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Prevalence of Vaginitis at the University Hospital Center of Yaounde (CHUY) and Effect of Plant Extracts Combinations and Conventional Antifungals on the Growth of Multidrug-Resistant *Candida albicans*

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Abstract

Background: The spread of vaginal infections in Cameroon and the resistance of the causative pathogens to antimicrobials require regular monitoring to establish new therapies. The objective of this study was to determine the prevalence of vaginitis among pregnant women at the University Hospital Center of Yaoundé (CHUY) and to evaluate the effects of combinations of plant extracts and conventional antifungals on the growth of *Candida albicans* isolates. **Methods:** Cervicovaginal samples collected from women were used to identify the involved pathogens and determination of infection prevalence. The effects of aqueous extracts of *Alchornea cordifolia*, *Antrocaryon klaineanum*, and *Cylicodiscus gabunensis* were then evaluated on the growth of five *Candida albicans* isolates, all resistant to at least three antifungals. This involved determining their sensitivity to the plant extracts and identifying inhibition parameters (MIC and MFC). Subsequently, combinations of plant extracts and plant extracts/conventional antifungals were prepared and tested on the isolates' growth. The determination of the FICI (Fractional Inhibitory Concentration

Index) highlighted the effects of the combinations on *C. albicans*. **Results:** The prevalence of genital infections at CHUY was 70% during the study period, comprising 30% for vulvovaginal candidiasis (VVC) and 40% for bacterial vaginosis. From the samples collected, nine *C. albicans* isolates were obtained, five of which were multi-resistant to at least three antifungals. A high resistance rate was recorded with azoles, notably Fluconazole at 88.88%, compared to 77.77% for Miconazole and Econazole. The plant extracts showed inhibitory properties on the growth of *C. albicans*, with inhibition diameters ranging from 6 to 15 mm. Both the plant extracts and the conventional antifungal (Amphotericin B) inhibited *C. albicans* growth with MIC values ranging from 0.097 to 6.25 mg/mL. The combination of *A. cordifolia* and *A. klaineanum* extracts exhibited a synergistic effect, especially on isolates Cab2, Cab3, and SR ATTCP 37037 (FICI = 0.498; 0.372; and 0.186). The combination of *A. cordifolia* and Amphotericin B also demonstrated a synergistic effect on *C. albicans* isolates Cab3 and Cab8 (FICI = 0.380; 0.505). These results indicate that combinations of *A. cordifolia* and *A. klaineanum*, as well as *A. cordifolia* and Amphotericin B, could be exploited in developing effective drugs against genital infections caused by *C. albicans*.

Keywords

Plant Extracts, Conventional Antifungals, Combinations, Inhibition, *C. albicans*

1. Introduction

A vaginal infection is the presence and abnormal proliferation of micro-organisms in the vagina, causing an inflammation known as vaginitis. Vaginal infections currently constitute a significant public health concern among women in general, and particularly among pregnant women or those of childbearing age [1] [2]. The WHO estimates over 374 million new cases of genital infections occur worldwide each year [3]. They occur in 85 to 90% of cases following infection by fungi belonging to the genus *Candida* [4]. Other causes include bacteria (*Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris, Shigella spp*) and parasites (*Trichomonas vaginalis*) [5] [6]. In Cameroon, studies conducted in the city of Douala on the prevalence of pathogens involved in vaginal infections and risk factors have shown a prevalence of 28% [7]. Others, carried out by Ngaba et *al.* [8] and Nsagha et *al.* [9] in the cities of Douala and Yaounde, showed higher prevalences of around 70.5% and 68.7%, respectively.

The treatment of infections caused by drug-resistant *Candida* faces several major challenges, exacerbated by the scarcity of therapeutic options and the rapid evolution of resistance mechanisms. To date, the management of *C. albicans* infections relies on four available classes of conventional antifungals (azoles, echinocandins, polyenes, and pyrimidines) [4] [9]. Despite the efficacy of these drugs, cases of recurrence are regularly reported. The improper use of anti-infective

agents, combined with the genetic variability of microbes, promotes the emergence of resistance and the development of uncommon infections [10]. Infections caused by resistant *Candida* can be difficult, sometimes impossible to cure, and are on the rise. Meanwhile, research activities aimed at developing effective antimicrobials are particularly lengthy and costly, especially in developing countries [11]. Moreover, aside from their long-term toxicity, conventional drugs can cause numerous side effects. In light of this, it is imperative to seek effective control methods that are especially low-cost, less toxic, and have a broad spectrum of action to offer alternatives to conventional therapy [12]. Indeed, in developing countries, nearly 80% of the rural population relies on medicinal plants for healthcare [13] [14]. These plants offer several advantages due to their active natural compounds; they help prevent and combat fungal infections. Moreover, they are often easy to cultivate or source and can be utilized in various forms (infusions, decoctions, macerations, essential oils, capsules), enabling a natural, personalized, and organism-friendly approach to treatment [15].

Alchornea cordifolia, *Antrocaryon klaineanum* and *Cylicodiscus gabunensis* are African medicinal plants traditionally used in Cameroon either individually or in combination with other plants to treat infectious diseases, including genital infections [16]. Studies have shown that these plants also possess antioxidant and anti-inflammatory properties [17]. Furthermore, research conducted by Adedayo et *al.* [18] demonstrated that combinations of these plants can not only broaden their spectrum of activity but also prove more effective by reducing the required concentration of each extract. These findings could have significant implications for drug development. Further investigation is warranted to evaluate the effects of these plant combinations on resistant pathogens and explore the combination of plant extracts with conventional antifungals in the same context.

This study aims to determine the prevalence of vaginal infections caused by *C. albicans*, evaluate the susceptibility of multidrug-resistant *C. albicans* isolates to combinations of plant extracts of *Alchornea cordifolia*, *Antrocaryon klaineanum* and *Cylicodiscus gabunensis* and reference antifungal agents.

2. Material and Methods

2.1. Determining the Prevalence of Vaginitis

The collection of information related to the study of the prevalence of vaginitis was carried out over a period of five months, from October 2021 to February 2022, during an internship at the Bacteriology Laboratory of the University Hospital Center of Yaounde (CHU-Y). The prevalence was calculated by dividing the number of cases by the size of the exposed population. The target population consisted of pregnant women of all ages who came for gynecological consultation at CHU-Y and exhibited symptoms of a vaginal infection diagnosed by a physician during the consultation. The inclusion criteria were not to be under antibiotic or antifungal treatment during the sampling period and to agree to sign informed consent. In total, 50 pregnant women were selected for sampling.

$$\text{Prevalence} = \frac{\text{Number of cases}}{\text{Study population}} \times 100 \quad (1)$$

2.2. Collection of Plant Material

The barks of *Alchornea cordifolia*, *Antrocaryon klaineanum*, and *Cylicodiscus gabunensis* were collected in the locality of Kometou, Yaounde (Latitude: 4˚03'00" N, Longitude: 11˚33'00" E, Cameroon) for the first plant, and in the locality of Ebolowa (Latitude: 2˚54'59.99" N, Longitude: 11˚08'60.00" E, Cameroon) for the latter two. The collected plant samples were identified at the National Herbarium of Cameroon by comparison with the specimen identification numbers 9657/SRF/Cam, 21574/SRF/Cam, and 1742/SRF/Cam, respectively for *Alchornea cordifolia, Antrocaryon klaineanum*, and *Cylicodiscus gabunensis.*

2.3. Isolation of Pathogenic Candidates

The fungal pathogens consisted of the reference strain *C. albicans* ATCC 37037 and clinical isolates of *Candida albicans* obtained from the sample population of 50 pregnant women suffering from vaginal infections, who came for consultation at CHUY during the period from October 2021 to February 2022.

