Research Article

# Antiyeast Activity of Methanolic Extracts and Compounds from *Jacaranda mimosifolia* (D.) Don and *Kigelia africana* (Lam.) Benth

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**ABSTRACT**

The aim of this study was to evaluate the anticandidal activity of fruit, root, stem bark extracts and compounds from *Jacaranda mimosifolia* and *Kigelia africana* (Bignoniaceae) on four *Candida albicans* strains ATCCL26, ATCC12C, ATCCP37039, and ATCCP37037. The extracts from different plants part were prepared using Methanol and Methanol/Methylene Chloride. Column chromatography was used for the fractionation and isolation of compounds. Agar disc diffusion method was used to screen anticandidal activity of the extracts and compounds. Micro dilution method was used to determine the Minimum Inhibitory Concentration of the selected extracts/compounds. The compounds Benzoic acid 1, 5-carboxy-1,2,3,4,4a,7,8,8a-octahydro-1,2,4a-trimethyl-, [1S-(1α,2β,4aβ,8aα)] 2, betulinic acid 3, and ursolic acid 5 were obtained from *Jacaranda mimosifolia*. Also, 2-(4-hydroxyphenyl) ethyl ester 11, oleanolic acid 14, *β*-friedelinol 15 and pomolic acid 16 were obtained from *Kigelia africana*. The inhibition zone diameters varied from 0 to 10 mm on the four tested *C. albicans* strains. The Minimum Inhibitory Concentration ranged from 2.045 to ≥ 8.3 mg/mL. The compounds showed better activity than the extracts. The results of this study suggest that the medicinal plants represent an untapped source of compounds with anti-*C. albicans* activity that could be a resource in the development of new therapeutic natural products.

**Keywords:** Anti yeast activity, Bignoniaceae, Jacaranda mimosifolia, Kigelia Africana.

## INTRODUCTION

The AIDS pandemic has resulted in large numbers of immune compromised patients susceptible to opportunistic fungal infections.[1] These persistent infections have become important causes of morbidity and mortality in immune compromised patients in the last two decades.[2] Most cases of yeast infection result from *Candida albicans*, which is an opportunistic fungus as it does not induce disease in immune competent individuals but only in those with impaired host immune defenses. In Cameroon, North West region *C. albicans* strains are highly prevalent amongst HIV/AIDS patients with 73% causing oral thrush.[3] In the Littoral region, forty (40.8%) subjects had *C. albicans* infection at more than one collection site.[4] The treatment of these infections is limited due to the increased resistance development against available antifungals, the toxicity of currently used drugs, such as amphotericin B which causes hepatotoxicity. Additionally, the cost of effective antifungals plays a vital role in their availability, mainly in developing countries.[5]

For all above reasons, most of the people in rural and urban areas of the world depended on the medicinal plants for the treatment of infectious diseases.[6] Today, there is wide spread interest in drugs derived from plants. The plants of the *Bignoniaceae* family especially *Jacaranda mimosifolia* are used in traditional medicine in different countries to cure wounds, ulcers, and as an astringent in diarrhoea and dysentery.[7-8] *Kigelia africana* (Lam) Benth is commonly used by traditional healers to treat a wide range of skin ailments like, fungal infections, boils, psoriasis and eczema. It is also used in the treatment of dysentery, malaria, diabetes, and pneumonia.[9-11] The medicinal properties of *K. africana* were attributed to the presence of various classes of secondary metabolites among which iridoids, flavonoids, naphthoquinones and volatile constituents.[12-13] Previous investigation of the ethanolic extract of *Kigelia* has been showed to possess antioxidant activity using DPPH test.[14-15] The stem bark and fruit extract showed activity against melanoma and carcinoma cell lines.[11] Extracts of root bark and stem bark exhibited antitrypanosomal activity. Thus, the main objective of this study was to evaluate the anticandidal activity of the fruit, root, stem bark extracts and compounds isolated from *Jacaranda mimosifolia* and *Kigelia africana* (*Bignoniaceae*).

## MATERIALS AND METHODS

### Plant Material

The stem barks and root of *J. mimosifolia* were collected at Melen (February 2010), while the stem bark and fruit of *K. africana* was collected at Dschang (November 2012), Centre and West areas of the Republic of Cameroon, respectively. These two plants were identified by Mr. Victor NANA of the Cameroon National Herbarium (HNC) where voucher specimens of *J. mimosifolia* (N° 50081/HNC) and *K. africana* (N° 157/HNC) were deposited.

### Extraction and isolation

The air-dried stem barks (2 kg) and root (1kg) of *J. mimosifolia* were exhaustively macerated with 12 L and 8 L of mixture $CH_2Cl_2$-MeOH (1:1) at room temperature for 72h respectively. The macerate were filtered through Whatman no.1 filter paper, the solvents were evaporated under reduced pressure in a rotary evaporator at 40ºC to afford dark crude extract (JMB, 300 g) and (JMR, 120 g) respectively.

