

Potentiating Antifungal Activity of Fluconazole or Nystatin with Methanol Bark Extract of *Harungana madagascariensis* Stem Bark

Ebelle R E¹, Mouokeu R S², Assam J PA³, Hopogap M L³, Masoohe A M³, Hiol M C, Tientcheu R, Ngono N R A^{3*}, Etoa F X⁴

¹Institute of Medical Research and Medicinal Plant Studies, (IMPM), PO Box 6163, Yaoundé, Cameroon

²Institute of Fisheries and Aquatic Sciences, University of Douala, PO Box 7236, Douala, Cameroon

³Faculty of Sciences, University of Douala, PO Box 24157, Douala, Cameroon

⁴University of Yaoundé I, Faculty of Sciences, PO box 812, Yaoundé, Cameroon

Received: 3rd March, 17; Revised 21st April, 17, Accepted: 14th May, 17; Available Online: 25th May, 2017

ABSTRACT

The therapeutic failures and the increasingly high costs of treating resistant bacterial infections calls for alternative means of care. The present study was initiated to evaluate the antifungal properties of the *Harungana madagascariensis* methanol barks extract and potent interactions with some usual drugs. The extract was prepared by maceration of the dry stem bark powder in methanol. Phytochemical analysis was carried out by colorimetric assays. Antifungal activity as well as interactions between extract, Nystatin or Fluconazole was evaluated by broth microdilution method. The therapeutic efficacies of *H. madagascariensis* extract and Fluconazole – extract mixture as compared to Fluconazole extract was studied in experimental models of oral and vulvovaginal candidiasis in rats by oral route. Results showed that methanol extract of *H. madagascariensis* stem barks had antifungal activity ranging from 128 to 1024 µg/ml. This extract also had a fungicidal activity on all of the tested yeasts. Moreover, the extract presented *in vitro* synergetic and additive interactions with Nystatin and Fluconazole. In both oral and vaginal infection model, all the treatment significantly reduced ($P < 0.05$) the number of colony formant unit (UFC) of *C. albicans* compared with untreated control. Moreover, significant decrease in the percentage of animals showing positive cultures was observed in rats treated with Fluconazole-extract mixture. In both therapeutic efficacy studies, the histological findings confirmed the microbiological results. The results of this study constitute a base for the usage of *H. madagascariensis* stem barks in association with Fluconazole to overcome yeast infection.

Keywords: *Harungana madagascariensis*, antifungal activity, synergy, oral candidiasis, vulvovaginal candidiasis.

INTRODUCTION

Over the past two decades, the prevalence of fungal infections has increased significantly due to the growing number of populations at high risk. *Candida* species are the most commonly isolated fungal pathogens causing morbidity and mortality in patients with impaired immunity, accounting 80% of fungal infections¹. These infections ranged from mucosal candidiasis, including oropharyngeal and esophageal candidiasis, which is frequently observed in immunocompromised patients, to vulvovaginal candidiasis, which affects a large number of women². Oropharyngeal candidiasis is the most common opportunistic infection strongly correlated with impairment of the immune system³. Vaginal candidiasis is widespread because it has been estimated that 75% of all women will experience an episode of *Candida* vaginitis once in their lifetime, with up to 5% showing recurrence⁴. In recent years, azole agents have become the drugs of choice for treating oropharyngeal and vulvovaginal candidiasis. Recent studies indicated the possibility of treatment failures associated with some *C. albicans* and related species by expression of efflux pumps that reduce

drug accumulation, alteration of the structure or concentration of antifungal target proteins, and alteration of membrane sterol composition⁵. For a long time, polyene drugs including Amphotericin and Nystatin were the only therapeutic options for invasive fungal infections. Nowadays, Nystatin remains the more available antifungal drugs in more developing countries. The still unacceptably high morbidity rate associated with some resistant mycosis indicates that alternatives to existing therapeutic options are needed. Plants are a major source of biomolecules with pharmacological properties recognized worldwide including antimicrobial activity. They could exhibit beneficial interactions with conventional drugs in the treatment of microbial infections.

