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# COMPARATIVE STUDIES OF THE ANTIFUNGAL ACTIVITIES OF THE LEAF EXTRACTS OF LOCALLY GROWN *CYMBOPOGON CITRATUS* AND CONVENTIONAL ANTIFUNGAL DRUGS AGAINST *CANDIDA ALBICANS* ISOLATED FROM PATIENTS ATTENDING A TERTIARY HOSPITAL IN ABAKALIKI, EBONYI STATE

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**ABSTRACT**
*Candida albicans* is a common commensal fungus that colonizes the oropharyngeal cavity, gastrointestinal tract, vaginal tract, and skin of healthy. One hundred (100) clinical samples of urine were collected from patients attending Alex Ekwueme Federal Teaching Hospital Abakaliki, Ebonyi state using sterilized clinical urine container out of which 20 samples of male patients tested, 5 (16.13%) were positive and 80 samples of female, 26 (83.17%) were positive. From the distribution, the female had more number of *Candida albicans* than male as observed. The antifungal susceptibility test was carried out using the Kirby-Bauer disc diffusion method. The following antifungal drugs were used: Flucytosine (1µg), Amphotericin B (10µg), Fluconazole (100µg) and Griseofulvin (10µg) The zone of inhibition of the equally tested extracts of *Cymbopogon citratus* showed that the 100 mg/ml extract showed more inhibition than the 50mg/ml and 25mg/ml with the highest IZD being 18mm and lowest 2mm. The organism showed high susceptibility rate to flucytosine, accounting 77.42% susceptibility rate, 9.68% resistance and 12.90% no inhibition. The organism also showed varying percentage of susceptibility to amphotericin B and griseofulvin, at 38.71% and 3.23% respectively, the resistance rate of 22.58% and 16.13% respectively and the percentage rate of no inhibition is 38.71% and 80.65% respectively. Fluconazole showed the highest rate of susceptibity at 74.19%, 25.81% were resistance. This shows that comparatively that the extract of *Cymbopogon citratus h*olds a promising note in microbial remedies using natural products.

**KEYWORDS:** Herbal plant, antimicrobial susceptibility, inhibition zones, antifungal.

## INTRODUCTION

Antifungal drug is any substance that acts selectively against a fungal pathogen (disease-causing organism) in the treatment of fungal infection (mycosis). The major groups of antifungals are the polyenes, the azoles, and the allyamines. These groups are distinguished primarily by chemical structure and mechanism of action. Important drugs that do not fall within these groups but that are used in the treatment of fungal infections include griseofulvin and flucytosine. Polyenes, such as amphotericin B and nystatin, are macrolide antibiotics made up of alternating conjugated double bonds.[4,7] The polyene drugs work by interacting with ergosterol, a type of steroid that is found in fungal membranes; this binding causes channels to form in the fungal membrane, resulting in the loss of membrane-selective permeability and of cytoplasmic components. Amphotericin B is used primarily in the treatment of serious fungal diseases, such as cryptococcal meningitis, histoplasmosis, and blastomycosis.[19,16] It is not absorbed from the gastrointestinal tract and is only used orally or topically for the treatment of infections of the skin and mucous membranes caused by *Candida albicans*. The antifungal agents of azoles are further divided into the imidazoles and triazoles, according to the number of nitrogen molecules in their organic ring structure, exert their effects by binding to fungal membranes and blocking the synthesis of fungal lipids, especially ergosterol. The azoles have broad antifungal activity and are active against fungi that infect the skin and mucous membranes and those that cause deep tissue infections. Clotrimazole, econazole, miconazole, and triconazole are given topically and are used for treating oral, skin, and vaginal infections. Introduction of the triazoles (fluconazole and

itraconazole) provided an alternative to amphotericin B in the treatment of endemic mycoses. Griseofulvin binds to keratin, thus depositing high levels in the skin. Griseofulvin affects the fungus by binding to microtubules, structures responsible for forming mitotic spindles during cell division and for processing cell wall components needed for growth. Flucytosine (5-FC) is unique in that it becomes active only when converted to 5-fluorouracil (5-FU) by an enzyme, cytosine deaminase, found in fungi but not present in human cells. Flucytosine inhibits RNA and DNA synthesis. 5-FC is used primarily in the treatment of systemic cryptococcal and Candida infections and chronomycosis. Because drug resistance may emerge against 5-FC, the agent often is used in combination with other antifungals, particularly amphotericin B.[3]

