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Anti-yeast activity of extracts and fractions from *Uvariodendron calophyllum* (Annonaceae)

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ABSTRACT

The resistance to available antifungals highlights the urgent need for innovative drugs to treat yeasts infections. This study aimed at evaluating the activity of extracts and fractions from *Uvariodendron calophyllum* against pathogenic yeasts. The ethanolic and aqueous extracts obtained by maceration were liquid-liquid-partitioned using organic solvents and screened against isolates of *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Cryptococcus neoformans* and *Candida albicans* reference strains NR-29445, NR-29444, NR-29451, and NR-29450 from BEI Resources using the broth micro-dilution method. Time kill kinetic, inhibition of germ-tube, filamentation and chlamydosporulation, and biofilm formation were assessed using the best sub-fraction. Overall, the most interesting sub-fraction (FS: 237–253) showed an MIC value of 0.0625 mg/mL with cidal effect against *C. albicans* NR-29450 and NR-29445 at 0.25 mg/mL after 12-16 hours and 24 hours respectively. Moderate inhibitory effects were observed at 0.25 mg/mL against germ-tube formation, filamentation and chlamydosporulation of all *C. albicans* strains. Also, very moderate inhibition of biofilm formation by *C. albicans* NR-29450 at 0.25 mg/mL was obtained. The results obtained support *U. calophyllum* as a potential source of compounds with anti-yeast activity. Further studies will confirm its potential as source of anti-yeast drugs.

Keywords: *Uvariodendron calophyllum*, anti-yeasts activity, time kill kinetics, biofilm.

INTRODUCTION

Candida spp. are the most common cause of fungal infections leading to a range of diseases from muco-cutaneous to invasive life-threatening systemic infections that can spread via the bloodstream to organs throughout the body (Jacqueline and Bettina, 2010 ; Moran et al., 2012; Al-Ahmadey and Mohamed, 2014). Candidiasis has emerged as an alarming opportunistic disease as there is an increase in number of patients who are immuno-compromised, aged, receiving

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prolonged antibacterial and aggressive anticancer chemotherapy or undergoing invasive surgical procedures, organ transplantation and HIV patients (Mohandas and Ballal, 2011). The number of clinical infections worldwide has risen considerably in recent years with about 9.5 million HIV patients suffering from oral candidiasis (The Fungal Research Trust, 2011). Other studies have shown that approximately 90% of HIV/AIDS infected persons have at one point demonstrated oropharyngeal colonization by *Candida* species (De Repentigny et al., 2004). Epidemiological studies showed that in Cameroon, the prevalence of candidiasis amongst HIV/AIDS patients is 67.8% with *C. albicans* being responsible for 42.8% of cases (Njunda et al., 2012; Njunda et al., 2013). Furthermore, mortality in patients with candidemia is high, ranging from 40% to 60%, with reported attributable mortality of 20–40% (Méan et al., 2008). Although *C. albicans* is still considered the most prevalent pathogen within the *Candida* clade, non-*albicans Candida* species are increasingly being isolated from patients, with *C. glabrata*, *C. parapsilosis* and *C. tropicalis* being the most represented ones (Peng and Lu, 2013). In addition, *Cryptococcus neoformans* is a human opportunistic fungal pathogen causing severe disseminated meningoencephalitis, mostly in patients with cellular immune defects. Overall, significant part of this prevalence is due to the increase incidence of resistance to conventional antifungal therapies (Lai et al., 2008). In addition, drug-related toxicity and high cost of available antifungals has encouraged the search for alternative treatment from natural products (Duraipandiyan and Ignacimuthu, 2008). Many plant-derived products have gained interest for use as alternatives to traditional antimicrobials, since these extracts are widely believed to be safe and have a long history of use in folk medicine for the prevention and treatment of diseases (Das et al., 2010).

Plants from the Annonaceae family are used in traditional medicine to treat many infectious diseases including microbial infections. The fruits and seeds of some of these plants are used as decoction for the treatment of dermatitis and liver disorders in the southwest and littoral regions of Cameroon (Jiofack et al., 2010). Specifically, *U. calophyllum* is used in traditional medicine for the treatment of infectious diseases and has been shown to possess antifungal and antioxidant activities (Zeuko'o et al., 2012). Based on this background, this study was designed to assess the activity of extracts of leaves, twigs, stem, and stem bark of *U. calophyllum* against isolates of *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Cryptococcus neoformans* and *Candida albicans* reference strains NR-29445, NR-29444, NR-29451, and NR-29450 obtained from BEI Resources following a bio-guided fractionation strategy. Inhibition of the morphological stages of *C. albicans* NR-29445 and NR-29450 was also assessed for the most promising extract sub-fraction.

MATERIALS AND METHODS

Collection and extraction of the plant materials

The leaves, twigs, stem bark, and stem of *U. calophyllum* were collected at Mount Kalla in Yaoundé (Cameroon) on 11th September 2011 and a voucher specimen deposited at the National Herbarium of Cameroon, Yaoundé under the identification number 28734/SFR/CAM. The plant parts were individually dried at room temperature, and then ground to fine powder. Five hundred grams (500 g) of each sample were macerated with regular stirring in 2L of 95% Ethanol for 72 h. The filtrate was evaporated using a rotary evaporator (Rotavapor BÜCHI 011). The plant residues from ethanol extraction

were dried and subsequently macerated in distilled water for 72 hours and the filtrate dried at Room Temperature (25-28 °C) under ventilation. The yields of extraction were calculated in percentage relative to the weight of the starting plant material.

Bio-guided fractionation of crude extracts

Extracts fractionation

The dried ethanolic and aqueous extracts were individually dissolved in water at 10 g/100 mL and subsequently partitioned as previously described (Bolou et al., 2011). Briefly, the extracts dissolved in water were successively washed using equal volumes (100 mL) of hexane, methylene chloride, and methanol to afford hexane fraction, methylene chloride fraction, methanolic fraction, and H_2O/CH_2Cl_2 interfaces for aqueous extracts. The solvents were separately evaporated under reduced pressure and the extracts screened against yeasts. Following our progression criteria, the methylene chloride fraction of the leaf ethanolic extract (UCl EtOH CH_2Cl_2) which showed the overall best anti-yeast activity was selected and subjected to silica gel column chromatography coupled with biological screening as described below. The column elution was done using solvent systems of increasing polarities, Hexane-Ethyl Acetate [100:0 – 0:100] and Ethyl Acetate - Methanol [95:5- 0:100]. Two hundred and fifty-three (253) sub-fractions of 100 mL each were collected and evaporated to dry, and subsequently pooled on the basis of their thin layer chromatography (TLC) profiles into nineteen (19) major sub-fractions FA-FS (FA: 1–11; FB: 12–17; FC: 18–28; FD: 29–35; FE: 36–50; FF: 51–57; FG: 58–64; FH: 65–81; FI: 82–86; FJ: 87–98; FK: 99–111; FL: 112–117; FM: 118–126; FN: 127–133; FO: 134–155; FP: 156–164; FQ: 165–178; FR: 179–236 and FS: 237–253).

Determination of the anti-yeast activity

C. albicans, *C. glabrata*, *C. parapsilosis*, and *Cr. neoformans* isolates obtained from HIV positive patients presenting at the Yaoundé Central Hospital (Cameroon), and developing various candidiasis (vaginal, oro-pharyngeal, intestinal, urinary) and cryptococcal meningitis were used for the experiments. No additional data or samples other than those collected during routine activity at the Yaoundé Central Hospital (YCH) laboratories were used during this study. Therefore, neither ethical approval nor patient consensus was considered necessary. Briefly, isolates were identified at YCH using routine conventional methods including macroscopic and microscopic morphology in conjunction with biochemical analyses. In addition, four *C. albicans* reference strains NR-29445, NR-29444, NR-29451, and NR-29450 provided by BEI Resources NIAID, NIH were used for the study.