H. madagascariensis is a plant of Hypericaceae family. It is used in traditional medicine for the treatment of microbial and non-microbial diseases. Previous studies confirm the use of aqueous root extract in the treatment of drug-induced hepatotoxicity⁶. They also highlighted the antibacterial activity⁷⁻⁹. The present findings were conducted to explore the antifungal activity of *H.*

madagascariensis stem bark and potent potentiating activity with Fluconazole and Nystatin.

MATERIALS AND METHODS

Plant material

Fresh *H. madagascariensis* stem barks were collected at Mount Khalla, Yaoundé (Cameroon) in April 2014. The identification of the plant was ascertained morphologically at the Cameroon National Herbarium where voucher sample were deposited under the registration number N° 4224 HNC.

Microorganisms

Six yeast strains including *Candida albicans* ATCC 1663, *Candida albicans* 9003, *C. krusei*, ATCC 6258, *Candida glabrata* CIP35, *Candida parapsilosis* ATCC 22019, *Cryptococcus neoformans* IP 96026 and one clinical isolate of *Candida albicans* and *Cryptococcus neoformans* were considered in the study. The isolates were obtained from the Pasteur Center, Cameroon.

Experimental animals

In vivo experiments were performed using Wistar albino adult rats of both sexes, 10 to 12 weeks old (200 ± 30 g). They were fed with a standard diet. Food and water were given *ad libitum* throughout the experimental period. Animals were maintained at room temperature ($22 \pm 2^\circ\text{C}$) and were handled according to standard protocols for the use of laboratory animals as recommended by the Guide for the Care and Use of Laboratory Animals, Washington.

Extract preparation

H. madagascariensis stem bark were air-dried for three weeks and powdered to coarse particles. Two hundred and fifty grams of powder were macerated during 48 hours, using 2.5 l methanol. Upon filtration, the extract was concentrated under reduced pressure at 45°C using Rota vapor Buchi R205 to yield a paste of 14.50%. The paste was further incubated 48 hours at 45°C .

Preliminary phytochemical screening

The secondary metabolite classes such as alkaloids, anthocyanins, quinones, flavonoids, phenols, saponins, tannins, sterols and triterpenes were screened according to phytochemical methods previously described by Harbone¹⁰.

In-vitro antifungal susceptibility testing

Antifungal susceptibility tests were performed by broth microdilution method in 96 microtitre plates¹¹. In short, stock solution of the plant extract was prepared in 5% Dimethylsulfoxide (DMSO). Serial two-fold dilutions of the extract were performed to obtain a final concentration of $1024\mu\text{g/ml}$ in the first well. Fungal suspension ($100\ \mu\text{l}$) in Sabouraud broth culture medium was added in each well and the plates were incubated 48 h at 35°C . Minimum Inhibitory Concentrations (MIC) were defined as the lowest concentrations of extract required to prevent the visual growth of the fungi at the end of the incubation time. Minimum Fungicidal Concentrations (MFC) were determined by sub-culturing $10\ \mu\text{l}$ aliquots of the medium drawn from wells which did not show any growth during MIC assay and incubated further for 48 hours at 35°C . The lowest concentration from which negative growth was

recorded was considered as MFC. Fluconazole and Nystatin were used as reference antifungal drugs.

The extract was further tested in association with Nystatin and Fluconazole at MIC/2 and MIC/4, MIC/8, MIC/16 and MIC/32 using checkerboard method^{12,13}. All assays were performed in triplicate and repeated thrice. Fractional Inhibitory Concentration (FIC) was calculated as the ratio of MIC_{Extract} in combination with reference drug/MIC_{Extract} alone and the interpretation made as follows: synergistic (<0.5), indifferent (0.5 to 4), or antagonistic (>4).