*Cymbopogon citratus* is a perennial grass that has been intentionally introduced in tropical and subtropical regions of the world for the essential oil extracts from its leaves and to be used as a culinary and medicinal herb[12, 17] It is a tall grass with rhizomes and densely tufted fibrous roots, which tillers profusely and has the potential to escape from cultivation.[13] Once established it can spread fairly rapidly, becoming weedy and invasive in disturbed areas. Currently it is listed as a weed in Mexico and as an invasive species on the island of St. Lucia. On this island, *C. citratus* is an invader which, due to its high oil content[15], has the potential to increase fire risk in areas such as Pigeon Island and Dennery quarry where it forms monotypic stands.[1,2]

Fungi are single celled or very complex multicellular organisms. They are found in just about any habitat but mostly live on the land, mainly in soil or on plant material rather than in sea or fresh water. A group called the decomposers grow in the soil or on dead plant matter where they play an important role in the cycling of carbon and other elements. Some are parasites of plants causing diseases such as mildews, rusts, scabs or canker. A very small number of fungi cause diseases in animals. In humans these include skin diseases such as athletes' foot, ringworm and thrush. A yeast-like fungus commonly occurring on human skin, in the upper respiratory, alimentary and female genital tracts. This fungus has a dimorphic life cycle with yeast and hyphal stages. The yeast produces hyphae (strands) and pseudohyphae. The pseudohyphae can give rise to yeast cells by apical or lateral budding. Spores are similar to seeds as they enable the fungus to reproduce. Yeasts are small, lemon-shaped single cells that are about the same size as red blood cells. They multiply by budding a daughter cell off from the original parent cell. Scars can be seen on the surface of the yeast cell where buds have broken off. Yeasts are also one of the most widely used model organisms for genetic studies, for example in cancer research. Other species of yeast such as *Candida* are opportunistic pathogens and cause infections in individuals who do not have a healthy immune system.

*Candida albicans* is a common commensal fungus that colonizes the oropharyngeal cavity, gastrointestinal and vaginal tract, and healthy individuals' skin. In 50% of the population, *C. albicans* is part of the normal flora of the microbiota. The various clinical manifestations of *Candida* species range from localized, superficial mucocutaneous disorders to invasive diseases that involve multiple organ systems and are life-threatening. From systemic and local to hereditary and environmental, diverse factors lead to disturbances in *Candida'*s normal homeostasis, resulting in a transition from normal flora to pathogenic and opportunistic infection.[22] Of all the species, *Candida albicans* is the most common causative agent of mucosal infections and systemic infection, and it is responsible for about 70% of fungal infections around the world.[21] It has been the leading cause of life-threatening invasive infections for the past several decades. Despite treatment, the mortality rate is close to 40%, especially in hospital conditions.[14]

## MATERIALS AND METHODS

## MATERIALS

### Ethical clearance

The approval for this study was obtained from the research and ethical committee of Alex Ekwueme Federal Teaching Hospital Abakaliki, Ebonyi state.

### Equipment and Instruments

The following equipment/instrument were used: forceps, wire loop, Bunsen burner, autoclave, oven, incubator, weighing balance, spatula, masking tape, cotton wool, nose mask, syringe, hand glove and aluminum foil. The following glass wares were used in this study: pipette, petri dish, beakers, conical flasks, slides, test tubes, glass rod, measuring cylinder**.**

### Chemicals and Reagents

The following reagents and chemicals that were used in this study includes: sterile water, Dimethyl Sulphur Oxide, normal saline.

### Media

The following media were used for this research work. Sabouraud Dextrose agar, Nutrient broth, Nutrient agar and Muller Hinton agar.

NB: All media were prepared aseptically according to the manufacturers' instruction 3.1.5 Antifungal drugs.

The following antifungal drugs were used, flucytosine, