NMR, HSQC and HMBC spectral data of compound A.
This study was conducted to optimize the antifungal activity of a hydro-ethanolic extract of *T. superba* against *C. albicans*. Analysis of results revealed that *C. albicans* is sensitive to all the tested extracts and shows a significant *in vitro* inhibition (figure 1, table 1). The results showed that the sensitivity of *C. albicans* to the extracts is concentration dependent. However the levels of these antifungal activities are variable from one extract to another, revealing that they do not have the same content of active principles. Analysis of MFC values (table 1) reveals that among the extracts obtained from partition of X₀ (the crude extract), X₁-₁ was the less active because it generated the highest MFC values (195 µg/mL). This weak antifungal potency of X₁-₁ is noticeable in figure 1. Conversely, X₁-₂ produced the lowest MFC values (48.75 µg/mL) meaning the strongest anticanidiosic activity. According to the activity classification scale of Kra et al. (2014), both extracts exhibited a very high level of antifungal activity.

Moreover, on the basis of MFC values, the comparison of the antifungal activities shows that X₁-₂ was respectively 4 times more active than X₁-₁ and 2 times more active than X₀ (MFC=97.5 µg/mL). So results revealed that the liquid-liquid partition permitted to optimize the anticanidiosic activity of X₀. Similar type of finding was reported by Zeidan et al. (2013) while working on *C. albicans*; Ouattara et al., (2013a, Ouattara et al., 2013b; Ouattara et al., 2013c; Majgaine & Verma, 2013; Kra et al., 2014). Furthermore, Ackah (2009) has been reported that hydroethanolic extract of *T. catappa* trunk bark inhibit the *in vitro* growth of *C. albicans*. However comparison of the anticanidiosic activity of this extract (MFC=190µg/mL) with that of TEKAM₂-X₀, reveals that the hydro-ethanolic extract (MFC=97.5µg/mL) from *T. superba* is 1.9 times more active. TEKAM₂-X₁-₂ is also 2.5 to 41 times more active than extracts from *Terminalia avicennioides* tested by Baba-Moussa et al. (1999) on *C. albicans*.

Analysis of data related to fractions obtained by chromatography shows that F₃ (MFC=12.1875 µg/mL and IC₅₀=3 µg/mL) is singularly the most active, because it produced the lowest MFC value against *C. albicans* compared with the other extracts. On the basis of MFC values, it is respectively 2 times more active than F₂ (MFC=24.375 µg/mL), 4 times more active than X₁-₁, 8 times more active than F₁ and X₀ (MFC=97.5 µg/mL), and, 16 times more active.
than X₀₁. Finally F₁ was reported 32 times more active than ketoconazole the reference antifungal drug. So this extractive method including successively a hydro-ethanolic extraction of the crude extract, a liquid-liquid partition (hexane/water) and a gel filtration chromatography on Sephadex-LH₂₀ gel, is a good process to optimize the antifungal activity of extracts from *T. superba*. This method permitted us to achieve the F₃ fraction which is the extract that contains the active principles.

Moreover, from the comparison of the whole data indicated that all the six extracts of *T. superba* are noticeably more active than ketoconazole. As a matter of fact, ketoconazole (MFC=390 µg/mL) was respectively two times less active than X₀₁, four times less active than X₀ and F₁, eight times less active than X₁₂ and sixteen times less active than F₂. In addition all the 6 extracts of *T. superba* exerted a very high level of antifungal activity. These obtained data highlighted the significant antifungal potency of extract from *T. superba*. The elucidation of the relatively high MFC value (390 µg/mL) of ketoconazole against *C. albicans* was revealed by results of works carried out on the mechanism of its weak sensitivity to azole. This resistance to azole is characterized by several stratagems including activation of multiple efflux pumps repressing the antifungal drug (Hitchcock, 1993; Sanglard et al., 1995; White et al., 2002), a modification of the target (such as the alteration of the enzyme 1.4-a-demethylase) and overproduction of the target (Hitchcock, 1993; Kelly et al., 1995; Albertson et al., 1996).