In-vivo antifungal susceptibility testing

Oral candidiasis

Oral infection in male rats was induced basically as reported by Martinez et al.¹⁴ with slight modifications. Rats were immunosuppressed one week before the first inoculation and continued throughout the experiment by administering dexamethasone (Sigma-Aldrich, German) in drinking water at 0.5 mg per liter. Also, a 0.1% aqueous solution of tetracycline hydrochloride (Sigma-Aldrich, German) was given to animals 7 days before inoculation. Experimental infection was established by inoculation of the oral cavities of the rats three times in 48 hours interval with 0.1 ml of yeast suspension containing 5×10^8 cells of *C. albicans* ATCC 1663. Oral inoculation was performed by means of a cotton swab rolled twice over all parts of the mouth.

Vulvovaginal candidiasis (VVC)

The animal model of VVC was established based on previously described models¹⁵ with slight modifications. Briefly, estrus was induced with intraperitoneal administration of estradiol (Sigma-Aldrich, German) at 4 mg/kg daily for 9 days before inoculation. Furthermore, animals were immunosuppressed with dexamethasone as described earlier. Aqueous solution of tetracycline hydrochloride 0.1% was given to experimental animals throughout the experimental period. Intravaginal infection was established with 10^7 yeast cells (*C. albicans* ATCC 1663) per 0.1 ml of sterile physiological saline water after three time inoculation at 48 hours intervals.

Treatment

In both cases, infected animals were randomly distributed into five groups of 5 animals each two days after the last inoculation. For oral candidiasis model, rats were treated by topical application in the oral cavity twice a day for eight consecutive days with 0.5 ml plant extract (2 mg/ml) (Group I), mixture of plant extract and Fluconazole (1 mg/ml; $\frac{1}{2}$, $\frac{1}{2}$) (Group II) and Fluconazole (1 mg/ml) (Group III) in viscous 0.8% agar solution as excipient.

In the VVC model, treatment was administered by gavage, twice daily. The first group received no treatment. The second group was treated with *H. madagascariensis* bark extract at 200 mg/kg, the third group with 50 mg/kg *H. madagascariensis* bark extract and 50 mg/kg Fluconazole, and the last group was treated with Fluconazole at 200 mg/kg. In both cases, control group consisted of infected and untreated animals.

Evaluation of therapeutic efficacy

The evaluation of the therapeutic efficacy of the treatment was assessed by microbiological and histopathological evaluation. Oral and vaginal samples were collected each

Table 1: Minimal Inhibitory Concentrations (MIC), Minimal Fungicidal Concentrations (MFC) and MFC/MIC ratios of *H. madagascariensis* methanol bark extract

Extract and antibiotics yeasts	Extract		Fluconazole			Nystatin			
	Antimicrobial parameters ($\mu\text{g/ml}$)								
	CMI	CMF	CMF/CM I	CMI	CMF	CMF/C MI	CMI	CM F	CMF/CM I
<i>C. albicans</i> ATCC 1663	256	256	1	1	4	4	1	1	1
<i>C. albicans</i>	1024	1024	1	4	16	4	4	16	4
<i>C. albicans</i> ATCC 9003	512	/	/	/	/		8	16	2
<i>C. neoformans</i>	1024	1024	1	4	8	2	4	2	2
<i>C. neoformans</i> IP 96026	256	256	1	1	4	4	4	16	4
<i>C. glabrata</i> CIP 35	256	256	1	1	16	16	1	1	1
<i>C. parapsilosis</i> ATCC 22019	256	512	2	2	4	2	2	16	8
<i>Candida krusei</i> ATCC 6258	128	512	4	/	/	/	4	16	4
<i>C. albicans</i> ATCC 9003	512	/	/	/	/	/	1	1	1

/= undetermined

Table 2: Fractional Inhibitory Concentrations (FIC) of *H. madagascariensis* bark methanol extract associated with Fluconazole or Nystatin.