Inoculum of each yeast isolate and strain was prepared from a 2 days old culture on Sabouraud Dextrose Broth (SDB) at 37 °C. The suspension was adjusted to 2.5x 10^3 cells/mL using sterile 0.9% sodium chloride solution (normal saline) and Malassez counting chamber under an optical microscope (HumaScope Light, Human) as previously described (Ngono et al., 2000).The broth micro-dilution method was used to assess yeast susceptibility to extracts according to the CLSI M27-A3 methodology (CLSI, 2008). Briefly, each extract residue obtained from partition and column chromatography was dissolved at 100 mg/mL in 10% DMSO in SDB and serially diluted using SDB in 96-well plates. Positive control consisted of Nystatin (Novadina Pharmaceutical Ltd, London, United Kingdom) at 10 mg/mL. 20 µL of inoculum standardized at 2.5×10^3 cells/mL were added into each well to achieved a final volume of

120 µL. The final tested plant product concentrations ranged between 0.029 - 30 mg/mL for the crude extracts, 0.0097-10 mg/mL for fractions, and 0.00195 – 2 mg/mL for column chromatography sub-fractions. Nystatin was tested at 0.0029 - 3 mg/mL.

Negative control wells consisted of inoculum with equivalent volume of 10% DMSO and no drugs added. After 48 h of incubation at 37 °C, the lowest concentration with no visual change in turbidity (indicating no growth of microorganism) was considered as the Minimum Inhibitory Concentration (MIC). The Minimum Fungicidal Concentration (MFC) was determined by sub-culturing 25 µL aliquots of the clear wells into 100 µL of freshly prepared broth medium and incubating at 37 °C for 48 h. The lowest concentration of test sample showing no turbidity change was considered as MFC. . Wells without inoculum or extract were included in each plate to control the background sterility and growth.

The type of antifungal effect of extract or fraction was deduced from the calculated MFC/MIC ratio, and identified as fungicidal when MFC/MIC $\leq$ 4, or fungistatic when MFC/MIC > 4 (Carbonnelle et al., 1987). Based on activity criteria (MIC, MFC), sub-fraction FS: 237-253 was selected and submitted to further detailed studies as described below.

Mechanisms of anti-yeast action of sub-fraction FS: 237 -253

Time-kill kinetic assay

The time-kill kinetic assay of sub-fraction FS: 237-253 against *C. albicans* NR-29445 and NR-29450 was performed as described by (Klepser et al., 1998), with some modifications. Inocula were prepared from two days-old cultures using Sabouraud Dextrose Broth culture medium and adjusted to 5 x 10^5 CFU/mL using the Malassez counting cell.

One milliliter of fungal suspension was added to 9 mL of broth with or without the plant sub-fraction at concentrations of 0.03125, 0.0625, 0.125, and 0.25 mg/mL. Test solutions were placed on an orbital shaker and incubated with agitation at 37 °C and for various time periods (0, 2, 4, 8, and 24 h). At predetermined time points, 50 µL aliquots were taken from each culture, and mixed with 50 µL of methylene blue at 1mg/mL for determination of viable colony forming units (CFU) using the Malassez counting cell. Experiments were performed in duplicate. The $\log_{10}$ CFU/mL was plotted on a graph as function of time and used to compare the rate of fungal death at various concentrations of plant sub-fraction. The effect of the sub-fraction was considered fungicidal when the number of cells was lower than or equal to 3 $\log_{10}$ CFU/mL of the initial inoculum, corresponding to reduction of $\geq$ 99.9% CFU/mL. When the number of cells was greater than 3 $\log_{10}$ CFU/mL, the effect was considered fungistatic (Lewis et al., 2000). In addition, the effects of the sub-fraction on *C. albicans* morphology were observed.

Effect of sub-fraction FS: 237 -253 on germ-tube formation

The same concentrations used for time-kill kinetics assay were prepared in sterile fetal bovine serum and 50 µl of *C. albicans* NR-29445 and NR-29450 suspensions (1 × 10^3 CFU/mL) were individually added. Nystatin was used as positive control and serum without inhibitor as negative control. The so-prepared mixtures were incubated at 37 °C for 4 h, and germ-tubes formation and morphological changes were subsequently observed using an optical microscope (Kim and Lee, 2012). Germ-tubes were considered positive when they were at least as long as the blastospores. Protuberances showing a constriction at the point of connection to the mother cell, typical for pseudohyphae, were excluded.

Effect of sub-fraction FS: 237 -253 on filamentation and chlamydosporulation

The effect of sub-fraction FS: 237 -253 on filamentation and chlamydosporulation of *C. albicans* NR-29445 and NR-29450 was determined by agar dilution method. Briefly, Potatoes Carrot Bile (PCB) and Rice Agar Tween were prepared and individually distributed at 15 mL each in test tubes and sterilized at 121 °C for 15 min. After sterilization, culture media were maintained in water bath at 40 °C and equivalent volumes of sub-fraction FS : 237 -253 in distilled water were introduced to achieve final concentrations of 0.03125, 0.0625, 0.125, and 0.25 mg/mL. The mixtures were homogenized and poured in Petri Dishes and allowed to solidify for 15 min. Nystatin was used as positive control. After solidification, the inoculum solution prepared at 2.5 $x10^3$ cells/mL was seeded in semi-deep manner and incubated at 25 °C for 72 h. Cultures without inhibitor were used as negative control. Filamentation and chlamydosporulation were thereafter determined by observation using the light microscope. The experiments were performed in duplicate.

Determination of the antibiofilm properties of sub-fraction FS: 237 -253

Biofilm quantification and inhibition

Biofilm forming ability of *C. albicans* NR-29450 was assessed using yeast nitrogen base (YNB) medium supplemented with 5% glucose. Quantification of biofilm was performed through total biomass using crystal violet (CV) staining on microtiter plates. After washing, biofilms were fixed with 200 µl of 99% methanol, which was removed after 15 min of contact. The microtiter plates were allowed to dry at room temperature, and 200 µl of CV (1% v/v) added to each well and incubated for 5 min. The wells were then gently washed with sterile double-distilled water (DD-water) and 200 µl of acetic acid (33% v/v) added to wash out the stain. The absorbance of the obtained solution was recorded using a microtiter plate reader (BioTek Synergy HT, Izasa, Lisbon, Portugal) at 590 nm. Optical density was directly proportional to the quantity of biofilm in each well (Silva et al., 2009). The experiments were performed in duplicate**.**

The inoculum of *C. albicans* NR-29450 was prepared from a 2 days-old culture on SDA at 37 °C. The suspension was adjusted to 1 x 10^3 cells/mL using YNB medium supplemented with 5% glucose from 0.5 MacFarland standards.

Briefly, the sub-fraction was serially diluted in YNB in flat-bottomed 96-well plates (MBEC^{TM} Biofilm Inoculator innovotech product panel P and G panel lot: 14040004) and tested at concentrations of 0.03125, 0.0625, 0.125, and 0.25 mg/mL. Eighty µL of inoculum standardized at 1×10^3 cells/mL were added into each well to achieve a final volume of 230 µL. *Candida* biofilms were formed by immersing the pegs of the cover lid into the biofilm growth plate, followed by incubation at 37 °C for 20 h-24 h without shaking. Peg lids were rinsed three times in sterile DD-water, placed onto new flat-bottomed microtiter plates containing 150 µL of YNB per well, and incubated for 18 h to 20 h at 37 °C. After incubation, peg lids were rinsed again three times in sterile DD-water and placed into extract-free YNB in a flat-bottomed microtiter plate (biofilm recovery plate). To transfer biofilms from pegs to wells, each plate was sonicated at room temperature for 20 min using a Bransonic 220 sonicator (BransonCo. Shelton, Conn.). The peg lid was discarded and replaced by a standard lid. The plates were then incubated at 37 °C for 24 h, visually observed and optical density read. A growth control containing microorganism and culture medium and a blank with only medium were included. Directly proportional optical densities were recorded in each well as

described above and plotted versus corresponding sub-fraction concentrations.