Furthermore, on the basis of the MFC values, the comparison with the anticandidosis activity of fraction MISCA-F₈ (MFC=781 µg/mL) from *M. scaber* prepared by Kporou et al. (2010), shows that TEKAM₄-F₁ (MFC=12.1875 µg/mL) is 64 times more active. The comparison also shows that TEKAM₄-F₁ has an antifungal activity potency 1.79 times greater than that of fraction TEKAM₄-F₀ (CMF=12.1875 µg/mL; IC₅₀=5.37 µg/mL) prepared by Yayé et al. (2012) from *T. mantaly*. On the same basis of comparison, the anticandidosis activity of TEKAM₄-F₁ is 2020 times greater than that of flavonoids (MFC=2500µg/mL) isolated from the *Olea cuspidata* dichloromethane extract tested by Maigjare & Verma (2013).

On the other hand, TEKAM₄-F₃ is 2.4 times less active than TEKAM₄-F₁₂ (MFC=5 µg/mL) prepared by Ackah et al. (2008) from a trunk bark extract of *T. catappa*. Fraction F₈ (MFC=6.09 µg/mL; IC₅₀=1.8 µg/mL) prepared by Ouattara et al. (2013b) from the stem bark of *T. ivorensis* is 2 times more active than TEKAM₄-F₁. In the chemical aspect, fractions F₈ of *T. ivorensis* (triterpenes and flavonoids) are different from that of TEKAM₄-F₁. Ultimately, the F₁ fraction shows the highest antifungal activity of TEKAM₄-X₀. Furthermore, the activities of X₀, X₁, X₁₂ and the three fractions (F₁, F₂, F₃) tested could be considered as very important since the *C. albicans* strain tested was resistant to commonly used antifungal drugs (Kah, 2011 ; Hope et al., 2012).

With respect to the physico-chemical aspect, after extraction and purification analysis, two compounds A and B were isolated. Compound A was obtained as yellow crystals and was soluble in methanol. The ESIMS spectrum (Annex 6) of compound A revealed a quasi-molecular ion peaks [M-H]⁻ at m/z 329 indicating a molecular weight of 330 Da. Its ultraviolet (UV) spectrum revealed the presence of α and β-unaturated Ketones (λₖₐ₅ 245.93 nm) and phenolic ring (λₖₐ₅ 369 nm). This spectrum (λₖₐ₅ 245.93 nm, 369 nm) was similar to that of ellagic acid derivatives (Khac et al., 1990), suggesting that compound A has an ellagic acid skeleton. These data suggested a molecular formula of C₁₄H₁₀O₆.

The ¹H-NMR spectrum (figure 3a) of compound A revealed two protons as singlet at δₗ 7.51 ppm, assignable to aromatic protons H-5 and H-5' respectively, by comparing with the ¹H-NMR data of ellagic acid (Nduji & Okwute, 1988; Khac et al., 1990; Adigun et al., 2000 ; Serafin et al., 2007; Thitilertdecha et al., 2010). The ¹H-NMR spectrum of compound A also showed methoxy protons at δₗ 4.04 ppm (6H, s), which gave correlations, in the HSQC spectrum (figure 3d), with carbon signal δc 60.94ppm. The HMBC (figure 3c) correlation between methyl group at δₗ 4.04 ppm (O-CH₃) and carbon δc 140.19ppm (C-4) confirmed the presence of methoxy groups. So, cross analysis of spectra of HMBC and HSQC clearly confirmed the linkage of the methoxy groups to carbons C-4 and C-4'. The ¹³C-NMR spectrum (figure 3b) of compound A showed two signal at δc 158.46 and 152.19ppm assignable to carbonyl carbons C-7 and C-7' respectively. Finally, analysis of the whole data indicates unequivocally that A is 4,4'-di-O-methyl-ellagic acid (figure 2).

Compound B was also obtained as yellow crystals and was soluble in methanol. The ESIMS spectrum of compound B revealed a quasi-molecular ion peaks [M-H]⁻ at m/z 315 indicating a molecular weight of 316. Analysis of its spectral data reveals that B and showed almost identical ¹H-NMR and ¹³C-NMR data, suggesting that compound B also has an ellagic acid skeleton. These data showed that the corresponding molecular formula of B is C₁₄H₁₀O₆.

The ¹H-NMR spectrum (figure 4a) of compound B revealed two protons as singlets at δₗ 7.55 and 7.50 ppm, assignable to aromatic protons H-5 and H-5' respectively. The ¹H-NMR spectrum of compound B showed also methoxy protons at δₗ 4.09ppm (3H, s), which gave correlations, in the HSQC spectrum, with carbon signal δc 60.93ppm. The HMBC spectrum showed the correlation between methyl group at δₗ 4.09 ppm (O-CH₃) and carbon δc 140.17ppm (C-4) confirmed the position of methoxy group. So, HMBC (figure 4d) and HSQC (figure 4c) experiments clearly showed it to be linked to C4 of the ellagic skeleton.

The ¹³C-NMR spectrum of compound B showed two signal at δc 159.09 and 158.44ppm assignable to carbonyl carbons C-7 and C-7' respectively. From the analysis of the whole NMR
and MS data, it result that compound B is 4-O-methylellagic acid (C_{13}H_{20}O_5) and has molecular weight of 316 Da (figure 2).

Finally the active principles of fraction F_1 are 4,4′-di-O-methylellagic acid (compound A) and 4-O-methylellagic acid (B). Compounds A and B are reported here for the first time from the Ivorian _T. superba_ species. These A and B compounds were identified as other active principles of plants from the genus _Terminalia_ by the present study. A number of ellagic acid derivatives close to A and B were also isolated from the stem bark of _T. superba_ by some authors (Kamthouing et al., 2006 ; Tabopda et al., 2008 ; Kuete et al., 2010).

In addition, antimicrobial properties of _O_-methylellagic acid derivatives were already proved by previous reports such as works of Adigun et al. (2000) and Kuete et al. (2010) whose investigations proved 3,4′-di-O-methylellagic acid, 3′-O-β-D-xylopyranoside and 4′-O-galloy-3,3′-di-O-methylellagic acid 4-O-β-D-xylopyranoside isolated from a _T. superba_ stem bark methanolic extract to have a large spectrum of both antibacterial and antifungal activities. As regard their antimicrobial properties the results from this study are in accordance with works of Kuete et al. (2010).

The antimicrobial activity has also been revealed for a number of _Terminalia_ species such as _T. catappa_ (Kloucek et al. 2005 ; Ackah et al., 2008), _T. chebula_ (Bonjar 2004), _T. glaucescens_ (Magassouba et al., 2007), _T. ivorensis_ (Ouattara et al., 2009; Ouattara et al., 2013a; Ouattara et al., 2013b ; Ouattara et al., 2013c), _T. mantaly_ (Yaye et al., 2011; Yaye et al., 2012 ; Ackah et al., 2014), _T. macroptera_ (Conrad et al., 1998 ; Silva et al., 2000), _T. sericea_ (Eldeen et al., 2006 ; Steenkamp et al., 2007), _T. superba_ (Ahon et al., 2011 ; Ahon et al., 2012 ; Kra et al., 2014).

Concerning their mode of action, ellagic acid and its derivatives have been found to complex proteins (Scalbert, 1991 ; Haslam, 1996 ; Stern et al., 1996), inactivate microbial adhesions (Haslam, 1996) enzymes and cell envelope transport proteins (Scalbert, 1991 ; Haslam, 1996 ; Stern et al., 1996).

**Conclusion**

In this study, it has been demonstrated that it is possible to obtain extracts with high levels of antican didosic activity and to optimize this activity by means of partitions followed by column chromatography separation. This study also showed that _C. albicans_ is sensitive to all the extracts in dose-response relationship. All the 6 extracts exerted antican didosic activity with more or less raised performance levels. All extracts from _T. superba_ produced very high antifungal activity levels. It proves and allows the understanding of the foundation of the use of several organs of _T. superba_ in traditional recipes against infections. Among these extracts, fraction _F_3 was not only the most active but it also contains the active principles that are 4,4′-di-O-methylellagic acid (compound A) and 4-O-methylellagic acid (B). These compounds allow fraction _F_3 to be 32 times more active than Ketoconazole. These molecules could expand the therapeutic arsenal and make it more effective. They could also serve as templates for synthesizing more active molecules. The overall results provide promising baseline information for the potential use of fractions _F_1 as well as the two isolated compounds in the treatment of candidiasis, fungal and other bacterial infections. However, this will further be confirmed by pharmacological and toxicological studies.

**Acknowledgements**

The authors are grateful to:

- Professor Ou hon jean and members of the Laboratory of Parasitology and Mycology of the Faculty of Medical Sciences of the Felix Houphouët-Boigny University for having provided the germs used in this study.
- Members of the Laboratory of Biochemistry-Pharmacodynamic for their valuable contribution.

The authors acknowledge the technical support of the Herbarium of the floristic national center of the Felix Houphouët-Boigny University (Abidjan, Côte d’Ivoire), that of Professor Laurent Aké-Assi of the Botany Laboratory (Abidjan, F.H.B University).