Yeasts	Plant extract + Fluconazole					Plant extract + Nystatin				
	FIC2	FIC4	FIC8	FIC 16	FIC 32	FIC2	FIC4	FIC8	FIC 16	FIC 32
<i>C. albicans</i> ATCC 1663	0.5	0.5	–	–	–	0.5	4	–	4	–
<i>C. albicans</i>	0.125	0.125	–	–	–	0.0625	0.125	1	–	–
<i>C. neoformans</i>	0.125	0.125	–	1	1	0.125	0.125	1	1	–
<i>C. neoformans</i> IP 96026	0.5	0.5	4	4	4	0.25	0.5	4	4	4
<i>C. glabrata</i> CPI 35	0.5	0.5	1	1	1	0.5	1	4	4	4
<i>C. parapsilosis</i> ATCC 22019	0.25	0.25	4	4	2	0.25	0.25	4	4	4

FIC 2, 4, 8, 16, 32= Fractional inhibitory concentrations of *H. madagascariensis* bark methanol extract in addition with Fluconazole or Nystatin at MIC/2, MIC/4, MIC/8, MIC/16, MIC/32 –= could not be found. Synergy activity ($FIC \leq 0.5$), additive activity ($FIC 0.5 < FIC < 1$), indifferent ($1 < FIC < 4$) and antagonism ($FIC > 4$).

two days by rolling a sterile cotton swab over the oral cavity or within the vagina. Samples were then suspended in 4 ml of sterile physiological saline water and cultured in triplicate on Sabouraud dextrose agar supplemented with 0.05 mg/ml actidione and gentamycin (Sigma-Aldrich, German). Plates were incubated at 37°C for 48 h. The yeast count was expressed as log₁₀ CFU per milliliter. Twenty-four hours after the last treatment, experimental rats were anesthetized with diazepam and sacrificed. Tongues or vaginas were aseptically removed. These organs were fixed by immersion in neutral buffered 10% formalin solution. Serial cross sections were further made in formalin for 12 h and small pieces were subjected to haematoxylin-eosin staining¹⁶. Pathological observations were performed on gross and microscopic basis. Histological plates were encrypted for analysis by a histopathologist.

RESULTS

Phytochemical analysis

Freshly prepared *H. madagascariensis* bark extract were subjected to a preliminary phytochemical screening. The results revealed the presence of phenols, alkaloids, flavonoids, tannins, sterols, saponins, anthocyanins and quinones. However, triterpenes could not be detected.

In vitro antifungal activity

All tested yeasts were sensitive to the methanol extract of *H. madagascariensis* bark with MIC values ranging from

128 mg/ml to 1024 mg/ml (Table 1). *C. krusei* was found to be more sensitive (128 $\mu\text{g/ml}$). *C. albicans* ATCC 1663, *C. neoformans* IP 96026 and CIP 35, *C. glabrata* and *C. parapsilosis* ATCC 22019 have comparable sensitivity with value equal to 256 $\mu\text{g/ml}$.

The methanol extract of *H. madagascariensis* bark in combination with Fluconazole and Nystatin revealed on all tested yeasts, additive or synergistic interaction with dilutions of these antifungal corresponding to MIC/2 and MIC/4. Beyond these dilutions, the interaction was either additive or indifferent on all the microorganisms (Table 2).

In vivo antifungal activity

Therapeutic efficacies of *H. madagascariensis* extract in association with Fluconazole against experimental oral *C. albicans* infections

The therapeutic efficacies of the *H. madagascariensis* extract in association with Fluconazole against experimental oral *C. albicans* infections were investigated by microbiological and pathological studies. Infected and untreated animals showed extensive colonization of the epithelium of the dorsal surface of the tongue by numerous hyphae (Figure 1). Treated animals showed multiple regenerative areas of the covering epithelium, and no histological evidence of *C. albicans* within the epithelium of the tongue was seen in animals treated with Fluconazole or in association with *H. madagascariensis* extract.

