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## RESEARCH ARTICLE

**EVALUATION OF OINTMENT ACTIVITY BASED ON *TERMINALIA MANTALY* EXTRACT****Soumahoro Ibrahima aimé<sup>1</sup>, d'Almeida Kayi Marie-Anne<sup>1,2</sup>, GNAHOUE Goueh<sup>4</sup>, KRA Adou Koffi Mathieu<sup>1</sup>, DJAMAN Allico Joseph<sup>1,3 2</sup>**<sup>1</sup> Laboratory of Biochemical Pharmacodynamic, UFR Biosciences, University of Felix Houphouet Boigny 22 B.P. 582 Abidjan 22 Côte d'Ivoire<sup>2</sup> Laboratory of molecular biology UFR of Biosciences, Université Felix Houphouet Boigny 22 B.P. 582 Abidjan 22 Côte d'Ivoire<sup>3</sup> Departement of clinical and fundamental Biochemistry Unit of Pasteur Institute (Côte d'Ivoire) 01 BP 490 Abidjan 01<sup>4</sup> Laboratory of Biochemistry Microbiology Ecole Nationale Supérieur ENS\*Correspondance and drawn with share, Email [soumibraime@yahoo.fr](mailto:soumibraime@yahoo.fr) Cell: 22505247000

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DOI: <http://dx.doi.org/10.22270/jddt.v6i5.1314>URI: <http://jddtonline.info/index.php/jddt/article/view/1314>**ABSTRACT**

In Côte d'Ivoire pharmacopoeia the antifungal virtues of the bark of *Terminalia mantaly* are known. Its bark is used to cure affections such as cutaneous candidiasis, gingivitis and diarrhoea. This work aims to compare the anticandidosic activities of the crude extract of *T. mantaly* and an ointment containing shea butter and the crude extract of *T. mantaly*. The anticandidosic activity of the crude Shea butter was also evaluated and ketoconazole was used as standards for antifungal assay.

Shea butter, ketoconazole, crude extract of *T. mantaly* and the ointment were separately incorporated to Sabouraud agar using the agar slanted double dilution method. The anti-fungal tests were performed by sowing 1000 cells of *Candida albicans* on a previously prepared medium culture. Antifungal activity was determined by evaluating of anti-fungal parameters values (minimal fungicidal concentrations MFC and IC50).

The results of the tests indicate that *C. albicans* is sensitive to each substance tested, however crude Shea butter has only fungistatic activity and the ointment (MFC = 1.874 µg/mL) was the most active. This ointment is 52 times more active than the crude extract of *T. mantaly* (MFC is of 97.5 µg/mL), 208.11 more active than ketoconazole (MFC = 390 µg/mL).

**Keywords:** *Terminalia mantaly*, Activity, ointment**INTRODUCTION**

In the world, the infectious diseases knew higher fresh outbreak during the last decades (<sup>1</sup>Dromer *et al.*, 2013). The diseases due to pathogenic resistant species are in great progression (<sup>2</sup>Assob and Nsagha, 2014) and mycosis are among these pathologies (<sup>3</sup> Hitchcock, 1993; <sup>1</sup>Dromer *et al.*, 2013; <sup>2</sup>Assob and Nsagha, 2014). This fresh outbreak of mycosis comes from several factors. Among them, change in the clinical spectrum of classic pathogenic, apparition of diverse resistances in the usual antifungals and the strong progress of immunodépressive affections such as HIV-AIDS (<sup>3</sup> Chabasse, 1994; <sup>4</sup> Chabasse *et al.*, 2009; <sup>1</sup>Dromer *et al.*, 2013). Their frequency, their recurring character and their gravity did not cease growing in West Africa generally and in Côte d'Ivoire in particular (<sup>5</sup>Akakpo-Akue, 2009; <sup>6</sup>Attoh-Toure *et al.*, 2009).