Qualitative phytochemical analysis and thin layer chromatography (TLC) of the promising extract, fraction, sub-fraction

To identify some of the classes of compounds present in the promising plant extracts and that might be individually or collectively responsible for the anti-yeast activity observed, a phytochemical screening was performed. This study was conducted following the protocols previously described (Sofowora, 1993; Trease and Evans, 1996; Oloyede, 2005), to screen the presence of biologically active classes of secondary metabolites, including alkaloids, phenols, tannins, saponins, flavonoids, glycosides.

Besides, the crude extract (UCl), fraction (UCl EtOH CH_2Cl_2), and sub-fraction (FS: 237-253) were eluted on a TLC format using Hexane/Ethyl Acetate (Hex/EtOAc) and Methylene chloride/Methanol (CH_2Cl_2/MeOH) solvent systems and visualized under UV (254-365 nm) to achieve their profiles.

Statistical analysis

The data were statistically analysed using the software SPSS 17.0 for Windows and analysis of variance (ANOVA) coupled with turkey test. A $p<0.05$ was considered as statistically significant.

RESULTS

Plant extraction and fractionation

The yields of plant extraction ranged from 1.96% to 6.77% for the aqueous extracts and from 7.79% to 10.67% for the ethanolic extracts. From the 4 ethanolic and 4 aqueous crude extracts, partition using hexane, methylene chloride and methanol afforded 12 and 16 fractions/interfaces respectively.

All the extracts and fractions were screened for biological activity against yeasts, and the methylene chloride fraction (UCl EtOH CH_2Cl_2) from the leaf ethanolic extract with the best anti-yeast potency was selected for further bio-guided study. The fractionation of UCl EtOH CH_2Cl_2 led to 19 sub-fractions: FA: 1–11; FB: 12–17; FC: 18–28; FD: 29–35; FE: 36–50; FF: 51–57; FG: 58–64; FH: 65–81; FI: 82–86; FJ: 87–98; FK: 99–111; FL: 112–117; FM: 118–126; FN: 127–133; FO: 134–155; FP: 156–164; FQ: 165–178; FR: 179–236 and FS: 237–253 that were similarly screened against the tested yeasts.

Anti-yeast activity of extracts, fractions and sub-fractions

The results obtained from the *in vitro* anti-yeast screening of aqueous and ethanolic crude extracts of *U. calophyllum* indicated concentration-dependent activity as shown in Figure 1. This Figure 1 summarizes the results obtained for the more promising extract (UCl EtOH), fraction (UCl EtOH CH_2Cl_2), and sub-fraction (Sub-fraction FS: 237-253). The complete dataset is provided in Table 1. Overall, the MIC values ranged from 0.63 to 30 mg/mL with the best potency being for the ethanolic extract of *U. calophyllum* leaf that showed cidal effect against all the tested yeasts, with the exception of *C. glabrata* and *Cr. neoformans***.**

Fractions afforded from crude extracts showed MIC values ranging from 1.25 to >10 mg/mL on the tested yeasts, with the methylene chloride fraction of the leaf ethanolic extract (UCl EtOH CH_2Cl_2) exhibiting the best activity on all the tested yeasts (MIC values ranging from 1.25-2.5 mg/mL). This fraction was selected and submitted to further bio-guided fractionation.

From the results obtained, the afforded sub-fractions exhibited varying levels of activity, with MIC values ranging from 0.0625 to ≥2 mg/mL with respect to the microorganism tested. Sub-fraction FS: 237-253 (obtained with 100% Methanol) exhibited the overall best activity on all the tested microorganisms with MIC values ranging

from 0.0625 mg/mL to 0.25 mg/mL. The *C. albicans* isolate showed particular sensitivity to this sub-fraction with a MIC of 0.0625 mg/mL. Overall, following the criteria stated above (Carbonnelle et al., 1987), it exerted cidal effect at concentrations tested against 7 out of the 8 yeasts, with the exception for *C. parapsilosis* where the action was fungistatic. Based on this activity profile of sub-fraction FS: 237-253, it was submitted to studies of phenotypic mechanisms of action.

Time-kill Kinetics and inhibition of phenotypic changes

Time-kill Kinetics

Figure 2 shows the induced concentration-dependent effect of sub-fraction FS: 237-253 on the variation of colony forming units of *C. albicans* NR-29445 (A) and *C. albicans* NR-29450 (B) within 24 h. Sub-fraction FS: 237-253 at 0.125 mg/mL and 0.25 mg/mL exerted significant inhibitory effects on *C. albicans* NR-29450 within 24 h. However, the cidal effect could be observed only at 0.25 mg/mL around 12-16 h of incubation. On the other hand, *C. albicans* NR-29445 showed to be relatively less sensitive to the sub-fraction than *C. albicans* NR-29450, as otherwise proven by the MICs of this sub-fraction against the two strains (0.125 mg/mL and 0.25 mg/mL respectively). Fungicidal effect was however apparent at 0.25 mg/mL at the edge of 24 h.

Inhibition of phenotypic changes: germ-tube formation (GTF), filamentation and chlamydosporulation, biofilm formation

The inhibitory effect of sub-fraction FS: 237-253 on the ability of *C. albicans* NR-29445 and NR-29450 to form germ tubes was assessed. From the results obtained, no germ tube was formed by *C. albicans* NR-29450 in the presence of sub-fraction FS: 237-253 at 0.125 mg/mL and 0.25 mg/mL. However, germ tubes were present at lower concentration, but less apparent than in the negative control. Concerning *C. albicans* NR-29445, inhibition of GTF could be observed only at 0.25 mg/mL. The inhibition of filamentation was observed with sub-fraction FS: 237-253 at 0.25 mg/mL on *Candida albicans* NR-29450 and 0.125 mg/mL on *C. albicans* NR-29445. Filamentation of yeast cells to hyphal growth is directly linked to its pathogenicity and cells that cannot undergo transition are known to be avirulent. In addition, inhibition of chlamydosporulation was observed at 0.0625 mg/mL, 0.125 mg/mL, and 0.25 mg/mL. The result obtained with the sub-fraction at 0.125 mg/mL against *C. albicans* NR-29445 is shown in Figure 3.

This figure shows a significant degradation of the hyphae-chlamydospores system of *C. albicans* NR-29445 in the presence of sub-fraction FS: 237-253 at 0.125 mg/mL, compared to the negative control.

The results obtained from biofilm inhibition assay (Figure 4) showed very moderate inhibition by the sub-fraction at 0.25 mg/mL relative to the control containing no drug. This is an indication that at concentrations tested the sub-fraction FS: 237-253 has no effect on biofilm production by *C. albicans* NR-29450.

Qualitative phytochemical composition of the promising extract, fraction, and sub-fraction

The qualitative phytochemical composition of the most promising extract (UCl EtOH), fraction (UC_l EtOH CH2Cl2) and sub-fraction (Sub-fraction FS: 237-253) obtained from the leaf of *U. calophyllum* is given in Table 2. Results indicate that all the screened classes of secondary metabolites are present in the crude ethanolic extract. Further fractionation of this extract led to a less complex sub-fraction (Sub-fraction FS: 237-253) containing only terpenoids, flavonoids, and glycosides.

The comparative TLC profiles of the crude extract (UCl), fraction (UCl EtOH CH_2Cl_2), and sub-fraction (FS: 237-253) of

the leaf extract of *U. calophyllum* are presented in Figure 5.

From this Figure 5, it appears that the crude extract and fraction are densely rich in secondary metabolites compared to the sub-fraction that presents only two clearly defined spots under UV visualization. It should also be noted that the sub-fraction was not significantly eluted by the solvent system Hex/EtOAc 25/75. Rather, this could be achieved with a more polar system (CH_2Cl_2/MeOH 20/80), suggesting that the secondary metabolites of *U. calophyllum* leaf extract responsible of the highest anti-yeast activity are likely polar.

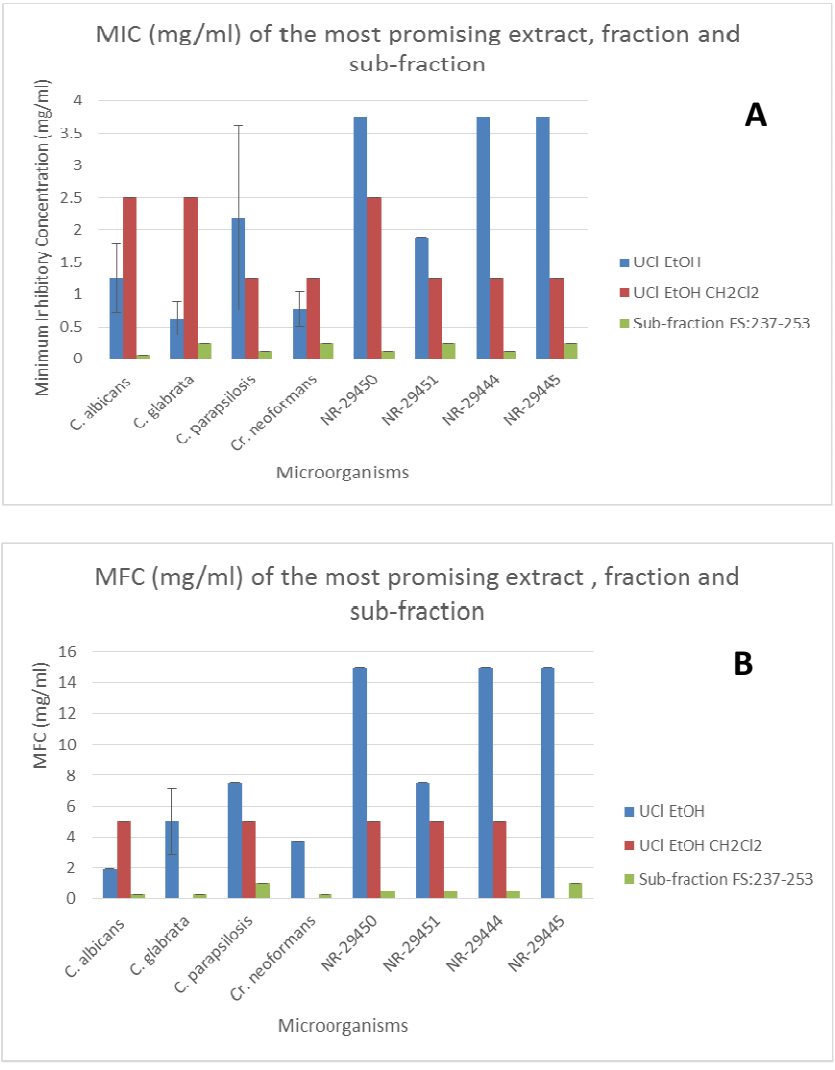


Figure 1: Activity parameters (MIC (A) and MFC (B)) of the most promising extract, fraction and sub-fraction. The plotted values represent means of duplicate experiments. Non prohibitive SD was obtained for UCl EtOH only on yeast isolates.

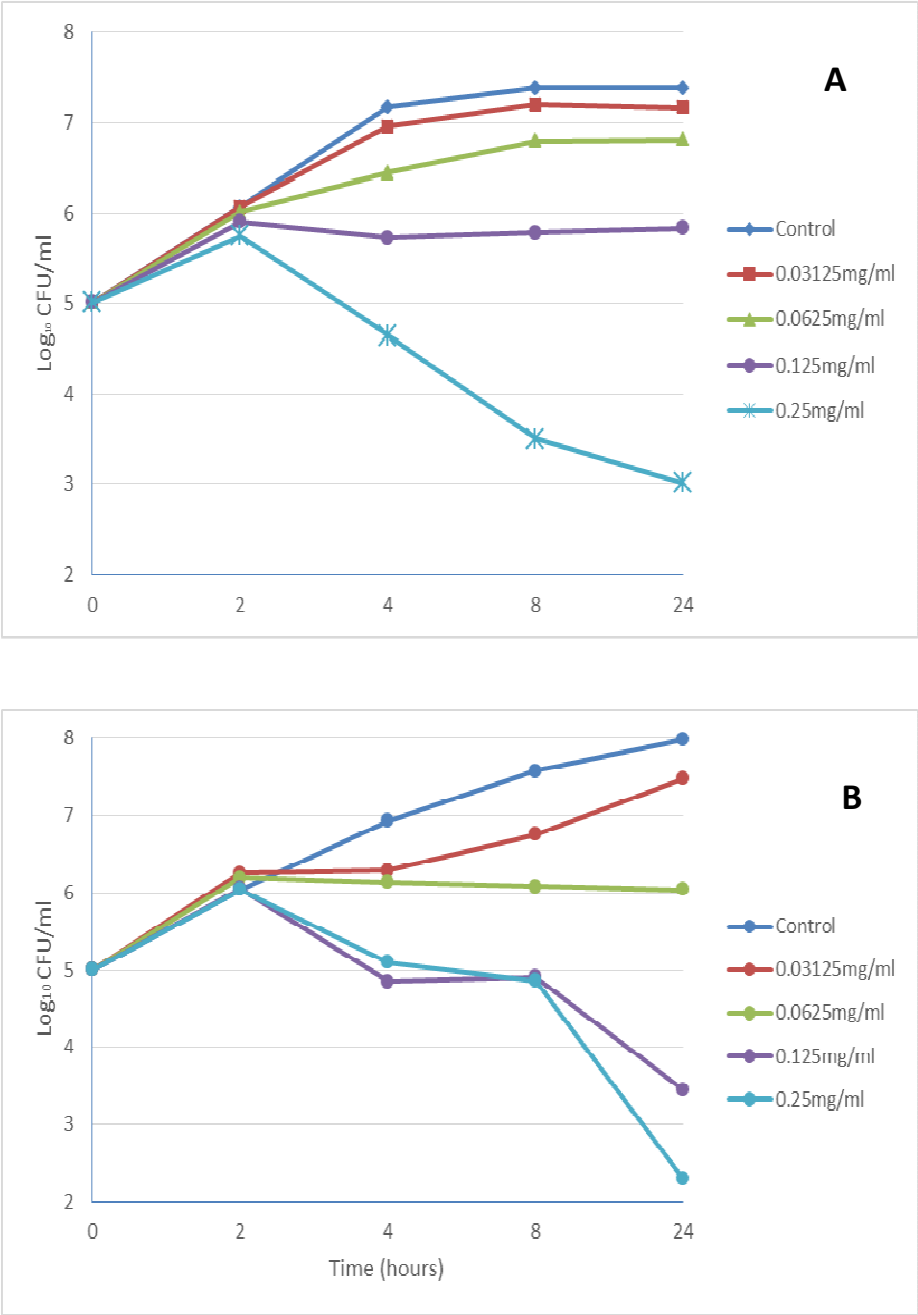
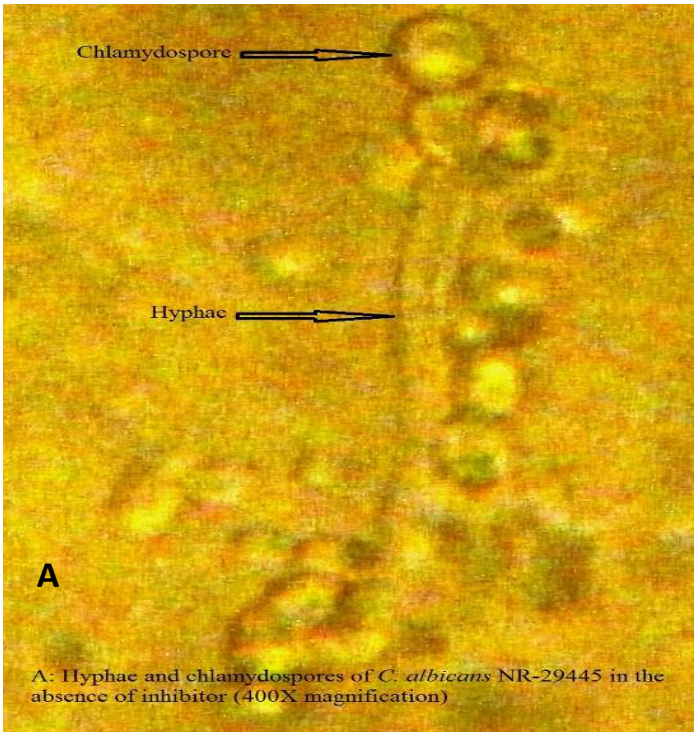
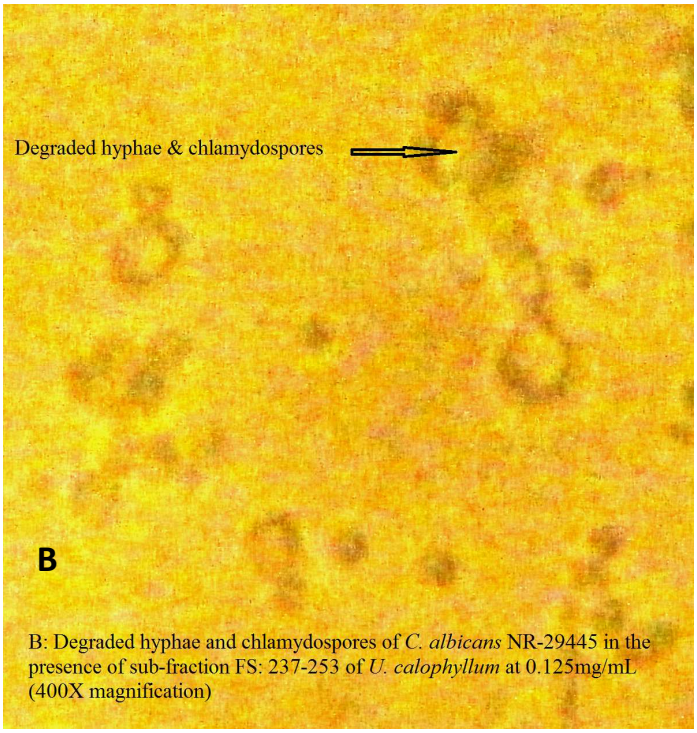


Figure 2: Time-dependent inhibition of *C. albicans* NR-29445 (A) and NR-29450 (B) at different concentrations of sub-fraction FS: 237 -253. The plotted values are means of results from duplicate experiments; the values of SD for individual datapoints were ≤0.2.



A: Hyphae and chlamydospores of *C. albicans* NR-29445 in the absence of inhibitor (400X magnification)



B: Degraded hyphae and chlamydospores of *C. albicans* NR-29445 in the presence of sub-fraction FS: 237-253 of *U. calophyllum* at 0.125mg/mL (400X magnification)

Figure 3: Hyphae and chlamydospores of *C. albicans* NR-29445 in the A: absence of inhibitor; B: presence of sub-fraction FS: 237-253 at 0.125 mg/mL.

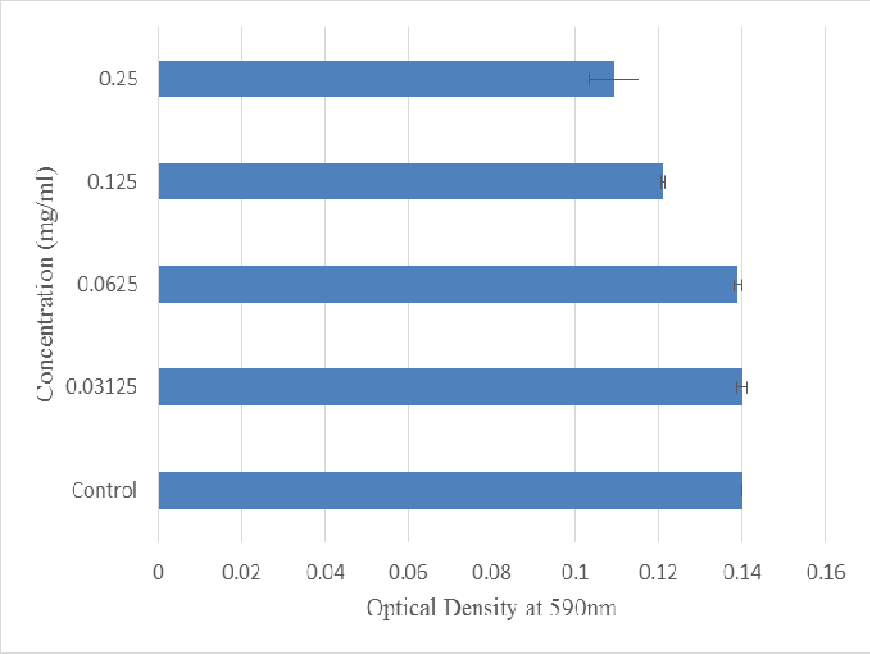


Figure 4: Effect of sub-fraction FS : 237 -253 against biofilm formation by *C. albicans* NR-29450. The plant extract was tested in duplicate at different concentrations. Average OD were plotted versus extract concentration. OD are directly proportional to biofilm density. The control represents yeast cultured with no extract added.

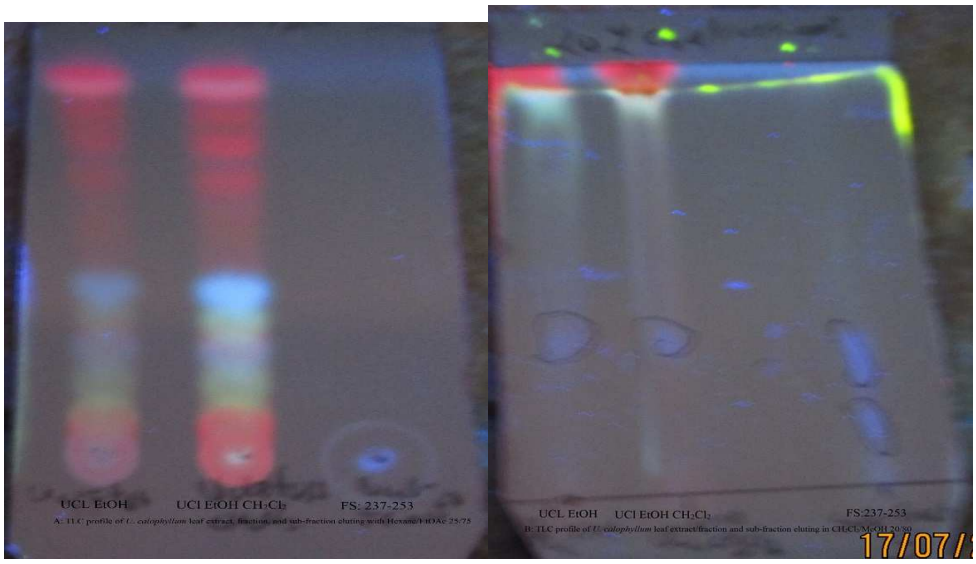


Figure 5: TLC profile of *U. calophyllum* leaf extract, fraction, and sub-fraction eluting with A: Hexane/EtOAc 25/75; B: CH_2Cl_2/MeOH 20/80. The TLC plates were visualized under UV.