The air-dried powdered stem barks (1.5 kg) and fruit (200 g) of *Kigelia africana* was separately extracted with MeOH (soxhelet) at 45°C for 3 days. The solvent was concentrated under suction to give dark crude extract (KAB, 160g) and (KAF, 50 g) respectively. Part of crude extract (JMR, 100g) of was exhaustively partitioned with n-hexane, DCM and ethyl acetate to give three fractions (JMR1, 20 g), (JMR2, 35 g) and (JMR3, 55 g) respectively.

Part of crude extract (JMB, 200 g) was treated by solubilizing with AcOEt followed by filtration to give after evaporation under reduce pressure AcOEt extract fraction (JMB1, 75 g). Part of this AcOEt, extract fraction (JMB1, 70 g) was subjected to column chromatography of silica gel 60 (0.063- 0.200 mm) (120 g) using n-hexane, n-hexane - ethyl acetate gradient and ethyl acetate. 150 fractions of 150 mL each were collected and concentrated under vacuum. The fraction JMB1 yielded four compounds: namely benzoic acid 1 (25 mg)[16], 1-naphthaleneacetic acid, 5-carboxy-1,2,3,4,4a,7,8,8a-octahydro-1,2,4a-trimethyl-,[1S-(1α,2β,4aβ,8aα)] 2 (23 mg)[17], betulinic acid 3 (15 mg)[16], lupeol 4 (15 mg)[18] and ursolic acid 5 (3 g)[19], obtained respectively in n-Hex-AcOEt, 93:7 (fraction 18-21), n-Hex-AcOEt, 90:10 (fraction 33-35), n-Hex-AcOEt, 75:25 (fraction 65-69), n-Hex-AcOEt, 70:30 (fraction 80-85) and n-Hex-AcOEt, 60:40 (fraction 110-117).

Part of crude extract (KAB, 140 g) was treated by Solubilizing successively with *n*-Hex-AcOEt, (1:1), AcOEt, and $H_2O$ followed by filtration to give 3 main fractions (KAB1, 35 g), (KAB2, 40 g) and (KAB3, 40 g) respectively. Part of the *n*-Hex-AcOEt fraction (KAB1, 20 g) was subjected to column chromatography of silica gel 60 (0.063- 0.200 mm) (40 g) using n-Hexane, n-Hexane - ethyl acetate gradient and ethyl acetate. 150 fractions of 150 mL each were collected and concentrated under vacuum. The fraction KAB1 yielded four compounds namely lapachol 6 (65 mg)[20- 21], dehydro-α-lapachone 7 (15 mg)[21], 2-acetylfuro-1,4-naphthoquinone 8 (20 mg)[22], *p*-coumaric acid 9 (30 mg)[23] and caffeic acid 10 (29 mg)[23], obtained respectively in n-Hex-AcOEt, 99:1 (fraction 26-33), n-Hex-AcOEt 98:2 (fraction 45-48), n-Hex-AcOEt 90:10 (fraction 70-74), n- Hex- AcOEt 55:45 (fraction 80-85) and (fraction 90-93).

Part of the ethyl acetate fraction (KAB2, 25 g) was applied on a column chromatography of silica gel 60 (0.063 - 0.200 mm) (40 g) using n-Hexane, n-Hexane-ethyl acetate gradient, ethyl acetate and ethyl acetate-methanol (AcOEt -MeOH) gradient. 198 fractions of 150 mL each were collected and concentrated under vacuum. The fraction KAB2 yielded six compounds namely 2-(4-hydroxyphenyl)ethyl ester 11 (25 mg)[24], *β*-sitosterol 12 (35 mg)[25], kigelinol 13 (25 mg)[26], oleanolic acid 14 (35 mg)[19], *β*-friedelinol 15 (20 mg)[27], pomolic acid 16 (15 mg)[28], obtained respectively in n-Hex-AcOEt 95:5 (fraction 26-33), n-Hex-AcOEt 90:10 (fraction 45-50), n-Hex-AcOEt 80:20 (fraction 64-67), n-Hex-AcOEt 80:20 (fraction 73-77), n-Hex-AcOEt 75:25 (fraction 96-100), AcOEt-MeOH 95: 5 (fraction 115-119). Part of aqueous fraction (KAB3, 25 g) was subjected to column chromatography of silica gel 60 (0.063 - 0.200 mm) (40 g) using ethyl acetate and ethyl acetate-methanol gradient. 60 fractions of 150 mL each were collected and concentrated under vacuum. The fraction KAB3 yielded one compounds namely kojic acid 17 (100 mg)[29] obtained in AcOEt-MeOH 90:10 (fraction 30-35).