On the therapeutic level, the number of proposed remedies remains limited. Moreover some molecules lost their effectiveness due to resistance phenomena and change (<sup>7</sup>Vanden, 1997; <sup>8</sup> Sanglard *et al.*, 1998; <sup>9</sup> Freiman and Saserville, 2006). To this is added the cost more and more raised of some medicine sold in the modern pharmacies. The financial difficulties of the populations direct them to the use of healing plants (<sup>10</sup>Aké-Assi, 1991; <sup>11</sup> Cowan, 1999). The determination of the pharmacological properties of the healing plants could bring a solution to their needs (<sup>12</sup>Hostettmann and Marston, 2002; <sup>2</sup> Assob and Nsagha, 2014), especially as several works have already proved the efficiency of molecules and extract from plants against diverse bacterial and fungal sorts (species) (<sup>13</sup> Aladesanmi *et al.*, 2007; <sup>14</sup> Nkomo and Kambizi, 2009; <sup>15</sup> Kuete *et al.*, 2010; <sup>16</sup> Ahon *et al.*, 2012; <sup>17</sup> Kra *et al.*, 2015).

Furthermore, at the end of an ethnobotanic survey realized by our research team in 1991 in the area of Issia in Côte d'Ivoire, *T. mantaly* was collected and identified among this plants species frequently used for their anti-infectious properties (<sup>18</sup> Zirihi, 1991). In Côte d'Ivoire, this plant is used against gastroenteritis, oral affections, skin disorders, genital candidiasis, arterial high blood pressure and diabetes (<sup>19</sup> Riviere et al., 2005, <sup>20</sup> Yayé et al., 2012). Otherwise, recent works proved that *T. mantaly* possesses excellent antibacterial and antifungal activities (<sup>15</sup> Kuéte et al., 2010; <sup>21-22</sup> Yayé et al., 2011 et 2012; <sup>23</sup> Ackah et al., 2014; <sup>24</sup> Kra et al., 2014). For better appreciation of the use of *T. mantaly* in human therapeutics, the present study was initiated to evaluate and compare the anticandidosic activities of the crude hydroethanolic extract of *T. mantaly* and to those of the ointment formulated from this extract.

## MATERIAL AND METHODS

### Plant Material

#### Bark of *Terminalia mantaly*

The Plant material was a powder obtained after crushing the dried bark pieces of *T. mantaly* codified TEKAM<sub>1</sub>.

### Shea Butter

The Shea butter (SB) was extracted from nuts of *Butyrospermum parkii* (G Don) Kotschy (Sapotaceae). This species spontaneously grows in the north of Côte d'Ivoire. The SB was heated and purified by decantation.

### Microorganism Studied

The tested fungus was the clinical and identified strain of *C. albicans* (13-298). This strain was provided by the Laboratory of Mycology of the Medical Sciences Faculty of the Felix Houphouët Boigny University (Abidjan, Côte d'Ivoire).

## METHODS

### Preparation of the vegetable extracts

#### Extraction

The plant bark was cut into small pieces and carefully air-dried for 2 weeks at room temperature in the laboratory, under continuous ventilation, away from sunlight and dust. After this step, the vegetal piece was crushed to a fine powder with an electric grinder IKA-MAG. The powder obtained were codified TEKAM<sub>1</sub>.

The hydro-ethanolic rough extracts were prepared from TEKAM<sub>1</sub>. Hundred grams (100g) of bark powder was extracted by homogenization in a blender with 1 L of mixture of ethanol 70% and distilled water 30%. After six cycles of homogenization, the homogenates obtained were first wrung out in a fabric square and then filtered twice successively with absorbent cotton and once with Whatman 3 mm filter paper. The resulting filtrate was concentrated under vacuum using a Büchi rotary evaporator at 60°C (<sup>25</sup> Zirihi et al., 2011). This powder was hermetically sealed in polyethylene bags and stored away from light and moisture until the time of

extraction. Dark powder obtained is the hydro-ethanolic crude extract codified TEKAM<sub>1</sub>-X<sub>0</sub>

### The shea butter (SB)

SB is a clear yellow product. It was used as excipient main of the ointment. Its melting point is between 33 and 42 °C. Its density at 15°C is between 0,915 and 0,920.

### Ointment Quality

#### Macroscopic Characters

The clear brown ointment obtained by the mixture of Shea and extract TEKAM 1- X<sub>0</sub> codified TEPO. It is an unctuous cream with soft consistency. The smell like of the Shea butter

#### Homogénéité

The ointment homogeneity was verified by applying on thin (confinement) layer a plane surface using a spatula. The regular distribution or not of the extracts in excipient was noted. It presents a very homogeneous without curds and smooth aspect to the touch.

#### pH Measure

The pH determined was that of a dilution to the tenth of the ointment in heater distilled water. The pH obtained is generally 5, 5.

#### Medium and ointment preparation

The anti-fungal tests were carried out on culture medium Sabouraud (Biomerieux / Ref: 180930; batch 401513b). The incorporation of plant extracts into the agar was made using the agar slanted double dilution method (<sup>26</sup> Ajello et al., 1963; <sup>27</sup> Holt, 1975; <sup>28</sup> Zirihi et al., 2003).

For each substance as TEKAM<sub>1</sub>, SB, and the ointment, serial tests were realized and each serie include 12 tests tubes. Ten test tubes contain mixture of vegetable extract and agar. The others 2 tubes constitute the pilot. Among these two tubes, one without vegetable extract was considered as witness of germs growth control while the second without germs and extract was constitute witness of culture medium sterility control growth. Extract concentrations range in the tubes from 2500µg/mL to 0,003µg/mL with geometrical connection of reason ½. All the tubes were pressure-sealed at 121°C during 15 min, and then tilted with small base at room temperature to allow their cooling and solidification of the agar (<sup>29</sup> Ackah, 2004; <sup>30</sup> Teas, 2008; <sup>31</sup> Kporou, 2009; <sup>22</sup> Yayé, et al., 2011; <sup>32</sup> Ouattara et al., 2013).

To obtain the ointment codified TEPO, 48g of Shea butter were added by small fractions to 2g of TEKAM<sub>1</sub>-X<sub>0</sub> and mixed until obtaining complete homogeneity (<sup>33</sup> Raoult, 1983; <sup>34</sup> Mathieu and Fonteneau, 2008; <sup>35</sup> Klusiewicz, 2008; <sup>36</sup> Charpentier 2008; <sup>37</sup> Mautrait and Raoult, 2009). Dimethylsulfoxide (DMSO with 1%) was used to facilitate the homogenization of both substances (<sup>38</sup> Boutet, 1967; <sup>39</sup> Bean et al., 1969).

#### Anti-microbial test

Fungal germs culture on slanted agar previously prepared was made by sowing 1000 cells of *C. albicans* (<sup>28</sup> Holt, 1975). These cultures were incubated at 30°C

for 48 h. At the end of the incubation time, colonies were counted out by direct counting with a colony counter pen (Ceinceware, number 23382). The growth in the 10 experimental tubes was expressed as survival percentage, calculated, compared to 100% of growth in the growth control tube. The formula to calculate this is shown below.

The processing of these data permitted to calculate the MFC values. (26Ajello *et al.*, 1963; 28Holt, 1975) In practice, the MFC is the extract concentration in the tube, which gave 99.99% inhibition compared to the control growth tube. It also made possible to plot the curves of activity of the extracts and the graphically determination of the IC<sub>50</sub> values.

Formula to calculate the survival percentage:

$$S = \frac{n}{N} \times 100$$

S: Survival (%)

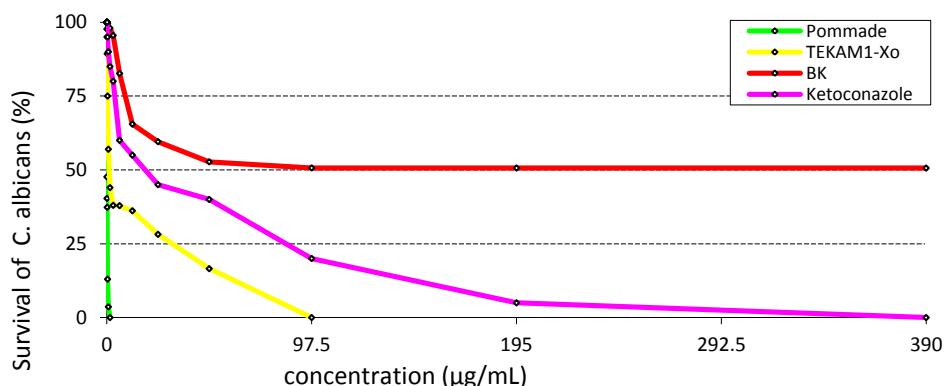
n: Number of colony in one experimental tube

N: Number of colony in the growth control tube.

## RESULTS

After 48 hours of incubation at 30°C, we observed comparatively to control tube a progressive decrease in the number of colonies gradually as the concentrations of the plant extract increased in the experimental tubes. The results were summarized in the form of curves of activity on Figure 1 and in table I containing the various values of MFC and IC<sub>50</sub>.

In general, all sensitivity curves showed a progressively decreasing pace with slopes that are stronger or not as strong according to the extracts. The TEPOm accentuated slope curve illustrates high anti-fungal potency. In contrast the Shea slope curve is less accentuated. It reveals low anti-fungal potency of this SB.



**Figure 1:** Sensitivity of *Candida albicans* to TEKAM<sub>1</sub>-X<sub>0</sub>, SB, TPOM and Kétoconazole

**Table I:** Values of anti-fungal parameters of TEPOm, TEKAM<sub>1</sub>- X<sub>0</sub> and Kétoconazole

Tested Substances	Antifungal parameters (μg/mL)	
	CI <sub>50</sub>	CMF
TEKAM <sub>1</sub> -X <sub>0</sub>	1,14	97,5
TEPOM	0,047	1,874
SB	417,85	Non enregistré
Kétoconazole	18,455	390

## DISCUSSION

The analysis of the whole results shows that *C. albicans* is sensitive to all substances tested namely TEKAM<sub>1</sub>-X<sub>0</sub>, SB, TEPOm, TEKAM<sub>1</sub>-X<sub>0</sub> and Kétoconazole according to relation dosis-dependent. However, the levels of these anti-fungal activities are variable from one substance to another.

According to the classification scale of the levels of the activities of <sup>24</sup>Kra *et al.*,(2014), TEPOm, TEKAM<sub>1</sub>-X<sub>0</sub> and Kétoconazole anti-fungal activities are classified as very high levels of activity. The anticandidosic activity of SB is very low. Among these substances, TEPOm is

most active on *C. albicans* for having generated the lowest values of MFC (1.874 μg/mL) and IC<sub>50</sub> (0.047 μg/mL). In contrast, of TEPOm, SB was the less active. It generated a fungistatic activity because none tested concentration completely inhibited the growth of *C. albicans*. On picture 1, its activity curve slope is very weak. The activities of TEKAM<sub>1</sub>-X<sub>0</sub> (MFC=97.5 μg/mL and IC<sub>50</sub> = 1.14 μg/mL) and Kétoconazole (MFC = 390 μg/mL and IC<sub>50</sub> = 18.455 μg/mL) range between these two extremes.

On the basis of CMF and IC<sub>50</sub> values, the comparison reveals that TEKAM<sub>1</sub>-X<sub>0</sub> is 4 times more active than Kétoconazole and 366.53 times more active than SB. Moreover, TEPOm is 52.02 times more active than TEKAM<sub>1</sub>-X<sub>0</sub>, 208.64 times more active than Kétoconazole and 8890.42 times more active than SB. So, incorporation of TEKAM<sub>1</sub>-X<sub>0</sub> extract in the Shea butter has generated a strong increase in the antifungal activity of this crude extract. This activity was multiplied by 52.02. The SB has noticeably maximized anticandidosic activity of TEKAM<sub>1</sub>-X<sub>0</sub>. This TEPOm can be a cure against skin mycosis. A similar product has