Cervico-vaginal samples were collected by swabbing according to the method described by Catalan et *al.* [19]. In the gynecological position, under lighting, the speculum was gently inserted in the closed position into the vagina until the fornix, where it was opened in a horizontal position. Swabs of the lateral walls and the posterior fornix of the vagina were taken. A sample was also taken from the endocervix after cleaning the exocervix with a sterile gauze pad. The presence of clinical signs was noted before each sampling.

2.4. Determining and Characterising the Type of Vaginal Flora

The determination of the type of flora was carried out according to the protocol of Donders et *al.* [20]. After collecting the samples, macroscopic examinations consisted of noting the appearance of leucorrhea and the odor of vaginal secretions after the removal of the speculum from the vagina, as well as the characteristics of the leucorrhea (color, smell, and appearance). Regarding microscopic observations, a direct fresh state examination was performed. Indeed, the vaginal secretions were diluted in a few drops of sterile physiological saline and then placed on a clean slide using a Pasteur pipette, while respecting sterilization rules. This slide was covered with a coverslip and examined under an optical microscope at 40x magnification. This examination allows for the detection of possible microorganisms, assessment of their morphology and abundance, observation of their motility, and the search for the presence of yeasts, epithelial cells, polymorphonuclear cells, and red blood cells.

The subsequent Gram staining allowed the bacteria to be colored and distinguished by their ability to retain gentian violet (Gram-positive) or fuchsin (Gram-negative), as well as to identify any imbalance in the vaginal flora. The flora can be classified as type I (abundant flora), type II (normal flora), type III (partially

destroyed flora), or type IV (completely destroyed flora) [21].

2.4.1. Culture of Samples

The culture was performed on the first day of sampling using two swabs. In a plate containing the culture medium, and with the help of one swab from each sample, we inoculated tightly in the first quadrant, moderately in the second, and loosely in the third to obtain isolated colonies, while respecting sterilization precautions. The cervical swab was used to inoculate the EMB (Eosin Methylene Blue) and chocolate agar with polyvitex media, and the swab from the vaginal wall was immediately inoculated onto Sabouraud agar with chloramphenicol and fresh blood agar [20]. For the blood agars (cooked and fresh), incubation was carried out at 37˚C for 24 to 48 hours under anaerobic conditions and in a humid atmosphere. The Sabouraud-chloramphenicol medium was incubated at 37˚C for 24 to 72 hours. Positive cultures on EMB, Chocolate + polyvitex, and blood agar media allowed the diagnosis of bacterial vaginosis, while positive cultures on SDA were used for the diagnosis and macroscopic identification of *Candida* species.

2.4.2. Microscopic Identification of Candidas

The identification of yeasts was performed by naked-eye observation of colonies grown on Sabouraud Dextrose Agar as described by Barantsevich [22]. The appearance of whitish, round, creamy colonies about 2 to 3 mm in size, with a convex elevation and regular contour indicated the presence of *Candida*.

To differentiate *C. albicans* from other Candida species, a filamentation test (germ tube test) was conducted by placing a few Candida colonies collected with a platinum loop into 0.5 mL of human serum. The resulting mixture was incubated at 37˚C for 3 hours. After incubation, a drop of the suspension was observed under a 40x objective between a slide and coverslip. The observation of a germ tube in approximately 50% of the yeasts present was indicative of *C. albicans*. Otherwise, the yeast was concluded to be a *Candida* species.

2.5. Evaluation of the Susceptibility of *C. albicans* to Conventional Antifungal Agents

The antifungal susceptibility test was performed using the diffusion method (Kirby-Bauer method) according to the protocol used by Alexyuk et *al.* [23]. This test allows the assessment of the sensitivity of a yeast to selected antifungal agents.

Inoculum preparation: The inoculum was prepared at a concentration equivalent to the 0.5 McFarland standard. First, a culture was grown on SDA medium and incubated at 37˚C for 24 to 48 hours. The next day, several colonies of similar morphology (if possible) were picked to avoid selecting an atypical variant. The colonies were then suspended in saline medium using a cotton swab and standardized with the 0.5 McFarland standard. The inoculum was finally adjusted by spectrophotometry. The fungal suspension was used optimally within 15 minutes.

Plate inoculation: The fungal inoculum was inoculated using the swabbing technique. This involved dipping a sterile cotton swab into the suspension, then

removing excess liquid by rotating the swab against the tube walls to avoid over-inoculation of the plates. The swab was then spread over the entire surface of the agar in three directions to form tight streaks.

Placement of antifungal disks: Five antifungal disks (Econazole, Miconazole, Fluconazole, Nystatin, Amphotericin B) were firmly placed on the surface of the inoculated agar.

Reading inhibition zone diameters: The edge of the inhibition zone was read with the naked eye, with the plate placed 30 cm from the eye. The inhibition zones were read from the back of the agar plates against a black background illuminated by reflected light. Results were measured by the diameter of the inhibition zone using a caliper. Interpretation of results was described as (S) for sensitive, (I) for intermediate sensitivity, and (R) for resistant.

The isolates of *C. albicans* that showed resistance to at least three ($R \geq 3$) conventional antifungals will be selected to test their susceptibility to plant extracts. The antifungal that demonstrated the best activity after the antifungal susceptibility test will be selected for further testing.