The results of oral wabs culture as a function of treatment and duration is presented in Table 3. The results from

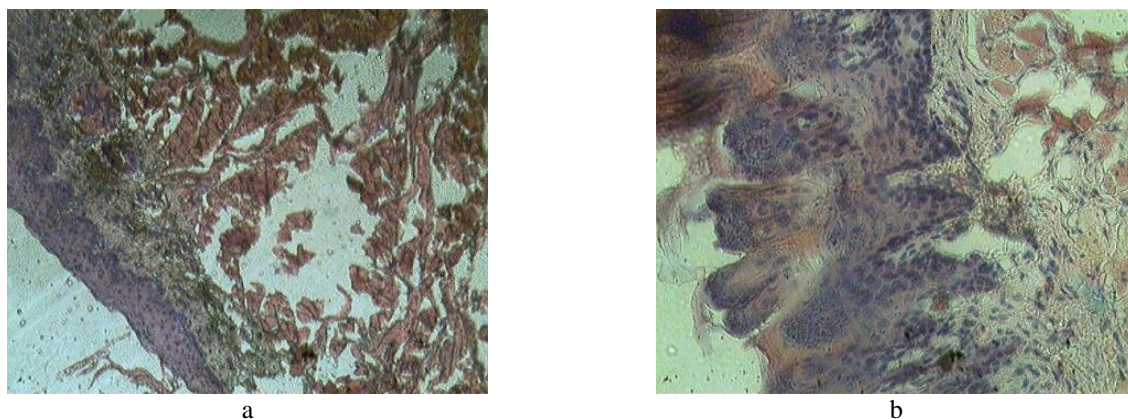


Figure 1: Histopathological analysis in rat's oral candidiasis following treatment with *H. madagascariensis* bark methanol extract.

- a) Control animals showing extensive colonization of the epithelium of the dorsal surface of the tongue by numerous hyphae. b) Treated animals showing multiple regenerative areas of the covering epithelium, and no histological evidence of *C. albicans* within the epithelium of the tongue.

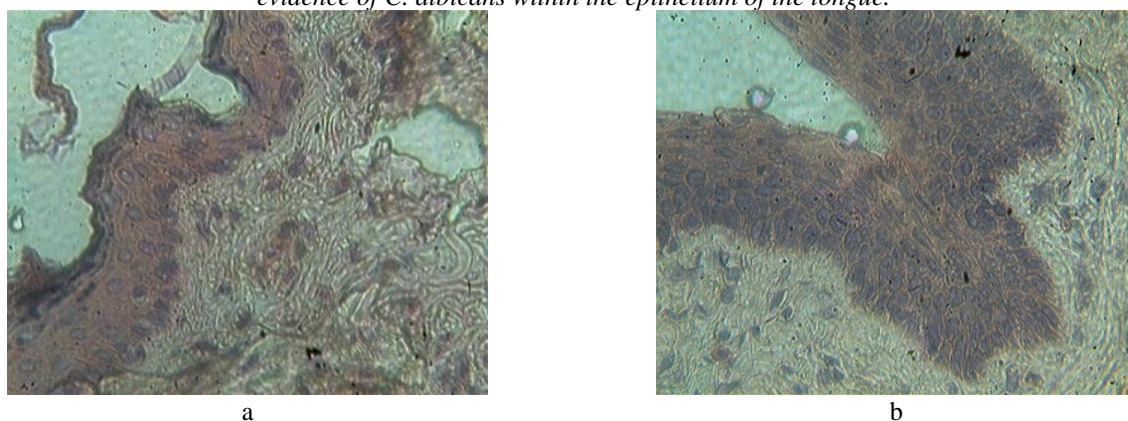


Figure 2: Histopathological analysis in rat's vulvovaginal candidiasis following treatment with *H. madagascariensis* bark methanol extract.

- a) Control showing abundant growth of *Candida* organisms, both as budding yeasts and in the pseudohyphal form b) Treated animal showing restoration of degraded structures and a lack of yeast.

infected rats indicate that all animals developed oral infection, with a log CFU per sample (mean \pm standard deviation) of 4.68 ± 1.04 . This infection remained in the untreated rats, although with a certain decrease in the number of organisms when evaluated 24 h after the end of the treatment (1.87 ± 0.70 log CFU/sample). In the treated groups, a significant and progressive decrease in yeast load was observed in the saliva. The decrease was more pronounced with rats treated with the extract associated with Fluconazole. In the 8th day of treatment, all the rats treated with the *H. madagascariensis* extract had positive yeast culture. Three out of five the rats treated with Fluconazole had positive culture while one out of five of those treated with the *H. madagascariensis* extract associated with Fluconazole had positive culture meaning that this association is more efficient compared to Fluconazole alone.