already been formulated by <sup>6</sup>Akakpo -Akué *et al.* (2009). These authors developed an antifungal cream using hydroethanolic extract of *T. catappa* and a neutral cream. This codified cream DAEL exhibited an excellent antifungal activity against *C. albicans* (MFC =3µg/mL) and *T mentagrophytes* (MFC = 2.4µg/mL). This DAEL cream was also used to cure diverse dermatosis such as the cropping trichophytic moths, the croppings microsporic moths, the intertrigo interdigito-plantar, the dermatophytic of the skin glabrous, the pityriasis versicolor. However, the comparison of the anticandidosic activities of TEPO with that of DAEL antifungal cream (MFC=3µg/mL) reveals that TEPO is 1.6 times more active than DAEL cream.

On the other hand, the qualities of the obtained ointment are similar to those obtained by <sup>40</sup>Rokia *et al.*, (2006) by its consistence, its pH (5.5) and its perfect homogeneity. However, the very strong anticandidosic activity of TEKAM1-X<sub>0</sub> has already been reported by<sup>24-18</sup> Kra *et al.*, (2014 and 2015) and <sup>21</sup>Yayé *et al.*, (2012) who worked in similar conditions with *T mantaly* barks. However the comparison of extract's performances reveals that TEKAM1-X<sub>0</sub> from the present study is respectively 2 times and 21.92 times more active than the extracts prepared by these authors.

The differences of performances of these extracts could be explained by the fact that we did not collect the barks in the same area, and we did not test the extracts on the same *C. albicans* strain. And each fungal strain has its own sensitivity to anti-fungal drugs.

Works of <sup>41</sup>Baba-moussa *et al.* (1999) also showed that extracts from *Terminalia avicennioides* produced a strong antifungal activity against *C albicans*. But the comparison reveals that TEKAM1-X<sub>0</sub> (MFC = 97.5 µG / ml) is 2.54 to 41 times more active than the extracts obtained by these authors. TEKAM1-X<sub>0</sub> is also 31,2 times more active than MISCA-X1.1 (CMF=3.125 mg/mL) prepared from *Mitracarpus villosus* by <sup>31</sup>Kporou *et al.*, (2009).On the other hand TEKAM1-X<sub>0</sub> is 8 to 128 times more active than methanolic and ethanolic extracts of the leaves and the fruits of *Rubus sanguineus* tested by <sup>42</sup>Zeidan *et al* (2013) on *C albicans*. In addition, the dichloromethane extracts of *Olea cuspidate* and *Olea glandulifera* are 256.41 to 512.82 times less active than TEKAM1-X<sub>0</sub> because they produced antifungal activities with values going from 25 mg/ml to 50 Mg / mL on *C albicans* (<sup>43</sup>Majgaine and Verma, 2013). TEKAM1-X<sub>0</sub> is respectively 16 times and 69 times less active than the hydroalcoholic extracts T3-X12 of *Terminalia catappa* tested by <sup>44</sup>Ackah *et al.*,(2008) on *C. albicans*.

Concerning SB, its anti-fungal activity is certainly due to the fact that it contains 3 to 17 % of insaponifiables which are the karitenes A, B, C and D, the β-amyrine, basseol, the butyrospermol, the parkeol, the luseol; Karistérols A and B and vitamins A and D which give her bacteriostatic antioxidant and fungistatic properties. The results of the present study confirm the very high antimicrobial potency extracts from plants in the

*Terminalia* genus. Moreover results of many previous studies are in agreements with this study concerning their strong antimicrobial activities.

## CONCLUSION

The present study focuses on the anti-infectious potency of *Terminalia mantaly*. The results of these experiences show that sensitivity of *C. albicans* is dose-response relationship. The extract effectiveness is more important when it is incorporated in ointment than used alone. TEPO activity being 52 higher times than isolated crude extract, then SB noticeably increased anticandidosic extract activity. Furthermore TEPO being much more active than the kétocoazole, this ointment can be a cure against skin mycosis. Finally from the present study it appears that using *Terminalia mantaly* as antimicrobial in rural area is justified.

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