### General experimental technique

Fractions were monitored by TLC and performed on precoated silica gel 60 F254 plates (Merck, Dramstadt, Germany) and spots were visualized using both ultra-violet light (254 nm and 366 nm) and 10% $H_2SO_4$ spray reagent. The structures of isolated compounds were elucidated by means of spectroscopic experiments mainly 1D and 2D NMR were performed on a 600 MHz Bruker Avance III-600 spectrometer equipped with a 5mm BBFO+ probe at 300K and ESIMS / HRESIMS analyses were recorded on a SYNAPT G2 HDMS (Waters) mass spectrometer and by comparison with literature data.

### Preparation of stock solution of plants crude extracts, fractions and compounds

The different crude extracts, fractions and Compounds were prepared by weighing 20 mg and 2 mg respectively and dissolving them in 1 mL of DMSO 10 % for a final concentration of 20 mg/mL and 2 mg/mL. 500 mg of Nystatin (Novadina Pharmaceutical Ltd) was dissolved in 250 mL of DMSO 10 % for a concentration of 2 mg/mL. After preparation, the different stock solutions were sterilized by heating at 60°C for 30 minutes.

### Microbial cultures

Four *Candida albicans* strains ATCCL26, ATCC12C, ATCCP37039, and ATCCP37037 were obtained from BEI Resources, NAID, NIH. Yeasts were grown and maintained on Sabouraud Dextrose Agar slants at 37°C until used. They were then stored under aerobic conditions. Before any test, yeasts were cultured 48 hours in Sabouraud Dextrose Broth.

### Inoculum preparation

A stock inoculum suspension was prepared from a 2 days old culture on Sabouraud Dextrose Agar at 37°C. The colonies were collected with inoculating loop and introduce in a tube with 5 mL sterile normal saline. The suspension was quantified using the Malassez counting chamber under a microscope and adjusted to 2.5 x $10^4$

cells/ mL using sterile 0.9 % sodium chloride (normal saline) solution.[30]

### Preliminary antiyeast activity by agar diffusion method

Sensitivity of different yeast strains to various extracts was measured in terms of zone of inhibition using agar diffusion assay (ADA).[31] The plates containing Sabouraud Dextrose Agar were spread with 0.1 mL of the inoculum. Discs (6mm diameter) were deposited on agar plates using sterilized pincers with 10µL of the extract/compound. The plates inoculated with different yeasts strains were incubated at 37°C up to 48 hours and diameter of any resultant zone of inhibition was measured. The zones of inhibition of more than 6 mm on all the strains were considered to be sensitive. Nystatin was used as the positive control.

### Determination of Minimal Inhibitory Concentration (MIC) and Minimum Fungicidal Concentrations (MFC)

The Minimum Inhibitory Concentration (MIC) was determined according to National Committee for Clinical laboratory Standards (NCCLS) M27-A3 microdilution method[32] using (12 x 8 wells) microtitre plates. In the well of the first line (1-12), 100 µL of culture medium Sabouraud Dextrose Broth was introduced and 100 µL in the other well of the plates. Later on, 100 µL of stock solution of crude extracts, fractions and compounds were added to the first well. The medium and extract/compound in the first well were mixed thoroughly before transferring 100 µL of the resultant mixture to the well of the second line. Serial two-fold dilutions of the test samples were made and 20 µL of inoculum standardized 2.5 × $10^4$ cells/mL were introduced in the entire well containing the test substances except the column of blank which constitute the sterility control. The concentrations ranged from 0.1296 to 8.3 mg/mL for crude extracts and fractions and 0.01295 to 0.83 mg/mL for compounds. In each microtiter plate, a column with broad-spectrum antibiotic (Nystatin) with the concentration range from 0.01295 mg/mL to 2 mg/mL was used as positive control. After an incubation period of 48 hours at 37° C, turbidity was observed as indication of growth. Thus the lowest concentration inhibiting the growth of yeast was considered as the Minimum Inhibitory Concentration (MIC).

The MFC was determined by transferring 25 µL aliquots of the clear wells into 100 µl of freshly prepared broth medium and incubating at 37°C for 48 hours. The MFC was regarded as the lowest concentration of test sample which did not produce turbidity as above, indicating no microbial growth. All tests were performed in triplicates. The anticandidal effect of extracts, fractions or compounds was evaluated by calculating the ratio of MFC/MIC. If MFC/MIC was ≤ 4, the extracts or compounds was defined as fungicidal, where as if MFC/MIC was > 4, the extract or fraction was defined as fungistatic.[33]