Therapeutic efficacies of H. madagascariensis extract in association with Fluconazole against experimental vaginal C. albicans infections

The vaginal histopathological analysis revealed two major findings (Figure 2). Untreated rats showed abundant growth of *Candida* organisms, both as budding yeasts and

in the pseudohyphal form, involving the stratum corneum and the luminal keratin debris accompanied by a thin epithelium, degraded and disorganized cells. On the other hand, treated animals regardless of treatment showed restoration of degraded structures and sometimes a lack of yeasts. The therapeutic effect of *H. madagascariensis* extract in association with Fluconazole, as compared to extract or Fluconazole on the *C. albicans* vaginal burden in infected rats is summarized in Table 4. A significant decrease in the percentage of animals showing positive vaginal cultures was observed in rats treated with both Fluconazole and Fluconazole -extract mixture. All the five animals treated with *H. madagascariensis* extract at 200 mg/kg body weight revealed positive cultures. Two-fifth of animals treated with Fluconazole at 200 mg/kg showed negative culture whereas three-fifth of animals treated with Fluconazole and extract mixture at 100 mg/kg showed positive *Candida* cultures. Animals still positive for vaginal *Candida* presence showed a statistically significant ($P < 0.05$) reduction in fungal burden compared to control as a function of treatment duration. This was more pronounced with Fluconazole and *H.*

madagascariensis extract mixture making it the most active.

DISCUSSION

The methanol extract of the bark of *H. madagascariensis* possess inhibitory properties on tested yeasts, and this could be justified by the presence of several classes of chemical compounds which was identified, since antimicrobial activity of compound from these groups have previously been mentioned¹⁷.

During the last twenty years, the frequency of invasive fungal infections and the risk of opportunistic fungal infections had increased especially in immunocompromised patients¹⁸. Among the opportunistic fungal pathogens, *C. neoformans* and *Candida* germs are commonly associated with fungal infections, causing most common infections. These microorganisms were susceptible to *H. madagascariensis* stem bark methanol extract, showing that the extract is a potential source of antifungal molecules capable of fighting against diseases caused by these microorganisms. Indeed, Iwalewa et al.¹⁹ reported antifungal activity of six extracts (hexane, dichloromethane, ethyl acetate, chloroform, acetone and methanol) bark of *H. madagascariensis* on various microorganisms including *C. neoformans* and *C. albicans*. Five of the eight tested yeasts exhibited MIC values below 500 µg/ml, and three of them out of six showed MICs values between 500 and 1500 µg/ml translating respectively strong and moderate activities of the methanol extract of *H. madagascariensis*. MFC/MIC ratios were in all cases <4 expressing the fungicidal activity of *H. madagascariensis*²⁰.

The continuing increase of resistance to antifungal drugs on one hand and the development of traditional medicine on other hand raise questions about the possible association for faster and effective struggling against infections. In combination with Fluconazole or Nystatin, the methanol extract of the bark of *H. madagascariensis* presented synergistic and additive effects on all the yeasts tested at MIC/2 and MIC/4. This suggests that these two antifungal reference drugs offer opportunity for association with the methanol extract of *H. madagascariensis* for potentiation of the antifungal activity. The presence of synergy between the methanol extract of *H. madagascariensis* bark and antifungal assumed that the latter would have molecules with complementary mechanisms of action or molecules with similar modes of action with these antifungal drugs. Indeed, Nystatin belongs to the polyene group. It exerts a physical-chemical interaction with membrane sterols, leading to the formation of aqueous pores in the fungal membrane. On the contrary, Fluconazole belongs to the group of azoles which exert their antifungal activity by inhibiting one of the steps in the synthesis of ergosterol, a major component of the fungal membrane²¹.