Table 1: Anti-yeast activity of the crude extracts, fractions, and sub-fractions of *Uvariodendron calophyllum.*

Extracts	Activity parameters	Field isolates				Reference *C. albicans* strains			
		C. albicans	***C. glabrata***	***C. parapsilosis***	***Cr. neoformans***	**NR-29450**	**NR-29451**	**NR-29444**	**NR-29445**
		Activity ± SD (mg/mL)							
Crude extracts									
UCl EtOH	MIC	1.25±0.54	0.63±0.27**	2.19±1.43	0.78±0.27*	3.75±0.00	1.88±0.00	3.75±0.00	3.75±0.00
	MFC	1.88±0.00*	5.00±2.16	7.50±0.00	3.75±0.00	15.00±0.00	7.50±0.00	15.00±0.00	15.00±0.00
	MFC/MIC	1.5	8	3.4	4.8	4	4	4	4
UCtw EtOH	MIC	1.88±0.00*	2.81±1.08	3.75±0.00	3.28±0.94	15.00±0.00	30.00±0.00	30.00±0.00	15.00±0.00
	MFC	3.75±0.00	15.00±0.00	15.00±0.00	15.00±0.00	>30.00±0.00	>30.00±0.00	>30.00±0.00	>30.00±0.00
	MFC/MIC	2	5	4	4.5	ND	ND	ND	ND
UCst EtOH	MIC	5.00±2.17	5.60±2.17	12.50±4.33	6.56±1.88	7.50±0.00	7.50±0.00	7.50±0.00	15.00±0.00
	MFC	7.50±0.00	7.50±0.00	12.50±0.00	30.00±0.00	>30.00±0.00	>30.00±0.00	>30.00±0.00	>30.00±0.00
	MFC/MIC	1.5	1.33	1	4.6	ND	ND	ND	ND
UCsb EtOH	MIC	3.75±0.00	2.50±1.08	6.25±2.17	5.63±2.17	7.50±0.00	15.00±0.00	7.50±0.00	15.00±0.00
	MFC	7.50±0.00	15.00±0.00	15.00±0.00	30.00±0.00	>30.00±0.00	>30.00±0.00	>30.00±0.00	>30.00±0.00
	MFC/MIC	2	6	2.4	5.3	ND	ND	ND	ND
UCl aqueous	MIC	7.50±0.00	3.75±0.00	7.50±0.00	1.88±0.00	15.00±0.00	15.00±0.00	15.00±0.00	15.00±0.00
	MFC	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	>30.00±0.00	>30.00±0.00	>30.00±0.00	>30.00±0.00
	MFC/MIC	1	2	1	3.9	ND	ND	ND	ND
UCtw aqueous	MIC	3.75±0.00	0.94±0.00	15.00±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	15.00±0.00
	MFC	7.50±0.00	7.50±0.00	15.00±0.00	15.00±0.00	>30.00±0.00	>30.00±0.00	>30.00±0.00	>30.00±0.00
	MFC/MIC	2	8	1	2	ND	ND	ND	ND
UCst aqueous	MIC	3.75±0.00	1.88±0.00	7.50±0.00	7.50±0.00	15.00±0.00	ND	7.50±0.00	15.00±0.00
	MFC	7.50±0.00	7.50±0.00	7.5±0.00	30.00±0.00	>30.00±0.00	ND	>30.00±0.00	>30.00±0.00
	MFC/MIC	2	4	1.5	4	ND	ND	ND	ND

UCsb aqueous	MIC	15.00±0.00	7.50±0.00	7.50±0.00	15.00±0.00	7.50±0.00	15.00±0.00	7.50±0.00	7.50±0.00
	MFC	15.00±0.00	7.50±0.00	7.50±0.00	30.00±0.00	>30.00±0.00	>30.00±0.00	>30.00±0.00	>30.00±0.00
	MFC/MIC	1	1	1	2	ND	ND	ND	ND
				Fractions					
UCl EtOH Hexane	MIC	7.60±0.00	>7.60±0.00	7.60±0.00	>7.60±0.00	3.80±0.00	1.90±0.00*	3.80±0.00	1.90±0.00*
	MFC	>7.60±0.00	>7.60±0.00	7.60±0.00	>7.60±0.00	3.80±0.00	7.60±0.00	3.80±0.00	3.80±0.00
	MFC/MIC	ND	ND	1	ND	1	4	1	2
UCtw EtOH Hexane	MIC	10.00±0.00	>10.00±0.00	>10.00±0.00	10.00±0.00	2.50±0.00	2.50±0.00	5.00±0.00	5.00±0.00
	MFC	10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
	MFC/MIC	1	ND	ND	ND	2	2	1	1
UCst EtOH Hexane	MIC	5.00±0.00	>10.00±0.00	5.00±0.00	10.00±0.00	5.00±0.00	2.50±0.00	2.50±0.00	2.50±0.00
	MFC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
	MFC/MIC	ND	ND	ND	ND	1	2	2	2
UCsb EtOH Hexane	MIC	10.00±0.00	5.00±0.00	5.00±0.00	10.00±0.00	5.00±0.00	5.00±0.00	2.50±0.00	2.50±0.00
	MFC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
	MFC/MIC	ND	ND	ND	ND	1	1	2	2
UCl EtOH CH_2Cl_2	MIC	2.50±0.00	2.50±0.00	1.25±0.00**	2.50±0.00	1.25±0.00**	1.25±0.00**	1.25±0.00**	1.25±0.00**
	MFC	5.00±0.00	>5.00±0.00	5.00±0.00	>5.00±.00	5.00±0.00	5.00±0.00	5.00±0.00	>5.00±.00
	MFC/MIC	2	ND	4	ND	4	4	4	ND
UCtw EtOH CH_2Cl_2	MIC	6.67±0.00	6.67±0.00	6.67±0.00	>6.67±0.00	3.33±0.00	3.33±0.00	6.67±0.00	3.33±0.00
	MFC	>6.67±0.00	>6.67±0.00	>6.67±0.00	>6.67±0.00	6.67±0.00	3.34±0.00	3.34±0.00	3.34±0.00
	MFC/MIC	ND	ND	ND	ND	2	1	1	1
UCsb EtOH CH_2Cl_2	MIC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>6.25±0.00	2.50±0.00	5.00±0.00	10.00±0.00	10.00±0.00
	MFC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>6.25±0.00	10.00±0.00	5.00±0.00	10.00±0.00	10.00±0.00
	MFC/MIC	ND	ND	ND	ND	4	1	1	1
UCtw aqueous CH_2Cl_2	MIC	>10.00±0.00	>10.00±0.00	10.00±0.00	>10.00±0.00	2.50±0.00	2.50±0.00	2.50±0.00	2.50±0.00
	MFC	>10.00±0.00	>10.00±0.00	10.00±0.00	>10.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
	MFC/MIC	ND	ND	1	ND	2	2	2	2

UCsb aqueous CH_2Cl_2	MIC	10.00±0.00	>10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00	5.00±0.00	5.00±0.00
	MFC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10±0.00	>10.00±.00	>10.00±.00	>10.00±.00	>10.00±.00
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND	ND
UCl EtOH MeOH	MIC	10.00±0.00	>10.00±0.00	5.00±0.00	>10.00±0.00	2.50±0.00	5.00±0.00	10.00±0.00	2.50±0.00
	MFC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND	ND
UCtw EtOH MeOH	MIC	10.00±0.00	>10.00±0.00	10.00±0.00	10.00±0.00	2.50±0.00	2.50±0.00	5.00±0.00	5.00±0.00
	MFC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
	MFC/MIC	ND	ND	ND	ND	2	2	1	1
UCst EtOH MeOH	MIC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>6.25±0.00	2.50±0.00	5.00±0.00	10.00±0.00	10.00±0.00
	MFC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>6.25±0.00	5.00±0.00	5.00±0.00	10.00±0.00	10.00±0.00
	MFC/MIC	ND	ND	ND	ND	2	1	1	1
UCsb EtOH MeOH	MIC	10.00±0.00	>10.00±0.00	>10.00±0.00	5.00±0.00	10.00±0.00	5.00±0.00	2.50±0.00	2.50±0.00
	MFC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	10.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
	MFC/MIC	ND	ND	ND	ND	1	1	1	1
UCl aqueous interface	MIC	5.00±0.00	10.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	10.00±0.00	10.00±0.00	5.00±0.00
	MFC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND	ND
UCtw aqueous interface	MIC	5.00±0.00	10.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	2.50±0.00	5.00±0.00
	MFC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND	ND
UCsb aqueous interface	MIC	10.00±0.00	5.00±0.00	5.00±0.00	10.00±0.00	1.25±0.00**	2.50±0.00	2.50±0.00	2.50±0.00
	MFC	10.00±0.00	10.00±0.00	>10.00±0.00	10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00
	MFC/MIC	1	2	ND	1	ND	ND	ND	ND
UCl aqueous MeOH	MIC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	10.00±0.00	>10.00±.00	>10.00±.00	>10±.00
	MFC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND	ND