## RESULTS

### Fractionation and isolation of compounds

The fractionation of *J. mimosifolia* and *K. africana* plant extracts by column chromatography lead to isolation and purification of five (1-5) and twelve compounds (6-17) respectively. The structures of the isolated compounds were established using spectroscopic analysis, especially, NMR spectra in conjunction with 2D experiments, COSY, HSQC, HMBC and direct comparison with reference data from available literature. The compounds isolated from stem bark of *J. mimosifolia* and *K. africana* (Figure 1) were terpenoids namely betulinic acid (3), lupeol (4), ursolic acid (5), oleanolic acid (14), *ϐ*-friedelinol (15), pomolic acid (16), quinones identified as lapachol (6), dehydro-α-lapachone(7), 2-acetylfuro-1,4- naphtoquinone (8), kigelinol (13), steroids namely *ϐ*-sitosterol (12), phenolic acids identified as benzoic acid (1), 1-naphthaleneacetic acid, 5-carboxy-1,2,3,4,4a,7,8,8a-octahydro-1,2,4a-trimethyl-[1S-(1α,2β,4aβ,8aα)] (2), *p*-coumaric acid (9), caffeic acid (10), nonacosanoic acid, 2-(4-hydroxyphenyl)ethyl ester (11) and kojic acid (17).

### Anti yeast activity

The Yeast inhibition zone diameters of crude extract, fractions and compounds are summarized in table 1 and table 2. The results of the MIC, MFC and MFC/MIC are represented in tables 3, 4, 5, 6 and 8 below.

The results (table 1 and 2) show that the inhibition zone diameters vary from 0 ± 0.00 -11 ± 0.01 mm on the tested yeasts strains. Ursolic acid 5 and $CH_2Cl_2$-MeOH extract **(**JMR**)** exhibited the highest inhibition with the inhibition zone diameters of 10 ± 0.01 mm on *C. albicans* ATCCL26 and *C. albicans* ATCC12C strains. Kojic acid 17 showed the best diameter zones 9 ± 0.00 mm on *C. albicans* ATCC P37037 and *C. albicans* ATCC 12C . The crude extracts, fractions and compounds having inhibition on the four yeasts strains were selected for the determination of the minimum inhibitory concentration and minimum fungicidal concentration.

The minimum inhibitory concentration ranged from > 0.83 ± 0.00 to > 8.3 ± 0.0 mg/mL on all the tested microorganisms. Amongst the crude extract and fractions, the best activities were observed with $CH_2Cl_2$-MeOH extract **(**JMR**)** on *C. albicans* ATCC P37039 and $CH_2Cl_2$-MeOH extract (JMB) (2.075 ± 0.00 mg/mL) on *C. albicans* ATCCP37039 and *C. albicans* ATCC 12C. MeOH extract **(**KAB**)** and n-Hex-AcOEt 1:1 fraction (KAB1) showed good activity on the tested *C. albicans* ATCC P37037 as shown in (Table 3). Whereas, amongst the compounds isolated from *J. mimosifolia* and *K. africana* plant extracts, the compounds *p*-coumaric acid (9) and kojic acid (17) are most active with the later greater than the first. This activity is better than crude extracts, fractions and Nystatin on the four strains.