The therapeutic efficacies of *H. madagascariensis* extract and Fluconazole mixture (100 mg/kg body weight) as compared to Fluconazole (200 mg/kg) and *H. madagascariensis* extract (200 mg/kg) was studied in experimental models of oral and vulvovaginal candidiasis

in rats. Oral and vaginal candidiasis models have been shown to afford a simple, reliable, and highly reproducible method for studying the efficacies of antifungal agents²².

Although oral candidiasis is not a dangerous disease in itself, it is the origin of morbidity among human. Its prevalence increases in immunocompromised patients whose prevalence is constantly growing. On the other hand, VVC also affects a large number of otherwise healthy women. It has been estimated that 75% of all women of childbearing age will experience an episode of VVC in their lifetime²³. Results point out that orally administered, the methanol extract of *H. madagascariensis* and Fluconazole as mixture have led to both candidiasis models studied, a significant reduction in fungal burden during treatment. This adequately showed that the extract of this plant had *in vivo* antifungal properties elucidated *in vitro*.

The extract of *H. madagascariensis* in combination with Fluconazole resulted after eight days treatment on both candidiasis models studied four healings against five compared to three against five with Fluconazole, used as a reference antifungal against candidiasis. This result shows that a complete cure of all animals could be acquired, the healing could be done much more quickly with this mixture compared to Fluconazole eventhough assessed at a reduced dose. The use of reduced doses for maximal activity seems useful because it reduces the risk of side effects following the administration of drug.

Administered at 100 mg/kg orally, the methanol extract of *H. madagascariensis* in combination with Fluconazole had a better result compared to Fluconazole given at 200 mg/kg. This result confirms the potentiator effect resulting in an additive or synergistic effect as observed *in vitro*. This result seems promising because beyond the antifungal activity highlighted, it provides the beneficial effects of the association between herbal and conventional medicines. This is particularly important since the resurgence of microorganisms to conventional antibiotics is growing and calls new challenges.

Animals given *H. madagascariensis* and Fluconazole mixture showed histopathological findings in line with normal mucosal recovery from the induced infectious process thereby, confirming the therapeutic efficiency of the treatment.

The results achieved with the *in vitro* synergetic and additive antifungal activity together with oral and vaginal candidiasis models strongly suggest that Fluconazole and *H. madagascariensis* extract mixture could be promising antifungal agents for the treatment of human oral and VVC *Candida* infections.

ACKNOWLEDGEMENTS

Authors are thankful to Cameroon National Herbarium (Yaounde) for plants identification and Jean Pierre Zoobo for English revision.

REFERENCES

1. Parisa B, Zahra H. Opportunistic invasive fungal infections: diagnosis & clinical management. Indian Journal of Medical Research 2014; 139(2): 195–204.