2.6. Preparation of Plant Extracts

Aqueous extracts were prepared according to the following protocol. Indeed, the plants were dried away from the sun for 2 weeks, then ground using a grinder and the various powders were obtained. For each plant the solvent used was water (H_2O). 100 g of powder from each plant material (*Alchornea cordifolia, Antrocaryon klaineanum*, and *Cylicodiscus gabunensis*) were immersed in 1000 mL of boiling water for 4 hours until exhaustion. The resulting decoction was left to rest to cool, then filtered using a 0.23 mm pore-size filter. The filtrate was then left to rest for 24 hours and subsequently dried in drying oven at 60˚C for 72 hours. The obtained dry extracts were weighed, labeled, and stored in a refrigerator at 4˚C. The extraction yield was determined according to the following formula (2):

$$\text{Yield}(\%) = \frac{\text{Extract mass obtained}}{\text{Mass of plant material}} \times 100 \quad (3)$$

2.7. Evaluation of the Antifungal Potential of Plant Extracts and Amphotericin B on *C. albicans* Using the Well Diffusion Method

The susceptibility of *C. albicans* isolates and strains was evaluated using the well diffusion method according to the CLSI [24]. The strains were sub cultured by the streak method on Sabouraud Dextrose Agar (SDA) to obtain pure and fresh colonies aged 48 hours. Then, isolated colonies were collected using a sealed Pasteur pipette and suspended in 5 mL of distilled water, adjusted to a density equivalent to the 0.5 McFarland standard, corresponding to a load of 10^8 cells/mL. Stock solutions of the extracts were prepared at a concentration of 100 mg/mL by dissolving the different extracts in 1 mL of distilled water to obtain the stock extract solution at 100 mg/mL. The stock solution of Amphotericin B was prepared by dissolving a 100 mg tablet in 100 mL of distilled water and making the appropriate

dilution to reach a concentration of 1 mg/mL.

After the media solidified, Petri dishes were first inoculated by swabbing with the inoculum of the different previously prepared strains. Then, wells of 6 mm diameter were made using a punch. Finally, a volume of 75 µL of the test extract solutions was introduced into each well. The inoculated Petri dishes were left for 15 minutes (pre-diffusion) under the hood and then incubated at 37˚C for 48 hours. After incubation, the inhibition zone diameters were measured, and the sensitivity of each strain to the extracts was classified by the diameter of the inhibition zones according to the following scale: Resistant (R) for a diameter less than 6 mm, Intermediate (I) for a diameter between 6 and 13 mm, Sensitive (S) for a diameter greater than 13 mm.

2.8. Evaluation of the Antifungal Potential of Plant Extracts and Amphotericin B on *C. albicans* Using the Well Dilution Method

The determination of the inhibition parameters of plant extracts on strains and isolates of *C. albicans* was carried out using the broth dilution method described by CLSI [24], with some modifications. To do this, in each well of a 96-well microplate, a volume of 100 µL of Sabouraud Dextrose Broth (SDB) liquid medium was added. Then, 100 µL of the stock solution of the extract to be tested or Amphotericin B (used as the reference antifungal) was added into the first four wells of the first column (rows A, B, C, and D). After mixing, a two-fold serial dilution was performed up to the eleventh well (starting from wells A, B, C, and D) by transferring 100 µL from the previous well to the next well after homogenization. This resulted in a concentration range of the extract from 25 to 0.0244 mg/mL for extracts of *A. cordifolia, C. gabunensis, A. klaineanum*, and from 250 to 0.0244 mg/mL for Amphotericin B. Finally, 100 µL of fungal inoculum with a density equivalent to the 0.5 McFarland standard (10^8 cells/mL) was added to each well. The final volume was 200 µL per well, and all tests were performed in triplicate. One row of the microplate was used as a negative control for the activity of the extracts, containing only the culture medium and the extracts at different concentrations. Additionally, some wells in the column containing only the culture medium and the inoculum were used as positive controls for microbial growth. The microplate was covered with its lid, sealed with cling film, and incubated at room temperature for 24 to 48 hours. Microbial growth was detected by adding 20 µL of Alamar Blue solution to the test wells, followed by incubation for 30 minutes. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of extract or Amphotericin B at which no visible microbial growth was observed with the naked eye. Growth was characterized by a color change from blue to red, and the absence of growth was indicated by the maintenance of the blue color.

For the determination of the minimum fungicidal concentration (MFC), 50 µL from each well with a concentration equal to the MIC was taken and added to 150 µL of Sabouraud broth. The mixture was incubated under the same conditions as for the MIC determination and revealed with Alamar Blue.

The fungicidal activity was evaluated according to the scale, which consisted of calculating the MFC/MIC ratios: if MFC/MIC < 4, the substance is fungicidal; if 4 ≤ MFC/MIC ≤ 16, the substance is fungistatic; and if MFC/MIC > 16, the substance is tolerant.

2.9. Study of Antifungal Effect of Plant Extracts and Amphotericin B Combinations on *C. albicans* Using the Checkerboard Method

The effect of combinations of plant extracts and Amphotericin B on strains and isolates of C. albicans was evaluated using the checkerboard method as described by Liu et *al.* [25]. For this, we formulated three pairs of combinations of extracts from *A. cordifolia*, *A. klaineanum*, *C. gabunensis*, and Amphotericin B in volume-to-volume proportions. The combinations tested were: *A. cordifolia-A. klaineanum*, *A. cordifolia-C. gabunensis*, and *C. gabunensis-A. klaineanum*. The plant extracts whose combination demonstrated the best activity were selected and combined with the reference antifungal (Amphotericin B) following the same protocol to evaluate their efficacy.

To do this, 50 µL of serial two-fold decreasing concentrations of substance A were added vertically, and 50 µL of serial two-fold decreasing concentrations of substance B were added horizontally so that each well contained a 50 µL cross of a concentration from the dilution range of each substance. Then, 100 µL of fungal inoculum at 10^8 CFU/mL was added to all wells. The MIC of each substance alone was determined in parallel, where 50 µL of the same dilution series received 50 µL of Sabouraud Dextrose Broth (SDB) and were inoculated with 100 µL of the fungal suspension. The 96-well microplate was then sealed and incubated at 37˚C for 18 - 24 hours. After incubation, 20 µL of Alamar Blue was added to each well to assess fungal growth and incubated at 37˚C for 30 minutes.

The MIC values of the extracts and their combinations allowed calculation of the fractional inhibitory concentrations (FIC) according to the following formulas (3):

$$FICI = FIC1 + FIC2 \quad (3)$$

FIC A = MIC of substance A in combination/MIC of substance A alone.

FIC B = MIC of substance B in combination/MIC of substance B alone.

According to Zainol et *al.* [26], the effects of combinations of antimicrobial substances are classified as: Synergistic if the sum of the FICI index or FICI ≤ 0.5; Additive if 0.6 ≤ FICI ≤ 1; Indifferent if 1 < FICI ≤ 4; Antagonistic if FICI > 4.

2.10. Statistical Analysis

Data regarding the type of genital infection, the distribution of flora, and the susceptibility of *C. albicans* to conventional antifungal drugs were entered into an Excel spreadsheet, and figures were generated accordingly. Concerning the effect of plant extracts on the growth of *C. albicans*, the experiment was performed in triplicate and the data were presented as mean standard deviation (±SD). Duncan's method was used to evaluate the significant differences between the data, and statistically

significant differences were set at $p < 0.05$. Graph of this part were performed with GraphPad Prism version 10.3.1 (GraphPad Software, Boston, MA, USA).