**Conflict of interest**

The authors declare no conflict of interest.

**References**

Abi-Said D, Anaissie E, Uzun O, Raad I, Pinzcowski H, Vartivarian S (1997) The epidemiology of hematogenous candidiasis caused by different Candida species. Clinical Infectious Diseases 24 : 1122-1128.

Ackah JAAB (2009) Evaluation et essai d’optimisation de l’activité antifongique de _Terminalia catappa_ Lin., une combrétagée de la pharmacopée ivoirienne sur la croissance in vitro de _Candida albicans_, _Aspergillus fumigatus_ et _Trichophyton mentagrophytes_. Thèse de Doctorat d’Université. UFR Biosciences, Université de Cocody Abidjan p. 241

Ackah JAAB, Kokora AP, Yaye YG, Bahi C, Loukou YG, Coulibaly A, Djaman AJ (2014) Action Spectrum of _Terminalia mantaly_ on the _in vitro_ Growth of _Pseudomonas aeruginosa_. International Journal for Pharmaceutical Research Scholars 3: 795-800.

Ackah JAAB, Kra AKM, Zirhi GN, Guédé-Guina F (2008) Evaluation et essais d’optimisations de l’activité antican didosique de _Terminalia catappa_ Linn. (TEKAM3), un extrait de com brétagée de la pharmacopée Ivoirienne. Bulletin de la Société Royale des Sciences de Liège 77:120-36.
Adigun JO, Amupitan JO, Kelly DR (2000) Isolation and investigation of antimicrobial effect of 3,4,3’-Tri-O-methylflavellagic acid and its glucoside from Anogeissus leocarpus. Bulletin of the Chemical Society of Ethiopia 14: 169-74.

Agouni A, El-Mellouki W, Chabaa L, Tigui H, Belmekki A, Louzi L, Linimouni B, Benjelloun L (2000) Étude de la prévalence des infections fongiques systémiques dans les services hospitaliers à risque. Espérance Médicale 7: 1-6.

Ahon GM, Kra AKM, Aw S, Zirhi GN, Ackah BAAJ, Siaka S, Kporou KE, Akapo-Akue MJ, Djaman AJ (2012) Improvement of the antifungal activity of the hydro-alcoholic extract of Terminalia superba on the in vitro growth of three pathogenic fungi. International Journal of Biology, Pharmacy and Allied Sciences 1: 1434-1442.

Ahon MG, Akapo-Akue JM, Kra MA, Ackah JB, Zirhi NG, Djaman JA (2011) Antifungal activity of the aqueous and hydro alcoholic extracts of Terminalia superba Engl. on the in vitro growth of clinical isolates of pathogenic fungi. Agriculture and Biology Journal of North America 2: 250-257.

Ajello L, George Lk, Kaplan W, Kaufman L (1963) Laboratory Manual for Medical Mycology 2nd ed. New York, USA: John Wiley and Sons, Inc 20-35.

Albertson GD, Niimi M, Cannon RD, Jenkinson HF (1996) Multiple efflux mechanisms are involved in Candida albicans fluconazole resistance. Antimicrobial Agents and Chemotherapy 40: 2835-2841.

Baba-Moussa F, Akpagana K, Bouchet P (1999) Antifungal activities of seven West African Combretaceae used in traditional medicine. Journal of Ethnopharmacology 66: 335-338.

Bonjar GH (2004) Inhibition of clotrimazole-resistant Candida albicans by plants used in Iranian folkloric medicine. Fitoterapia 75:74-6.

Chabasse D (1994) Les nouveaux champignons opportunistes apparus en médecine. Revue générale. Journal de Mycologie Médicale 4 : 9-28.

Conrad J, Vogler B, Klaiber I, Roos G, Walter U, Kraus W (1998) Two triterpene ester from Terminalia macroperata bark. Phytochemistry 48: 647-650.

Develoux M, Bretagne S (2005) Candidoses et levuroses diverses. Encyclopédie médico-chirurgicale. Maladies infectieuses 2:318-27.

 Eldeen IM, Elgorashi EE, Mulholland DA, Van SJ (2006) Anolignan B: A bioactive compound from the roots of Terminalia sericea. Journal of Ethnopharmacology 103:135-138.

Haslam E (1996) Natural polyphenols (vegetable tannins) as drugs: possible modes of action. Journal of Natural Products 59: 205-215.

Hitchcock CA (1993) Resistance of Candida albicans to azole antifungal agents. Biochemical Society Transactions 21: 1039-1047.