2. Sobel DMDJ, Chaim WMD RN, Leaman D. Recurrent vulvovaginal candidiasis associated with long-term tamoxifen treatment in postmenopausal women. *Obstetrics & Gynecology* 1996; 88 (4) : 704-706
3. MacCallum D, Odds F. Temporal events in the intravenous challenge model for experimental *Candida albicans* infections in female mice. *Mycoses* 2005; 48:151-161.
4. Achkar MJ, Fries CB *Candida* infections of the genitourinary tract. *Clinical Microbiology Reviews* 2010; 23(2): 253-273.
5. Sanglard D, Odds FC. Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. *The Lancet Infectious Diseases* 2002; 2(2):73-85.
6. Adeneye AA, Olagunju JA, Elias SO, Olatunbosun DO, Mustafa AO, Adeshile OI, et al. *Harungana madagascariensis* in acute and repeated acetaminophen hepatotoxic rats. *International Journal of Applied Research in Natural Products* 2008; 1(3): 29-42.
7. Kisangau D, Hosea K, Joseph C Iyaruu H. *In vitro* antimicrobial assay of plants used in traditional medicine in Bukoba district, Tanzania. *African Journal of Traditional, Complementary Alternative Medicines* 2007; 4(4): 510-523.
8. Afieroho OE, Izontimi SS, Okoroafor DO Caleb B. Antibacterial and phytochemical evaluation of *Harungana madagascariensis* L (Hypericaceae) seeds. *International Research Journal of Pharmacy* 2012; 3:11.
9. Kengni F, Fodouop SPC, Tala SD, Djimeli NM, Fokunang C, Gatsing D. Antityphoid properties and toxicity evaluation of *Harungana madagascariensis* Lam (Hypericaceae) aqueous leaf extract. *Journal of Ethnopharmacology* 2016; 6(2): 62-76.
10. Harbone SB. A guide to modern techniques of plant analysis. Chapman and Hall, London, 1984: 4-80.
11. Mouokeu RS, Ngono Ngane RA, Njateng GSS, Kamtchueng MO, Kuate JR. Antifungal and antioxidant activity of *Crassocephalum bauchiense* (Hutch) Mile-Redh ethyl acetate extract and fractions (Asteraceae). *BioMed Central Research Notes* 2014; 7:44.
12. Braga LC, Leite AAM, Xavier KGS, Takahashi JA, Bemquerer MP, Chartone-Souza E, et al. Synergic interaction between pomegranate extract and antibiotics against *Staphylococcus aureus*. *Canadian Journal of Microbiology* 2005; 51: 541-547.
13. Bajaksouzian SMA, Visalli MR, Appelbaum PCJ. Activities of levofloxacin, ofloxacin, and ciprofloxacin, alone and in combination with amikacin, against acinetobacters as determined by checkerboard and time-kill studies. *Antimicrobial Agents and Chemotherapy* 1997; 41:1073-1076.
14. Martinez A, Ferrer S, Santos I, Jimenez E, Spareowe J, Regadera J, et al. Antifungal efficacy of GM237354 and GW471558 in experimental model of oral and vulvovaginal candidiasis in immunocompromised rats. *Antimicrobial Agents and Chemotherapy* 2001; 45 (12): 3304-3309.
15. Cassone A, De Bernardis F, Pontieri E, Carruba G, Girmenia C, Martino P, et al. Biotype diversity of *Candida parapsilosis* and its relationship to the clinical source and experimental pathogenicity. *The Journal of Infectious Diseases* 1995; 171, 967-75.
16. Venkataranganna MV, Rafiq M, Gopumadhavan S, Peer G, Babu UV, Mitra SK. NCB-02 (standardized Curcumin preparation) protects dinitrochlorobenzene-induced colitis through down-regulation of NFkappa-B and iNOS. *World Journal of Gastroenterology* 2007; 13:1103-1107.
17. Marjorie MC. Plant products as antimicrobial agents. *Clinical Microbiology Reviews* 1999; 12(4):564-582.
18. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clinical Microbiology Reviews* 2007; 20:133-163.
19. Iwalewa OE, Suleiman MM, Mdee KL, Eloff NJ. Antifungal and antibacterial activities of different extracts of *Harungana madagascariensis* stem bark. *Pharmaceutical Biology* 2009; 47:9.
20. Oussou KR, Yolou S, Boti JB, Guessenn KN, Kanko C, Ahibo C, et al. Etude chimique et activité antidiarrhéique des huiles essentielles de deux plantes aromatiques de la pharmacopée ivoirienne. *European Journal of Scientific Research* 2008; 24(1):94-103.
21. Dismukes WE. Introduction to antifungal drugs. *Clinical Infectious Diseases* 2000; 30:653-657.
22. Samaranayake YH, Samaranayake LP. Experimental oral candidiasis in animal models. *Clinical Microbiology Reviews* 2001; 14(2):398-429.
23. Ghelardi E, Pichierra G, Castagna B, Barnini S, Tavanti A, Campa M. Efficacy of chromogenic candida agar for isolation and presumptive identification of pathogenic yeast species. *European Journal of Clinical Microbiology and Infectious Diseases* 2008; 14:141-147.