3. Results

3.1. Prevalence and Type of Genital Infection in the Study Population

Out of 50 PVC samples analyzed, 15 were diagnosed negative for infectious vulvovaginitis, while 35 were positive. This results in a frequency of infectious vulvovaginitis or genital infection in the study population of 70%. Infectious vulvovaginitis was then distributed according to the nature (bacterial, fungal, and parasitic) of each causative agent, and the results obtained, expressed as a percentage distribution, are shown in Figure 1 below. From this figure, it is noted that no cases of vaginal parasitosis (due to *Trichomonas vaginalis*) were observed, 30% of cases were vulvovaginal candidiasis (due to *Candida spp.* and *C. albicans*), and 40% were bacterial vaginosis (due to *Gardnerella vaginalis*).

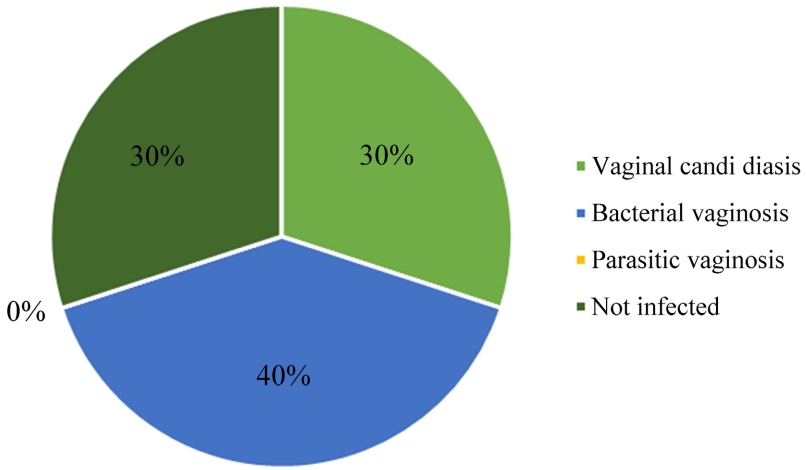

Figure 1. Distribution of the type of genital infection in population.

3.2. Distribution of the Type of Vaginal Flora According to the Types of Associated Infections

Out of a total of 50 women consulted in the context of this study, the analysis of the vaginal flora made it possible to establish the distribution of flora types according to the type of infection in the patients (Figure 2). It appears that, regarding cases of bacterial vaginosis, the majority (15 out of 18) of the patients present a disrupted vaginal flora (type IV). On the other hand, among patients with vaginal candidiasis as well as those who are not infected, the normal vaginal flora (type II), with a total of 10 out of 15 and 12 out of 15 respectively, is the most represented.

3.3. Distribution and Specification of Isolated Yeast Species

Figure 3 below shows the distribution of vulvovaginal candidiasis (VVC) accord-

ing to the Candida species involved. This figure reveals that, out of 15 isolates obtained, *C. albicans* was the species most frequently responsible for VVC, with a frequency rate of 60% (*i.e.*, 9/15 isolates).

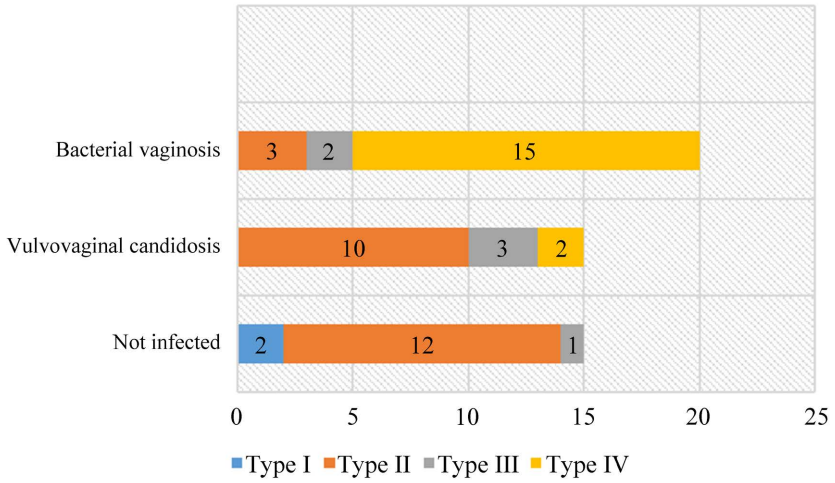

Figure 2. Distribution of flora type according to type of infection in patients.

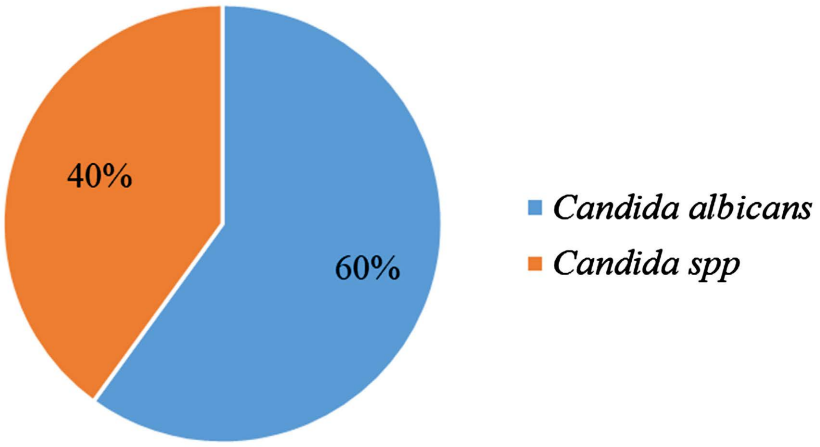

Figure 3. Breakdown of yeasts isolated by species.

3.4. Susceptibility Profile of *C. ablicans* Isolates to Conventional Antifungal Agents

The antifungigram test was performed with five conventional antifungals (Econazole, Miconazole, Fluconazole, Nystatin, Amphotericin B) on the nine *C. albicans* isolates obtained. Table 1 presents the sensitivity and resistance profile of the Candida albicans isolates to the five antifungals tested. This table shows that 8 *C. albicans* isolates were resistant to at least 1 conventional antifungals and are therefore multidrug-resistant. Isolates Cab2 and Cab8 were resistant to 4 antifungals; Cab3, Cab5, and Cab9 showed resistance to three antifungals; Cab4, Cab6, and Cab7 were resistant to two antifungals; and finally, isolate Cab1 was resistant to one antifungal.

Table 1. Sensitivity profile of different isolates of *C. albicans* to the five antifungal drugs tested.

Isolates	Species	Conventional antifungals					Total resistance
		Amphotericin B	Econazol	Fluconazol	Miconazol	Nystatin	
*Cab*1	*C. albicans*	S	S	R	S	S	**1**
*Cab*2	*C. albicans*	S	R	R	R	R	**4**
*Cab*3	*C. albicans*	S	R	R	R	S	**3**
*Cab*4	*C. albicans*	S	R	S	R	S	**2**
*Cab*5	*C. albicans*	S	R	R	R	S	**3**
*Cab*6	*C. albicans*	S	R	R	S	S	**2**
*Cab*7	*C. albicans*	S	S	R	R	S	**2**
*Cab*8	*C. albicans*	S	R	R	R	R	**4**
*Cab*9	*C. albicans*	S	R	R	R	S	**3**

R: Resistant; **S**: Sensitive ; ***Cab***: *C. ablicans* isolate.

Isolates of *C. ablicans* showing resistance to at least three conventional antifungal agents were selected to test their succeptibility to plant extracts. The histogram in Figure 4 below shows the results expressed as percentage sensitivity for each conventional antifungal on the 9 isolates after antifungigram testing. An analysis of this figure reveals that the sensitivity of *Candida* isolates varies depending on the different antifungals. Amphotericin B and Nystatin had the highest spectra of action with sensitivities of 100% and 77.77%, respectively. They were followed by Econazole and Miconazole, which demonstrated a sensitivity rate of 22.23%. Except for Cab4, all other isolates were resistant to Fluconazole, which therefore showed a low sensitivity (11.11%). In terms of intermediate sensitivity, we obtained 0% with all the antifungals tested. Regarding the resistance rate, Amphotericin B recorded 0%, Nystatin 22.23%, Econazole and Miconazole 77.78%, and Fluconazole 88.88%.

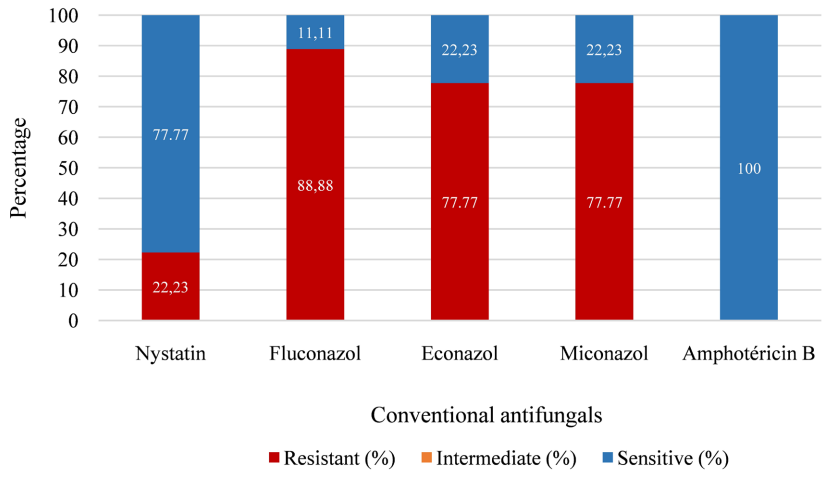

Figure 4. Distribution of *C. albicans* according to their susceptibility to conventional antifungal drugs.

3.5. Yields Extracts of Plants

The extraction yield of plants was 28.41 % for *A. cordifolia*, 12.65% for *A. klaineanum* and 8.3% for *C. gabunensis* (Table 2).

Table 2. Extraction yield of differents plants.

Yields extracts	*A. cordifolia*	*A. klaineanum*	*C. gabunensis*
	28.41%	**12.65%**	**8.3%**

3.6. Anti-Candida Activity of Individual Plant Extracts and Amphotericin B

The antifungal potential of *Alchornea cordifolia, Antrocaryon klaineanum, Cylicodiscus gabunensis*, and Amphotericin B was evaluated on five multidrug-resistant *Candida albicans* isolates (Cab2, Cab3, Cab5, Cab8, and Cab9) and on the reference strain *C. albicans* ATTCP 37037. Figure 5 below shows the inhibition diameters (mm) of the substances tested on six *C. albicans* yeasts. This figure reveals that the inhibition diameters generated by the plant extracts vary depending on the plant extract tested. At the tested concentration of 100 mg/mL, Amphotericin B was statistical more active than all the plant extracts ($p < 0.05$ according to Duncan's test). Also, the extract of *A. cordifolia* was statisticaly more active ($p < 0.05$) than the other extracts, with inhibition diameters of 14.7 mm on Cab2, 13.5 mm on Cab3, 13.8 mm on Cab5, 14.6 mm on Cab8, 13.2 mm on Cab9, and 13.5 mm on the reference strain *C. albicans* ATTCP 37037. This was followed by the extract of *A. klaineanum*, which showed an average inhibition diameter on all *C. albicans* isolates of 8.63 mm. Finally, the extract of *C. gabunensis* demonstrated an average inhibition diameter of 6.4 mm on all *C. albicans* isolates. From these observations, it can be concluded that all *C. albicans* isolates were sensitive

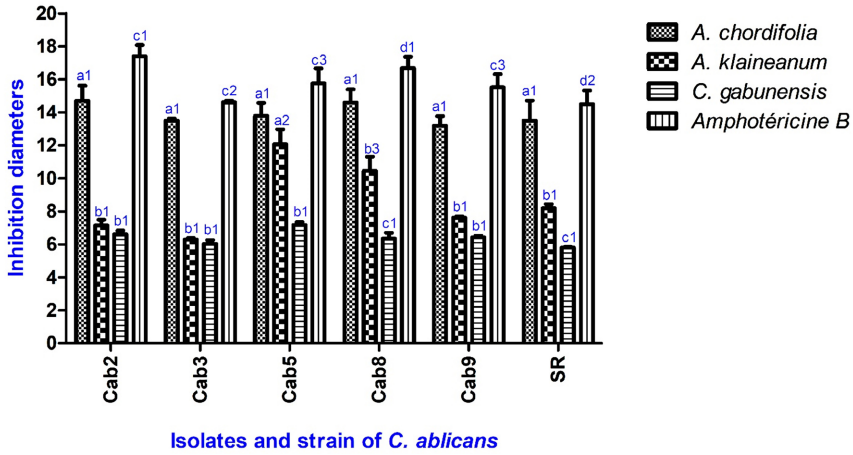

Figure 5. Effect of plant extracts on the growth (diameters in mm) of 6 *C. albicans* yeasts. For the same isolate and different plant extract activity, the histograms bearing different letters (a, b, c, d) present a significant difference at $p \leq 0.05$. For the same plant extract for different isolates, the histograms with different letters (1, 2, 3) are significantly different at $p \leq 0.05$ according to Duncan's text.

to the extract of *A. cordifolia* (inhibition diameter > 13 mm) and showed intermediate sensitivity to the extracts of *A. klaineanum* and *C. gabunensis* (6 < inhibition diameter < 13 mm).

3.7. MIC and CMF of Extracts on the Growth of *C. albicans* Isolates

The MICs and MFCs were determined by microdilution, and the results are recorded in Table 3 below. An analysis of this table shows that the MICs and MFCs vary from one extract to another and depending on the isolates. The MICs of the *A. cordifolea* extract were 0.39 mg/mL for isolate Cab5, 0.781 mg/mL for isolates Cab2, Cab3, Cab8, Cab9, and 1.56 mg/mL for the reference strain *C. ablicans* ATCC 37037. *A. klaieneanum* extract showed MICs ranging from 0.390 to 1.56 mg/mL on the growth of the strains. Also, the *C. gabunensis* extract presented MICs between 0.195 and 6.250 mg/mL. Regarding the MFCs, they ranged from 1.56 to 3.125 mg/mL for the *A. cordifolea* extract, from 6.250 to 12.5 mg/mL for *A. klaieneanum* extract, and 3.125 mg/mL for the *C. gabunensis* extract. The obtained MICs and MFCs allowed the calculation of the MFC/MIC ratio to determine the nature of inhibition of each extract (fungicidal, fungistatic, or tolerant). In this results, *A. cordifolea* extract demonstrated fungicidal activity on four isolates (Cab2, Cab8, Cab9, and ATCC 37037) (MFC/MIC < 4) and fungistatic activity on two isolates (Cab3 and Cab5) (4 ≤ MFC/MIC ≤ 16). Furthermore, *A. klaieneanum* extract showed fungistatic activity on Cab2, Cab8, and ATCC 37037. *C. gabunensis* extract, it exhibited tolerant activity on Cab8 (MFC/MIC > 16) and fungistatic activity on ATCC 37037. Amphotericin B showed fungicidal activity on Cab2 and Cab8, and fungistatic activity on Cab5, Cab9, and ATCC 37037.

Table 3. Inhibition parameters of plant extracts on clinical isolates of *C. ablicans*.

		Plant extracts			Antifungal
Isolates	**Parameters**	***A. cordifolia***	***A. klaieneanum***	***C. Gabunensis***	**Amphotéricine B**
Cab2	MIC (mg/ml)	0.781	0.781	3.125	**0.0156**
	MFC (mg/ml)	1.562	6.250	Nd	**0.0312**
	MFC/MIC	2	8	Nd	**2**
	Activity	**Fungicide**	**Fungistatic**	**Nd**	**Fungicide**
Cab3	MIC (mg/ml)	0.781	0.390	1.562	**0.0156**
	MFC (mg/ml)	3.125	12.50	Nd	**0.0312**
	MFC/MIC	4	32	Nd	**Nd**
	Activity	**Fungistatic**	**Tolerant**	**Nd**	**Nd**
Cab5	MIC (mg/ml)	0.390	0.390	0.781	**0.0039**
	MFC (mg/ml)	3.125	6.250	Nd	**0.0625**
	MFC/MIC	8	16	Nd	**16**
	Activity	**Fungistatic**	**Fungistatic**	**Nd**	**Fungistatic**

Continued

Cab8	**MIC (mg/ml)**	**0.781**	**0.781**	**0.195**	**0.0156**
	MFC (mg/ml)	1.562	Nd	3.125	**0.0312**
	MFC/MIC	2	Nd	32	**2**
	Activity	**Fungicide**	**Nd**	**Tolerant**	**Fungicide**
Cab9	MIC (mg/ml)	0.781	1.562	6.250	**0.0078**
	MFC (mg/ml)	1.562	Nd	Nd	**0.0625**
	MFC/MIC	2	Nd	Nd	**8**
	Activity	**Fungicide**	**Nd**	**Nd**	**Fungistatic**
SR	MIC (mg/ml)	1.562	1.562	0.781	**0.0039**
	MFC (mg/ml)	3.125	12.5	3.125	**0.0625**
	MFC/MIC	2	8	2	**16**
	Activity	**Fungicide**	**Fungistatic**	**Fungistatic**	**Fungistatic**

Legend: Nd: Not determined, MIC: Minimum Inhibitory Concentration, MFC: Minimum Fungal Concentration, Cab: *C. ablicans* isolate, SR: souche de référence ATTCP 37037.

3.8. Effect of Combinations of Different Plant Extracts and Amphotericin B on the Growth of *C. ablicans*

The effect of combinations of plant extracts *A. cordifolia*-*A. klaineanum*, *A. cordifolia*-*C. gabunensis* and *C. gabunensis*-*A. klaineanum* on isolates and strains of *C. albicans* is shown in Table 4. This table groups their individual MICs, the MICs of the combinations, the Fractional Inhibitory Concentrations (FIC), and the Fractional Inhibitory Concentration Indexes (FICI).

It appears from this table that the combination of *A. cordifolia*-*A. klaineanum* extract (Ac + Ak/v:v) demonstrated a synergistic effect on Cab2, Cab3, and SR ATTCP 37037 with respective FICI values of 0.498, 0.372, and 0.186; an additive effect on Cab5 and Cab8 (FICI = 0.748); and an indifferent effect on Cab9 (FICI = 1.499). Regarding the combination *A. cordifolia*-*C. gabunensis* (Ac + Cg/v:v), it showed a synergistic effect on SR ATTCP 37037 (FICI = 0.498); an additive effect on Cab2, Cab5, and Cab9 (FICI = 0.998, 0.747, and 0.747 respectively); and an indifferent effect on isolates Cab3 (FICI = 2.249) and Cab8 (FICI = 1.499). The combination *C. gabunensis*-*A. klaineanum* (Cg + Ak/v:v) presented an additive effect on Cab3 (FICI = 1.000); an indifferent effect on Cab8, Cab9, and SR ATTCP 37037 (FICI = 2.499, 2.500, and 2.249 respectively); and an antagonistic effect on Cab2 and Cab5 with FICI values of 5.001 and 5.005 respectively.

The plant extracts of *A. cordifolia* and *A. klaineanum* proved to be the most active, both in antifungal tests of individual extracts and in combination. These two plants were each combined with a reference antifungal (Amphotericin B) and tested on isolates and strains of *C. albicans*. The results of this test are presented in Table 5. It emerges from this table that the combination *A. cordifolia*-Ampho-

tericin B (Ac + AmB/v:v) demonstrated a synergistic effect on Cab3 and Cab8 (FICI: 0.380 and 0.505 respectively); a additive effect on Cab2 and Cab9 (FICI: 0.761 and 0.636); a indifferent effect on SR ATTCP 37037 (FICI: 1.275); and a antagonistic effect on Cab5 (FICI: 4.602). The combination *A. klaineanum*-Amphotericin B (Ak + AmB/v:v) showed an additive effect on SR ATTCP 37037 with a FICI value of 0.637; an indifferent effect on Cab2, Cab3, Cab8, and Cab9 (FICI: 1.274, 2.256, 2.512, and 1.512 respectively). From these results, it appears that the combination *A. cordifolia*-*A. klaineanum* was more active than all the extract–extract plant combinations. Also, the combination A. cordifolia-Amphotericin B proved more effective than the combination *A. klaineanum*-Amphotericin B. Furthermore, all combinations demonstrated better anticandidasis activity than the individual plant extracts. The sensitivity of the isolates and strains of *C. albicans* to the different combinations varied from one isolate to another and also depending on the type of combination.

Table 4. Interactions of plant extract-plant extract combinations on isolates and strains of *C. ablicans*.