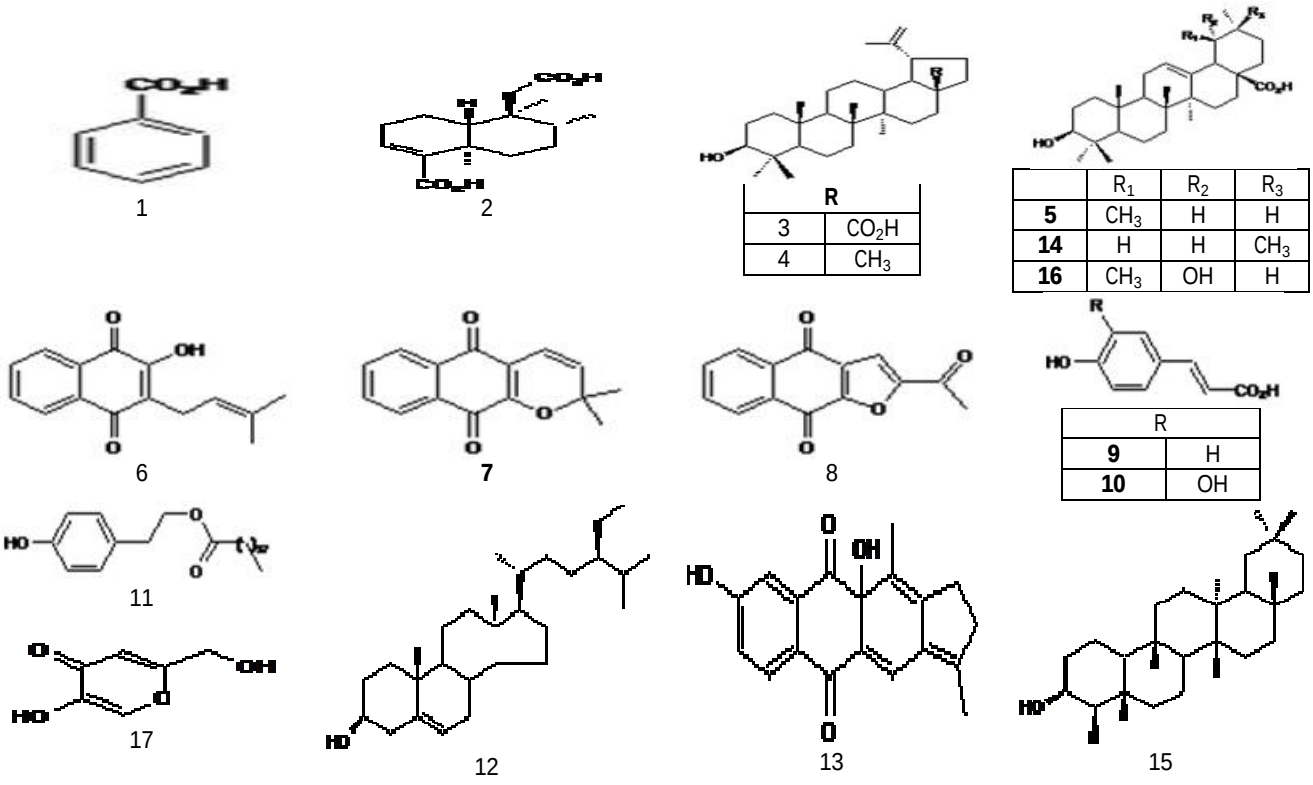


**Figure 1:** Chemical structures of compounds **1–5** isolated from *J. mimosifolia* and **6-17** isolated from *K. africana.*

**Table 1:** Yeast inhibition zone diameters of extracts and fractions from *J. mimosifolia* and *K. Africana*

| Test Microorganism[d] | Inhibition zone diameter ± S.D[a] (mm) | | | |
|---|---|---|---|---|
| | *C.albicans* | | | |
| | ATCCP37037 | ATCCP37039 | ATCCL26 | ATCC 12C |
| **Crude extracts** | | | | |
| JMB[b] | 6 ± 0.00 | 8 ± 0.00 | 7 ± 0.00 | 9 ± 0.00 |
| JMR[b] | 6 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 | 10 ± 0.1 |
| KAB[c] | 6 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 |
| KAF[c] | 0 ±0.00 | 6 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 |
| **Fractions** | | | | |
| JMB1[b] | 8 ± 0.00 | 7 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 |
| JMR1[b] | 8 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 |
| JMR2[b] | 6 ± 0.00 | 0 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 |
| JMR3[b] | 6 ± 0.00 | 0 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 |
| KAB1[c] | 6 ± 0.00 | 7 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 |
| KAB2[c] | 0 ± 0.00 | 6 ± 0.00 | 0 ± 0.00 | 6 ± 0.00 |
| KAB3c | 6 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 | 0 ± 0.00 |
| **Reference** | | | | |
| Nystatin | 10 ± 0.10 | 8 ± 0.00 | 10 ± 0.00 | 8 ± 0.00 |

[a]S.D: Standard deviation of three tests. [b] *J.mimosifolia* extracts JMB, JMR: $CH_2Cl_2$-MeOH extract, JMB1, JMR3: AcOEt extract fraction, JMR1: n-Hexane extract fraction, JMR2: $CH_2Cl_2$ extract fraction ; [c]*K. africana* extracts KAB, KAF: MeOH extract, KAB1: n-Hex-AcOEt 1:1 fraction, KAB2: AcOEt fraction, KAB3: aqueous fraction; [d]Microorganism: *Candida albicans* ATCCP37037, ATCCP37039, ATCCL26, ATCC 12C.

The MFC/MIC ratio (Table 7 and 8) ranged from 1 to 4 and others were not determined. Most extracts, fractions and compounds exhibited fungicidal activity on the tested microorganisms, highlight the ability of these plant extract and compounds to kill the microorganisms.

## DISCUSSION

The two plants chosen for this study are commonly used for treating infectious diseases in herbal therapy and are known to produce a wide range of bioactive compounds; including antimicrobials.[17] The present

study showed that an extract of *J. mimosifolia* and *K. africana* had an antifungal action against all the four strains of *Candida albicans*. Varying level of potency of the extracts and compounds were observed against the tested yeasts strains. On the other hand, these test strains may have different level of intrinsic tolerance to antimicrobials and thus the MIC values differ from strain to strain. The MIC of extracts from *J. mimosifolia* ranged from 2.075 ± 0.00 to 8.3 ± 0.0 mg/mL and that of *K. africana* varied from 4.15 ± 0.00 to 8.3 ± 0.0 mg/mL on the tested strains. The activities observed by *K. africana* could be attributed to the presence of various classes of secondary metabolites among which phenolic compounds, alkaloids, saponins, tannins, anthraquinons, steroids, flavonoids, naphthoquinones and volatile constituents. In fact, amongst the compounds isolated from stem bark of these plants, *p*-coumaric acid (9) and kojic acid (17) exhibited good antifungal properties with the MIC range from 0.415 ± 0.00 to 0.0259 ± 0.00 mg/mL as observed in this study.