UCtw aqueous MeOH	MIC	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00	5.00±0.00	10.00±0.00
	MFC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00	>10.00±0.00
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND	ND
UCsb aqueous MeOH	MIC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>6.25±0.00	5.00±0.00	5.00±0.00	10.00±0.00	10.00±0.00
	MFC	>10.00±0.00	>10.00±0.00	>10.00±0.00	>6.25±0.00	5.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00
	MFC/MIC	ND	ND	ND	ND	1	2	1	1
		Sub-fractions of UCl EtOH CH_2Cl_2 fraction							
FA : 1-11	MIC	2.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
	MFC	>2.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00
	MFC/MIC	ND	1	1	1	ND	ND	ND	1
FB: 12-17	MIC	2.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00	2.00±0.00	2.00±0.00	1.00±0.00	2.00±0.00
	MFC	>2.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00
	MFC/MIC	ND	1	1	1	ND	ND	ND	1
FC :18-28	MIC	2.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
	MFC	>2.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00
	MFC/MIC	ND	1	1	1	ND	ND	ND	1
FD : 29-35	MIC	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
	MFC	>2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00
	MFC/MIC	ND	1	1	1	ND	ND	ND	1
FE : 36-50	MIC	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	1.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00
	MFC	>2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	>2.00±0.00	2.00±0.00
	MFC/MIC	ND	1	1	1	2	1	ND	2
FF : 51-57	MIC	2.00±0.00	0.50±0.00	1.00±0.00	2.00±0.00	1.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00
	MFC	>2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00
	MFC/MIC	ND	4	2	1	ND	ND	ND	2
FG :58-64	MIC	2.00±0.00	1.00±0.00	1.00±0.00	0.50±0.00*	2.00±0.00	2.00±0.00	2.00±0.00	1.00±0.00
	MFC	>2.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00

	MFC/MIC	ND	2	1	2	ND	ND	ND	2
FH : 65-81	MIC	2.00±0.00	1.00±0.00	1.00±0.00	0.50±0.00	2.00±0.00	2.00±0.00	2.00±0.00	1.00±0.00
	MFC	2.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00
	MFC/MIC	1	2	1	2	ND	ND	ND	2
FI : 82-86	MIC	2.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	1.00±0.00
	MFC	>2.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00
	MFC/MIC	ND	1	1	1	ND	ND	ND	2
FJ :87-98	MIC	2.00±0.00	0.50±0.00*	0.125±0.00**	0.125±0.00**	1.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00
	MFC	ND	1.00±0.00	1.00±0.00	0.25±0.00*	>2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00
	MFC/MIC	ND	2	8	2	ND	ND	ND	2
FK: 99-111	MIC	1.00±0.00	0.25±0.00*	0.25±0.00*	0.25±0.00*	0.50±0.00*	1.00±0.00	0.50±0.00*	1.00±0.00
	MFC	ND	1.00±0.00	1.00±0.00	1.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00
	MFC/MIC	ND	4	4	4	ND	ND	ND	2
	MIC	2.00±0.00	2.00±0.00	2.00±0.00	0.50±0.00	2.00±0.00	2.00±0.00	2.00±0.00	1.00±0.00
FL:112-117	MFC	ND	2.00±0.00	2.00±0.00	1.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00
	MFC/MIC	ND	1	1	2	ND	ND	ND	2
FM:118-126	MIC	1.00±0.00	0.50±0.00*	0.25±0.00*	0.125±0.00*	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
	MFC	1.00±0.00	1.00±0.00	0.50±0.00*	1.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00	2.00±0.00
	MFC/MIC	1	2	2	8	ND	ND	2	2
FN: 127-133	MIC	1.00±0.00	0.50±0.00*	0.25±0.00*	0.25±0.00*	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
	MFC	>2.00±0.00	1.00±0.00	1.00±0.00	0.50±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00	2.00±0.00
	MFC/MIC	ND	2	4	2	ND	ND	2	2
FO:134-155	MIC	1.00±0.00	0.50±0.00*	0.50±0.00*	0.50±0.00*	1.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00
	MFC	>2.00±0.00	0.50±0.00*	1.00±0.00	0.50±0.00*	>2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
	MFC/MIC	ND	1	2	1	ND	1	1	2
FP:156-164	MIC	1.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	>2.00±0.00	2.00±0.00
	MFC	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00
	MFC/MIC	2	1	1	1	1	ND	ND	1

FQ:165-178	MIC	1.00±0.00	2.00±0.00	2.00±0.00	0.50±0.00	2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00
	MFC	2.00±0.00	2.00±0.00	2.00±0.00	0.50±0.00	2.00±0.00	>2.00±0.00	>2.00±0.00	2.00±0.00
	MFC/MIC	2	1	1	1	1	ND	ND	1
FR:179-236	MIC	2.00±0.00	1.00±0.00	>2.00±0.00	1.00±0.00	2.00±0.00	2.00±0.00	>2.00±0.00	2.00±0.00
	MFC	>2.00±0.00	2.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00	>2.00±0.00
	MFC/MIC	ND	2	ND	ND	ND	ND	ND	ND
FS:237-253	MIC	0.0625±0.00***	0.25±0.00*	0.125±0.00**	0.25±0.00*	0.125±0.00**	0.25±0.00*	0.125±0.00**	0.25±0.00*
	MFC	0.25±0.00*	0.25±0.00*	1.00±0.00	0.25±0.00*	0.50±0.00*	0.50±0.00*	0.50±0.00*	1.00±0.00
	MFC/MIC	4	1	8	1	4	2	4	4
Positive control (Nystatin)	MIC	0.0156±0.00	0.0078±0.00	0.0078±0.00	0.0156±0.00	0.0078±0.00	0.0039±0.00	0.0078±0.00	0.002±0.00
	MFC	0.0156±0.00	0.031±0.00	0.0156±0.00	0.0625±0.00	0.031±0.00	0.031±0.00	0.0156±0.00	0.031±0.00
	MFC/MIC	1	4	2	4	4.2	8	2.05	16

*indicate P≤0.05, **P≤0.01, ***P≤0.001

MIC: Minimum Inhibitory Concentration; MFC: Minimum Fungicidal Concentration; SD: Standard Deviation; ND: not determined, UCl EtOH : Ethanolic extract of *U. calophyllum leaves*; UCtw EtOH : Ethanolic extract of *U. calophyllum twigs;* UCst EtOH : Ethanolic extract of *U. calophyllum stem;* UCsb EtOH : Ethanolic extract of *U. calophyllum stem bark;* UCl aqueous: aqueous extract of *U. calophyllum* leaves; UCtw aqueous: aqueous extract of *U. calophyllum* twigs; UCst aqueous: aqueous extract of *U. calophyllum* stem; UCsb aqueous : aqueous extract of *U. calophyllum* stem bark; UCl EtOH Hexane: Hexane fraction of the ethanolic extract of *U.calophyllum leaves* ; UCl EtOH CH_2Cl_2 : Methylene Chloride Fraction of the ethanolic extract of of *U. calophyllum leaves* ; UCl EtOH MeOH : Methanol extract of the ethanolic extract of *U. calophyllum* leaves *;* UCtw EtOH Hexane : Hexane fraction of the ethanolic extract of *U. calophyllum* twigs ; UCtw EtOH CH_2Cl_2: Methylene Chloride Fraction of the ethanolic extract of of *U. calophyllum* twigs ; UCtw EtOH MeOH: Methanol fraction of the ethanolic extract of *U. calophyllum* twigs ; UCst EtOH Hexane : Hexane fraction of the ethanolic extract of *U. calophyllum* stem; UCst EtOH CH_2Cl_2 : Methylene chloride fraction of the ethanolic extract of *U. calophyllum* stem*;* UCst EtOH MeOH : Methanol fraction of the ethanolic extract of *U. calophyllum* stem ; UCsb EtOH Hexane : Hexane fraction of the ethanolic extract of *U. calophyllum* stem bark ; UCsb EtOH CH_2Cl_2 : Methylene chloride fraction of the ethanolic extract of *U. calophyllum* stem bark ; UCsb EtOH MeOH : Methanol fraction of the ethanolic extract of *U. calophyllum* stem bark; UCl aqueous interface: Interface fraction of the aqueous extract of *U. calophyllum* leaves ; UCl aqueous MeOH : Methanol Fraction of the aqueous extract of *U. calophyllum* leaves ; UCtw aqueous CH_2Cl_2: Methylene chloride fraction of the aqueous extract of aqueous extract of *U. calophyllum* twigs ; UCtw aqueous interface: Interface fraction of the aqueous extract of the aqueous extract of *U. calophyllum* twigs ; UCtw aqueous MeOH: Methanol fraction of the aqueous extract of the aqueous extract of *U. calophyllum* twigs ; UCsb aqueous CH_2Cl_2 : Methylene chloride of the aqueous extract of *U. calophyllum* stem bark ; UCsb aqueous interface: Interface fraction of the aqueous extract of *U. calophyllum* stem bark ; UCsb aqueous MeOH : Methanol fraction of the aqueous extract of *U. calophyllum* stem bark.