Isolates	plants extracts	MIC_{ES}	Ac +Ak (v:v)				Ac + Cg (v:v)				Cg + Ak (v:v)			
			MICco	FCI	**FICI**	**EFFECT**	MICco	FCI	**FICI**	**EFFECT**	MICco	FCI	**FICI**	**EFFECT**
	Ac	0.781	0.195	0.249			0.390	0.499			nd			
Cab2	Ak	0.781	0.195	0.249	**0.498**	**S**	nd		**0.998**	**A**	3.125	4.001	**5.001**	**An**
	Cg	3.125	nd				1.562	0.499			3.125	1.000		
	Ac	0.781	0.097	0.124			0.195	0.249			nd			
Cab3	Ak	0.390	0.097	0.248	**0.372**	**S**	nd		**2.249**	**I**	0.195	0.5	**1.000**	**A**
	Cg	1.562	nd				3.125	2.00			0.781	0.5		
	Ac	0.390	0.195	0.500			0.097	0.248			nd			
Cab5	Ak	0.390	0.097	0.248	**0.748**	**A**	nd		**0.747**	**A**	1.562	4.005	**5.005**	**An**
	Cg	0.781	nd				0.390	0.499			0.781	1.000		
	Ac	0.781	0.195	0.249			0.390	0.499			nd			
Cab8	Ak	0.781	0.390	0.499	**0.748**	**A**	nd		**1.499**	**I**	0.390	0.499	**2.499**	**I**
	Cg	0.195	nd				0.195	1.000			0.390	2.000		
	Ac	0.781	0.390	0.499			0.195	0.249			nd			
Cab9	Ak	1.562	1.562	1.00	**1.499**	**I**	nd		**0.749**	**A**	3.125	2.000	**2.500**	**I**
	Cg	6.25	nd				3.125	0.5			3.125	0.500		
	Ac	1.562	0.097	0.062			0.390	0.249			nd			
SR	Ak	1.562	0.195	0.124	**0.186**	**S**	nd		**0.498**	**S**	3.125	2.000	**2.249**	**I**
	Cg	0.781	nd				0.195	0.249			0.195	0.249		

Legend: Ac: *A. cordifolia*; Ak: *A. klaineanum*; Cg: *C. gabunensis*; SR: Reference strain ATTCP 37037; Cab: *C. ablicans* Isolate; S: Synergy; I: Indifferent; A: Additive; An: Antagonist; FCI: Fractional Inhibitory Concentration; FICI: Fractional Inhibitory Concentration Index; FIC: Fractional Inhibitory Concentration; MIC_{IE}: MIC of individual extracts; MICco: MIC of combinations.

Table 5. Effect of plants extracts and Amphotericin combinations on *C. ablicans.*

Isolates	Substance	CMI_{IS}	Ac + AmB (v:v)				Ak + AmB (v:v)			
			MICco	FCI	FICI	EFFECT	MICco	FCI	FICI	EFFECT
	Ac	0.781	0.195	0.249			nd			
Cab2	Ak	0.781	nd		**0.761**	**A**	0.195	0.249	**1.274**	**I**
	AmB	0.0156	0.008	0.512			0.016	1.025		
	Ac	0.781	0.097	0.124			nd			
Cab3	Ak	0.390	nd		**0.380**	**S**	0.781	2.002	**2.258**	**I**
	AmB	0.0156	0.004	0.256			0.004	0.256		
	Ac	0.390	0.195	0.500			nd			
Cab5	Ak	0.390	nd		**4.602**	**An**	0.390	1.000	**5.102**	**An**
	AmB	0.0039	0.016	4.102			0.016	4.102		
	Ac	0.781	0.195	0.249			nd			
Cab8	Ak	0.781	nd		**0.505**	**S**	1.562	2.000	**2.512**	**I**
	AmB	0.0156	0.004	0.256			0.008	0.512		
	Ac	0.781	0.0975	0.124			nd			
Cab9	Ak	1.560	nd		**0.636**	**A**	1.562	1.000	**1.512**	**I**
	AmB	0.0078	0.004	0.512			0.004	0.512		
	Ac	1.560	0.390	0.250			nd			
SR	Ak	1.560	nd		**1.275**	**I**	0.195	0.125	**0.637**	**A**
	AmB	0.0039	0.004	1.025			0.002	0.512		

Legend: Ac: *A. cordifolia*; Ak: *A. klaineanum*; AmB: Amphotéricine B; SR: SR: Reference strain ATTCP 37037; Cab: *C. ablicans* Isolate; S: Synergy; I: Indifferent; A: Additive; An: Antagonist; FCI: Fractional Inhibitory Concentration; FICI: Fractional Inhibitory Concentration Index; FIC: Fractional Inhibitory Concentration; MIC_{IE}: MIC of individual extracts; MICco: MIC of combinations.

4. Discussion

Cervicovaginal swabs (CVS) were obtained from women attending gynecological consultations at CHUY. These samples enabled the identification of isolates responsible for genital infections. The isolates obtained were categorized according to the type of infectious vulvovaginitis. Thus, fifteen CVS samples were diagnosed as negative for infectious vulvovaginitis, while 35 were positive. The prevalence of infectious vulvovaginitis or genital infection in the population was 70%, comprising 40% bacterial vaginosis (BV), 30% vulvovaginal candidiasis (VVC), and 0% vaginal parasitosis (VP) due to *Trichomonas vaginalis*. This prevalence of bacterial vaginosis closely aligns with that reported in Cameroon (42%) by Koueke [27]. The prevalence of vulvovaginal candidiasis obtained is similar to studies conducted in Tunisia (36.39%) also in Cameroon respectly by Anane et *al.* [28] and Okalla et *al.* [7] in Cameroon (32%). The prevalence of parasitic infections (0%) in this study differs from data reported by Tibaldi et *al.* [29] in Turin, where trichomoniasis accounted for 1.6%. Conversely, Okalla et *al.* [7] found, among 300 pa-

tients, 40.46% bacterial-origin vaginal infections and 51.45% fungal-origin infections, predominantly due to *Candida albicans* (86.46%). Furthermore, a study conducted in Pennsylvania, USA, on the effect of reusing vaginal contraceptives on vaginal microflora and infection risk identified three species among the pathogens: *Candida albicans, Escherichia coli* and *Staphylococcus aureus* [30]. These differences in prevalence may be explained by variations in the studied populations, the number of vaginal samples collected, and the sociodemographic conditions of the different study locations. The predominance of bacterial vaginosis among these infectious vulvovaginitides could be related to the fact that bacteria possess more virulence factors compared to yeasts to *Candida* genus. Moreover, the vaginal environment is not a selective medium for parasites, which accounts for the low rate of vaginal parasitosis observed.