**Table 2:** Yeast inhibition zone diameters of compounds isolated from *J. mimosifolia* and *K. africana*

| Test Microorganism[d] | Inhibition zone diameter ± S.D[a] (mm) | | | |
|---|---|---|---|---|
| | *C.albicans* | | | |
| | ATCCP37037 | ATCCP37039 | ATCCL26 | ATCC 12C |
| **Compounds** | | | | |
| 1[b] | 0 ± 0.00 | 8 ± 0.00 | 7 ± 0.00 | 6 ± 0.00 |
| 2[b] | 7± 0.00 | 8 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 |
| 3[b] | 6 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 |
| 4[b] | 6 ±0.00 | 6 ± 0.00 | 6 ± 0.00 | 8± 0.01 |
| 5[b] | 7 ±0.00 | 7± 0.00 | 10 ±0.01 | 0± 0.00 |
| 8[c] | 7 ± 0.00 | 7 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 |
| 9[c] | 6 ± 0.00 | 7 ± 0.00 | 7 ± 0.00 | 6 ± 0.00 |
| 10[c] | 8 ± 0.00 | 7 ± 0.00 | 7 ± 0.00 | 6 ± 0.00 |
| 13[c] | 6 ± 0.00 | 6 ± 0.00 | 6 ± 0.00 | 0 ± 0.00 |
| 15[c] | 7± 0.00 | 8± 0.00 | 7 ± 0.00 | 7 ± 0.00 |
| 16[c] | 0 ± 0.00 | 6 ± 0.00 | 7 ± 0.00 | 7 ± 0.00 |
| 17[c] | 7 ± 0.00 | 9 ± 0.00 | 7± 0.00 | 9 ± 0.00 |
| **Reference** | | | | |
| Nystatin | 10 ± 0.10 | 8 ± 0.00 | 10 ± 0.00 | 8 ± 0.00 |

[a] S.D: Standard deviation of three tests. [b] *J. mimosifolia* compounds: benzoic acid **1,** 1-naphthaleneacetic acid, 5-carboxy-1,2,3,4,4a,7,8,8a-octahydro-1,2,4a-trimethyl-[1S-(1α,2β,4aβ,8aα)] **2** , betulinic acid **3**, lupeol **4** ,ursolic acid **5** ; [c] *K. Africana* compunds :

2- acetylfuro-1, 4-naphthoquinone **8** , *p*-coumaric acid **9**, caffeic acid **10,** , kigelinol **13**, *δ*-friedelinol**15**, pomolic acid **16** and kojic acid **17**; [d] Microorganism: *Candida albicans* ATCCP37037, ATCCP37039, ATCCL26, ATCC 12C

**Table 3:** MIC value of the extracts and fractions from *J. mimosifolia*, *K. Africana*

| Test Microorganism[d] | MIC ± S.D[a] (mg/mL) | | | |
|---|---|---|---|---|
| | *C.albicans* | | | |
| | ATCCP37037 | ATCCP37039 | ATCCL26 | ATCC 12C |
| **Crude extracts** | | | | |
| JMB[b] | 8.3 ± 0.00 | 2.075 ± 0.00 | 4.15 ± 0.00 | 2.075 ± 0.00 |
| JMR[b] | 8.3 ± 0.00 | 2.075 ± 0.00 | 8.3 ± 0.00 | 4.15 ± 0.00 |
| KAB[c] | 4.15 ± 0.0 | 8.30 ± 0.00 | 8.30 ± 0.00 | 8.30 ± 0.00 |
| **Fractions** | | | | |
| JMB1[b] | 8.30 ± 0.00 | 8.30 ± 0.00 | 4.15 ± 0.00 | 8.30 ± 0.00 |
| KAB1[c] | 4.15 ± 0.00 | 4.15 ± 0.00 | 4.15 ± 0.00 | 8.30 ± 0.00 |
| **Reference** | | | | |
| Nystatin | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 |

[a]S.D: Standard deviation of three tests. [b] *J.mimosifolia* extracts JMB, JMR: $CH_2Cl_2$-MeOH extract, JMB1: AcOEt extract fraction, ; [c]*K. africana* extracts KAB: MeOH extract, KAB1: n-Hex-AcOEt 1:1 fraction, [d]Microorganism: *Candida albicans* ATCCP37037, ATCCP37039, ATCCL26, ATCC 12C