Holt RJ (1975) Laboratory tests of antifungal drug. Journal of Clinical Pathology 28: 767-774.

Hope WW, Castagnola E, Groll AH, Rolildes E, Akova M, Arendrup MC, Arikan-Akdagli S,Bassetti M, Bille J, Cornely OA, Cuenca-Estrella M, Donnelly JP, Garbino J,Herbrecht R, Jensen HE, Kullberg BJ, Lass-Flörl C, Lortholary O, Meerseman W, Petrikos G, Richardson MD, Verweij PE, Viscoli C, Ullmann AJ (2012) ESCMID Guideline for the diagnosis and management of Candida diseases 2012: prevention and management of invasive infections in neonates and children caused by Candida spp. Clinical Microbiology and Infections 18: 9-18 doi: 10.1111/1469-0691.12040.

Kah N (2011) Dermatophyties, candidoses et autres mycoses superficielles : rôles du pharmacien d’officine. Diplôme d’Etat de Docteur en Pharmacie. Faculté de Pharmacie, Université Henri Poincare-Nancy Pp 1-134.

Kamchouung P, Kahpu SM, Dzeufiet PD, Tédong L, Asongalem EA, Dimo T (2006) Anti-diabetic activity of methanol/methylene chloride stem bark extracts of Terminalia superb and Canarium schweinfurthii on streptozotocin-induced diabetic rats. Journal of Ethnopharmacology 104: 306-309.

Kelly SL, Lamb DC, Corran AJ, Baldwin BC, Kelly DE (1995) Mode of action and resistance to azole antifungals associated with the formation of 1,4-alpha-methylerygosta-8,24(28)-dien-3-beta,6alpha-diol. Biochemical and Biophysical Research Communications 207: 910-915.

Khac DD, Tran-Van S, Campos AM, Lallemand JY, Fetizon M (1990) Ellagic compounds from Diplopanax stachyanthus. Phytochemistry 29: 251-56.

Kloucek P, Polesny Z, Svobodova B, Vlkova E, Kokoska L. (2005) Antibacterial screening of some Peruvian medicinal plants used in Calléria District. Journal of Ethnopharmacology 99 : 309-312.

Kporou KE, Kra AKM, Ouattara S, Guédé-Guina F, Djaman AJ (2010) Amélioration par fraccionnement chromatographique de l’activité anticanidiosique d’un extrait hexanique de Mitracarpus scaber Zucc. sur la croissance in vitro de Candida albicans et Candida tropialis. Phytothérapie 8: 290-294.
Antifungal activity of Terminalia superba (Combretaceae).

Kra AKM, Ahon GM, Djo-Bi D, Ouattara S, Coulibaly A, Djaman AJ (2014) Antifungal activities of medicinal plants extracts of Ivoirian pharmacopeia. Journal of Intercultural Ethnopharmacology 3: 159-166.

Kuete V, Tabopda TK, Ngameni B, Nana F, Tshikalange TE, Ngadjui BT (2010) Antimycobacterial, antibacterial and antifungal activities of Terminalia superba (Combretaceae). South African Journal of Botany 76: 125-131.

Magassouba FB1, Diallo A, Kouyaté M, Mara F, Mara O, Bangoura O, Camara A, Traoré S, Diallo AK, Zaoro M, Lamah K, Diallo S, Camara G, Traoré S, Kéita A, CamaraMK, Barry R, Kéita S, Oularé K, Barry MS, Donzo M, Camara K, Tôté K, BergerDV, Tôté J, Pieters L, Vletinck AJ, Baldé AM (2007) Ethnobotanical survey and antibacterial activity of some plants used in Guinean traditional medicine. Journal of Ethnopharmacology 114; 44-53.

Majgaine P, Verma LD (2013) Antifungal Activity of Olea caspida and Olea glauclifera Linn. IOSR Journal of Pharmacy 3; 20-23.

Nduji AA, Okwute SK (1988) Co-occurrence of 3,3',4'-tri-O-methylflavellagic acid and 3,3'-di-O-methylenegall acid in the bark of Anogeissus schimperii. Phytochemistry 27; 1548-50.

Ouattara S, Kporou KE, Kra AKM, Yapi HF, Ziriih GN, N'guessan JD, Bidié AP, Djaman AJ (2013b) Optimization of the in vitro antifungal activity of hydroalcoholic extract of Terminalia ivorensis A. Chev. Journal of Natural Products and Plants Resources 3; 29-33.