Table 2: Qualitative phytochemical composition of the most promising extracts, fraction and sub-fraction.

Extracts	Alkaloids	Terpenoids	Tannins	Saponins	Flavonoids	Glycosides
UCl EtOH	+	+	+	+	+	+
UCl EtOH CH_2Cl_2	+	+	+	-	+	+
Sub-fraction FS:237-253	-	+	-	-	+	+

Legend: (+) Present, (-) Absent

DISCUSSION

The aqueous and ethanolic extracts of *U. calophyllum* showed significant anti-yeast potency against the tested isolates and strains. These results support the use of this plant by practitioners of indigenous medicine (Masoko et al., 2010). The MIC values of the ethanolic extracts of leaf and twig against *C. albicans* (1.25 and 1.88 mg/mL respectively) and *C. glabrata* (0.63 and 2.81 mg/mL respectively) showed slight difference from the results previously obtained with the same extracts (Zeuko' o et al., 2012) on the same yeasts (*C. albicans* 2.61; 2.08 and *C. glabrata* 2.08). These disparities could be due to differences in the inoculum size and methodological approaches used.

The essence of bioactive plant extract fractionation is to concentrate the active principle to result in activity improvement (Firas and Al-Bayati, 2008; Nwodo et al., 2011)**.** In the particular case of this study, the most active crude extract (UCl EtOH) showed MIC values ranging from 0.63-3.75 mg/mL against the tested yeasts. Partition of the crude extract led to the more promising fraction (UCl EtOH CH_2Cl_2) with the range of activity being 1.25-2.50 mg/mL. Further fractionation afforded sub-fractions with MIC ranging below 2 mg/mL, the most potent having an MIC as low as 0.0625 mg/mL, to say 10 fold more active than the original crude extract. It is therefore relevant to note that fractionation of *U. calophyllum* leaf crude extract has increased the activity.

Given the results obtained with sub-faction FS: 237-253, its effect was studied on the morphological stages of *C. albicans* (*C. albicans* NR-29450 and *C. albicans* NR-29445). The variation of colony forming units at different concentrations of the sub-fraction showed to be significant with more pronounced action against *C. albicans* NR-29450 at 0.25-0.0125 mg/mL and cidal effect at 0.25 mg/mL after 8 hours of contact. At these concentrations, *C. albicans* NR-29445 showed reduced sensitivity.

Establishing whether an agent has fungistatic or fungicidal properties provides valuable information on the potential action of antifungal agents *in-vitro*. However, it is necessary to combine this information with pharmacokinetic/pharmacodynamic data in order to provide more meaningful prediction of efficacy *in vivo***.**

On the other hand, the ability of *C. albicans* to undergo morphogenetic conversion from yeast to pseudo- hyphae then hyphae contributes to disease establishment and progression (Navarro-Garcia et al., 2001; Berman and Sudbery, 2002; Banerjee et al., 2008). In addition, this yeast to hyphae transition is necessary for biofilm formation and filamentation that is an essential component of mature biofilms. Biofilm formation and filamentation of *C. albicans* are being increasingly recognized as the main virulence factors contributing to the pathogenesis of candidiasis. The inhibition of filamentation and chlamydosporulation exerted by sub-faction FS: 237-253 therefore underscores its mechanism of action and emphasizes its potential for the development of new antifungal agents (Baillie and Douglas,

1999; Lopez-Ribot, 2005; Nobile et al., 2008). Moreover, sub-fraction FS: 237-253 inhibited germ tube formation by *C. albicans* NR-29450 at 0.125 mg/mL and 0.25 mg/mL. GTF is a morphological characteristic that increases the ability of the fungi to adhere and penetrate into infected tissue (Cutler, 1991). It has been suggested as a potential virulence factor in the pathogenesis of *C. albicans* (Sudbery et al., 2004), since it is the first stage of true hyphae development. The ability to change morphology from the yeast form to hyphae (and vice versa) is thought to be the primary cause of the *C. albicans* pathogenicity (Lee et al., 2005).

Additionally, the formation of hyphae (Liu et al., 1994) and biofilms (Fanning and Mitchell, 2012), added to the integrity of the cell wall (Kelly et al., 2004), the rapid adaptive capacity to external environmental conditions (Brown et al., 2012) all contribute to virulence. During morphogenesis, cells may switch to filamentous growth and develop elongated suspensor cells that give rise to chlamydospores (Martin et al., 2005). The functions of chlamydospores for the biology and possibly for the virulence of *C. albicans* are unclear. The inhibition of chlamydospores by sub-faction FS: 237-253 further emphasizes its ability to stop yeast cells switching to filamentous growth thereby blocking the production of biofilms (Sudbery, 2011).

Biofilm production could be moderately inhibited by sub-fraction FS: 237-253 at 0.25 mg/mL. Biofilm formation is a complex, multicellular process, consisting of cell adhesion, growth, morphogenic switching between yeast and filamentous states, and quorum sensing (Deveau and Hogan, 2011). Adhesion of *C. albicans* cells to materials or host cells is a prerequisite for biofilm formation, and cell-cell interactions may be important in the hierarchical organization of cells within the biofilm (Ramage et al., 2005).

Phytomedicines are the mainstay of traditional system of healing in developing countries, usually integrated to cultural believes. This approach is becoming part of the integrative healthcare system known as complementary and alternative system of medicines. Moreover, identification of the active principles of these phytomedicines is a critical step towards the standardization of their production. In addition, these active principles may act individually or collectively to produce the observed beneficial effects. The biological activities of some of these principles have been reported. The phytochemical analysis of the most interesting sub-fraction indicated the presence of terpenoids, flavonoids, and glycosides. The mechanism of action of terpenoids is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds (Cowan, 1999). Flavonoids have the ability to complex with extra-cellular and soluble proteins present on the microbial cell wall and leading to the inactivation of these proteins, thereby causing loss of their function (Mason and Wasserman, 1987). The glycosides are thought to act through the formation of ion channels in the microbial membrane (Terras et al., 1993; Zhang and Lewis, 1997) or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors (Sharon and Ofek, 1986). The cidal effect exerted by sub-fraction FS: 237-253 might be due to the mechanisms of these components.

Conclusion

This work has investigated the anti-yeast activity of extracts and fractions from the twig, stem, stem bark and leaf of *U. calophyllum.* Results showed that the leaf extract and subsequent fractions possess potent anti-yeast properties, more likely due to the quality of their secondary metabolites contents. Most of the promising extracts, fractions, and sub-fractions demonstrated fungicidal potential, emphasizing their potential as source of anti-yeast agents with innovative mode of action. Meanwhile, to

develop alternative therapies against mycoses based on these starting points, further detailed investigations are required to characterize the active ingredients and study their pharmacological and toxicological properties.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

EZM and FFB designed the study; team composed by EZM, RMTK, CDJM, and PVTF carried out the studies, acquired and analyzed the data, and drafted the manuscript. PTD carried out column chromatography fractionation. TNK provided the fungal isolates from HIV patients of the Yaoundé Central Hospital and revised the manuscript. FFB supervised the work and revised the final draft of the manuscript. All authors read and approved the final manuscript.

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