**Table 4:** MIC value of the pure compounds **3**, **5**, **9**, **10**, **15** and 17 compounds from *J. mimosifolia*, *K. africana*

| Test Microorganism[d] | MIC ± S.D[a] (mg/mL) | | | |
|---|---|---|---|---|
| | *C.albicans* | | | |
| | ATCCP37037 | ATCCP37039 | ATCCL26 | ATCC 12C |
| **Compounds** | | | | |
| 3[b] | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 |
| 5[b] | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 |
| 9[c] | 0.415 ± 0.00 | 0.2075±0.00 | 0.051875±0.00 | 0.10375 ±0.00 |
| 10[c] | 0.83 ± 0.00 | 0.83 ± 0.00 | 0.415 ± 0.00 | 0.83 ± 0.00 |
| 15[c] | > 0.8 3± 0.00 | > 0.83 ± 0.00 | 0.01295 ± 0.00 | > 0.83 ± 0.00 |
| 17[c] | 0.0259 ± 0.00 | 0.01295 ± 0.00 | 0.01295 ± 0.00 | 0.01295 ±0.00 |
| **Reference** | | | | |
| Nystatin | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 |

[a] S.D: Standard deviation of three tests. [b] *J. mimosifolia* compunds : betulinic acid **3**, ursolic acid **5** ; [d] *K. africana* compounds: : *p*-coumaric acid **9**, caffeic acid **10**, *ß*-friedelinol **15**, pomolic acid **16** and kojic acid **17**; [d] Microorganism: *Candida albicans* ATCCP37037, ATCCP37039, ATCCL26, ATCC 12C

**Table 5:** MFC value of the extracts, fractions from *J. mimosifolia*, *K. Africana*

| Test Microorganism[d] | MFC ± S.D[a] (mg/mL) | | | |
|---|---|---|---|---|
| | *C.albicans* | | | |
| | ATCCP37037 | ATCCP37039 | ATCCL26 | ATCC 12C |
| **Crude extracts** | | | | |
| JMB[b] | 8.3 ± 0.00 | 8.3 ± 0.00 | 8.3 ± 0.00 | 8.3 ± 0.00 |
| JMR[b] | 8.3 ± 0.00 | 8.3 ± 0.00 | 8.3 ± 0.00 | 8.3 ± 0.00 |
| KAB[c] | 8.3 ± 0.00 | 8.3 ± 0.00 | 8.3 ± 0.00 | > 0.83 ± 0.00 |
| **Fractions** | | | | |
| JMB1[b] | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 | 8.3 ± 0.00 |
| KAB1[c] | 8.3 ± 0.00 | 8.3 ± 0.00 | 8.3 ± 0.00 | 8.3 ± 0.00 |
| **Reference** | | | | |
| Nystatin | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 |

[a] S.D: Standard deviation of three tests. [b] *J.mimosifolia* extracts JMB, JMR: $CH_2Cl_2$-MeOH extract, JMB1: AcOEt extract fraction, ; [c] *K. africana* extracts KAB: MeOH extract, KAB1: n-Hex-AcOEt 1:1 fraction, [d] Microorganism: *Candida albicans* ATCCP37037, ATCCP37039, ATCCL26, ATCC 12C

**Table 6:** MFC value of pure compounds 3, 5, 9, 10, 15, and 17

| Test Microorganism[d] | MFC ± S.D[a] (mg/mL) | | | |
|---|---|---|---|---|
| | *C.albicans* | | | |
| | ATCCP37037 | ATCCP37039 | ATCCL26 | ATCC 12C |
| **Compounds** | | | | |
| 3[b] | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 |
| 5[b] | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 |
| 9[c] | > 0.83 ± 0.00 | 0.10375±0.00 | 0.83 ± 0.00 | 0.83 ± 0.00 |
| 10[c] | 0.83 ± 0.00 | 0.83 ± 0.00 | 0.83±0.00 | > 0.83 ± 0.00 |
| 15[c] | > 0.8 3± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 |
| 17[c] | 0.051875±0.00 | 0.10375 ± 0.00 | 0.0259 ± 0.00 | 0.01295 ±0.00 |
| **Reference** | | | | |
| Nystatin | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 | > 0.83 ± 0.00 |

[a] S.D: Standard deviation of three tests. [b] *J. mimosifolia* compunds : betulinic acid **3**, ursolic acid **5** ; [d] *K. africana* compounds: : *p*-coumaric acid **9**, caffeic acid **10**, *ß*-friedelinol **15**, pomolic acid **16** and kojic acid **17**; [d] Microorganism: *Candida albicans* ATCCP37037, ATCCP37039, ATCCL26, ATCC 12C