In our study, 60% of yeasts involved in vulvovaginal candidiasis (VVC) were *Candida albicans*. This high involvement of *C. albicans* in VVC could be explained by the strong adhesion of *C. albicans* to the vaginal mucosa, facilitated by the presence of vaginal cellular receptors for the *Candida* ligand. This interaction allows the expression of its virulence factors, germination and transformation from the saprophytic state in the form of blastospores to the pathogenic filamentous form. The distribution of different infectious vulvovaginitis types according to the flora type highlighted an imbalance in the vaginal flora. Type II flora was predominantly found with a rate of 66.66% in VVC, which contradicts the studies conducted by Sylla et *al.* [31] in Dakar, where type III flora was predominant at 43.8%. This result supports the hypothesis that candidosis vaginosis is associated with an imbalance and destruction of the vaginal flora.

Of the 9 *Candida albicans* isolates obtained, 5 were multidrug-resistant (resistant to at least three antifungal agents), namely Cab2, Cab3, Cab5, Cab8, and Cab9. These isolates were sensitive to Amphotericin B and Nystatin at rates of 100% and 77.77%, respectively, followed by Econazole and Miconazole, which showed sensitivity rates of 22.22%. Regarding resistance rates, Fluconazole led with 88.88%, followed by Econazole and Miconazole at 88.88%, then Nystatin at 22.23%, while Amphotericin B showed 0% resistance. These results are comparable to those reported by Kpongbo et *al.* [32] in Côte d'Ivoire, who demonstrated 100% sensitivity to Amphotericin B, as well as those of Badiee et *al.* [33] with 98.6% sensitivity to Nystatin. Conversely, these findings do not corroborate the work of de Gonsu et *al.* [34] in Cameroon, who reported a 99% sensitivity rate of *Candida* to Miconazole and a 95% resistance rate to Amphotericin B. This discrepancy may be related to the use of different diagnostic methods. The high resistance rates observed for Fluconazole and Miconazole could be attributed to their long-standing use and widespread self-medication among Cameroonian populations. A study conducted in the intensive care unit of CHU Grenoble between 2004 and 2009 showed that prior antifungal consumption altered the epidemiology and antifungal susceptibility of Candida species [35]. Furthermore, strains often acquire mutations in their genes over time, which can impact their

antifungal susceptibility profiles.

The results obtained in this study demonstrate that all evaluated extracts exhibited antimicrobial activity against the six tested strains of *Candida albicans*. Among the three tested extracts, *A. cordifolia* and *A. klaieneanum* showed strong activity with minimum inhibitory concentrations (MICs) ranging from 0.390 to 1.562 mg/mL against the isolates and strains of *C. albicans*. Numerous authors have reported the antibacterial, antiparasitic, and antifungal potential of *A. cordifolia, A. klaieneanum*, and *C. gabunensis* in combating vaginal infections [36]-[39]. Our results are similar with those of Domfeh et *al.* [40], who demonstrated the efficacy of *A. cordifolia* against *C. albicans* strains. Additionally, Agyare et *al.* [41] showed the anti-infectious properties of *A. cordifolia* extract on *C. albicans* isolates. Also, Amang à Ngnoung et *al.* [42] revealed the antimicrobial potential of secondary metabolites from *A. klaieneanum* on *C. ablicans*. Conversely, the extract of *C. gabunensis* exhibited the lowest activity, with MICs ranging from 0.195 to 6.250 mg/mL. This finding contrasts with the work of Ndjib et *al.* [16], who, in their ethnobotanical study of medicinal plants used in Cameroon for treating vaginal infections, demonstrated that *C. gabunensis* extract was highly active against *C. albicans*. The discrepancy between our results and those of other authors may be attributed to the extraction methods and drying temperatures used, which could inhibit certain active principles of the plant. The antifungal efficacy of these plants could be related to their chemical composition, which is rich in secondary metabolites such as shikimic acid, quercetin, myricetin, quercitrin, kaempferol, proanthocyanidins, phenolic acids, and flavonoids [43]-[45]. These secondary metabolites present in the plants are believed to act on the fungal microorganism membranes, inducing destabilization and destruction through turgescence [46].

Therapeutic combinations represent a critical avenue in the search for effective antimicrobial agents, as synergistic interactions can potentially broaden the spectrum of activity, minimize the emergence of antifungal-resistant microorganisms, and reduce toxicity and treatment duration (El Baz et al., 2025). According to this study's findings, the *A. cordifolia*-*A. klaineanum* combination (Ac + Ak, v:v) demonstrated synergistic effects against Cab2, Cab3, and *C. ablicans* SR ATTCP 37037, with enhanced antifungal properties and combined minimum inhibitory concentrations (MICco) ranging from 0.097 to 1.562 mg/mL. Similarly, the *A. cordifolia*-Amphotericin B combination (Ac + AmB, v:v) exhibited synergy against Cab3 and Cab8, with variant MICs of 0.008 - 0.195 mg/mL. The combined extracts produced larger inhibition zones, indicative of stronger antimicrobial activity and suggesting distinct mechanisms of action. Similar results were reported by Hlebová et *al.* [47], who observed synergistic effects of plant extracts on *C. albicans*, *C. glabrata* and *C. tropicalis* isolates. Ghandour et *al.* [48] also revealed synergistic activity of medicinal plant combinations against *C. albicans* isolates. Furthermore, Adelakun et *al.* [18] demonstrated synergistic interactions between *T. cordifolia* extracts and conventional antifungals (fluconazole) against *C. albi-*

cans. The observed synergy between plant-plant and plant-conventional antifungal combinations may stem from bioactive compound diversity, which potentiates overall inhibitory action on *C. albicans* isolates. High concentrations of active secondary metabolites in these combinations likely establish a coordinated mechanism that impedes pathogenic microorganisms from developing resistance [49]-[46].

5. Conclusion

This study focused on isolating resistant *Candida albicans* isolates and assessing their susceptibility to conventional antifungals and plant extracts from *A. cordifolia, A. klaineanum*, and *C. gabunensis*. Nine fungal isolates were obtained, five of which exhibited resistance to at least two antifungals (Cab2, Cab3, Cab5, Cab8, Cab9, and reference strain ATCC 37037). All plant extracts and amphotericin B inhibited *C. albicans* growth, with minimum inhibitory concentrations (MICs) ranging from 0.097 to 6.25 mg/mL. Fractional inhibitory concentration index (FICI) calculations identified two combinations with the most synergistic effects: *A. cordifolia-A. klaineanum* extract against Cab2, Cab3, and reference strain ATCC 37037 (FICI = 0.498, 0.372, and 0.186), and *A. cordifolia*-amphotericin B against *C. albicans* isolates Cab3 and Cab8 (FICI = 0.380 and 0.505). Thus, the combinations of *A. cordifolia-A. klaineanum* and *A. cordifolia-amphotericin B* can be recommended as alternatives to synthetic antifungals for treating genital infections caused by *C. albicans*, provided that their cytotoxicity on vaginal epithelial cells is evaluated in order to establish their safety profile for potential clinical use in topical applications.

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Conflicts of Interest

The authors declare that they have no conflict of interest regarding the publication of this article.

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