Ouattara S, Kporou KE, Kra AKM, Zirihi GN, N'guessan JD., Coulibaly A, Djaman AJ (2013a) Antifungal activities of Terminalia ivorensis A. Chev. bark extracts against Candida albicans and Aspergillus fumigatus. Journal of Intercultural Ethnopharmacology 2; 49-52.

Ouattara S, Kra AKM, Kporou KE, Guédé-Guina F (2009) Evaluation de l’activité antifongique des extraits de Terminalia ivorensis (TEKAM 2) sur la croissance in vitro de Aspergillus fumigatus. Bulletin de la Société Royale des Sciences de Liège 78:302-10.

Ouattara S, Kra AKM, Kporou KE, Zirihi GN, Yapi HF, N’guessan JD, Djaman AJ (2013c) Comparative study chromatographic fractions activities from Terminalia ivorensis and ketoconazole as standard antifungal on in vitro growth of trichophyton mentagrophytes var. interdigitale. Journal of Drug Delivery and Therapeutics 3; 18-21

Sanglard D, Kuchler K, Ischer F, Pagani JL, Monod M, Bille J (1995) Mechanisms of Resistance to Azole Antifungal Agents in Candida albicans isolates from AIDS Patients Involve Specific Multidrug Transporters. Antimicrobial Agents and Chemotherapy 39; 2378-2386.

Scalbert A (1991) Antimicrobial properties of tannins. Phytochemistry 30 : 3875-3883.

Serafin C, Nart V, Malheiros A, de Souza MM, Fischer L, Delle Monache G, Della Monache F, Cechinel Filho V (2007) Bioactive Phenolic Compounds from Aerial Parts of Plinia glomerata. Zeitschrift für Naturforschung 62:196-200.

Silva O, Gomes ET, Wolfender JL, Marston A, Hostettmann K (2000) Application of High Performance Liquid Chromatography Coupled with Ultraviolet Spectroscopy and Electrospray Mass Spectrometry to the Characterisation of Ellagitannins from Terminalia macroptera Roots. Pharmaceutical Research 17: 1396-1401.

Steenkamp V, Fernandes AC, Van Rensburg CEJ (2007) Antifungal activity of Venda medicinal plant. Fitoterapia 78: 561-564.

Stern JL, Hagerman AE, Steinberg PD, Mason PK (1996) Phorotannin-protein interactions. Journal of Chemical Ecology 22: 1887-1899.

Tabopda TK, Ngoupayo J, Liu J, Ali MS, Khan SN, Ngadjui BT, Luu B (2008) Alpha-Glucosidase inhibitors ellagic acid derivatives with immunoinhibitory properties from Terminalia superba. Chemical and Pharmaceutical Bulletin 56: 847-850.

Thitilertdecha N, Teerawutgulrag A, Kilbum ID, Rakariyatham N (2010) Identification of Major Phenolic Compounds from Nephelem lappaceum L. and Their Antioxidant Activities. Molecules 15: 1453-1465.

White TC, Holleman S, Dy F, Mirels LF, Stevens DA (2002) Resistance mechanisms in clinical isolates of Candida albicans. Antimicrobial Agents and Chemotherapy 46: 1704-1713.

Yayé YG, Ackah JAAB, Kra AKM, Djaman AJ (2012) Antifungal activity of different extracts of Terminalia mantaly H. Perrier on the in vitro growth of Aspergillus fumigatus. European Journal of Sciences Resources 82: 132-138.

Yayé YG, Kra AKM, Ackah JBAA, Djaman AJ (2011) Evaluation de l’activité antifongique et essais de purification des principes actifs des extraits de Terminalia mantaly (H. Perrier), une combrétique sur la croissance in vitro de Candida albicans. Bulletin de la Société Royale des Sciences de Liège 80:953-64.

Zeidan R, Oran S, Khleifat K, Matar S (2013) Antimicrobial activity of leaf and fruit extracts of Jordanian Rubus sanguineus Friv. (Rosaceae). African Journal of Microbiology Research 7: 5114-5118 DOI: 10.5897/AJMR2013.5770.
Zirihi GN, Kra AKM, Guédé-Guina F (2003) Evaluation de l'activité Antifongique de Microglossa pyrifolia (LAMARCK) O.KUNTZE (Asteraceae) "PYMI" sur la croissance in vitro de Candida albicans. Revue de Médecine et. Pharmacopées Africaines 17 : 11-18.