**Table 7:** MFC/MIC ratio of the extracts, fractions from *J. mimosifolia*, *K. africana*

| Test Microorganism[d] | MFC/MIC | | | |
|---|---|---|---|---|
| | *C.albicans* | | | |
| | ATCCP37037 | ATCCP37039 | ATCCL26 | ATCC 12C |
| **Crude extracts** | | | | |
| JMB[b] | 1 | 4 | 2 | 4 |
| JMR[b] | 1 | 4 | 1 | 2 |
| KAB[c] | 2 | 2 | 1 | nd |
| **Fractions** | | | | |
| JMB1[b] | nd | nd | nd | 1 |
| KAB1[c] | 2 | 2 | 2 | 1 |
| **Reference** | | | | |
| Nystatin | nd | nd | nd | nd |

[a]S.D: Standard deviation of three tests. [b] *J.mimosifolia* extracts JMB, JMR: $CH_2Cl_2$-MeOH extract, JMB1: AcOEt extract fraction; [c] *K. africana* extracts KAB: MeOH extract, KAB1: n-Hex-AcOEt 1:1 fraction, [d] Microorganism: *Candida albicans* ATCCP37037, ATCCP37039, ATCCL26, ATCC 12C

**Table 8:** MFC/MIC ratio of pure compounds 3, 5, 9, 10, 15, and 17

| Test Microorganism[d] | MFC/MIC | | | |
|---|---|---|---|---|
| | *C.albicans* | | | |
| | ATCCP37037 | ATCCP37039 | ATCCL26 | ATCC 12C |
| **Compounds** | | | | |
| 3[b] | nd | nd | nd | nd |
| 5[b] | nd | nd | nd | nd |
| 9[c] | nd | 1 | 1 | 1 |
| 10[c] | 1 | 1 | 1 | nd |
| 15[c] | nd | nd | nd | nd |
| 17[c] | 1 | 1 | 1 | 1 |
| **Reference** | | | | |
| Nystatin | nd | nd | nd | nd |

[a] S.D: Standard deviation of three tests. [b] *J. mimosifolia* compunds : betulinic acid **3**, ursolic acid **5** ; [d] *K. africana* compounds: : *p*-coumaric acid **9**, caffeic acid **10**, *ϕ*-friedelinol **15**, pomolic acid **16** and kojic acid **17**; [d] Microorganism: *Candida albicans* ATCCP37037, ATCCP37039, ATCCL26, ATCC 12C

According to the World Health Organization, infectious diseases are a significant cause of worldwide morbidity with increasing hospitalization rates over time and are associated with substantial mortality, approximately 50 % of all deaths in tropical countries with high economic consequences.[34] Additionally, antimicrobial resistance to antibiotics is emerging as a serious health issue and alternatives to treat infectious diseases in the future need to be developed.[35] A number of studies have voiced the necessity of developing alternative antimicrobial drugs.[36] Plant antimicrobials would appear to be an excellent choice.[31] Our study revealed that, kojic acid (17) isolated in this study produced strong antifungal and may offer prospective new treatments for fungal infections.

In fact, kojic acid is a biologically important natural antimicrobial also produced by various fungal or bacterial strains such as *Aspergillus oryzae*, *Penicillium* or *Acetobacter spp*. in an aerobic process from a wide range of carbon sources.[37-38] It plays an important role in iron-overload diseases such as β-thalassemia or anemia, since it possesses iron chelating activity.[38-40] Also, it forms stable complexes of metal kojates via reaction of kojic acid with metal acetate salts such as tin, beryllium, zinc, copper, nickel, cobalt, iron, manganese, chromium, gold, palladium, indium, gallium, vanadium, and aluminum.[41] They were used as new drugs in the therapy of some diseases such as diabetes, anemia, fungal infections and neoplasia.[38-42] Kojic acid shows a competitive inhibitory effect on monophenolase activity and a mixed inhibitory effect on the diphenolase activity of mushroom tyrosinase. The ability of kojic acid to chelate copper at the active site of the enzyme may well explain the observed competitive inhibitory effect. In addition, kojic acid known as the inhibitor of tyrosinase is an additive to prevent browning of food materials such as crab, shrimp, and fresh vegetables in food industry (e.g., as an antioxidant or antibrowning agent) in order to preserve their freshness and to inhibit discoloration.[43]

The fact that some compounds did not display good antifungal activity does not mean that they may have the same effect *in vivo*; thus, it should be noted that they only demonstrated weak activity *in vitro*. Additionally, weak activity might mean that there are used to treat the symptoms rather than the disease itself.

## CONCLUSION

The good antifungal properties of *J. mimosifolia* and *K. africana* crude extracts and isolated compounds found in this study form a good basis for further pharmacological investigation such as antiyeast evaluation against infectious non *Candida albicans* species, experiments on the toxicity and mechanism of action of these compounds. These results validate the traditional use of these plants in the treatment of infectious diseases Africa.

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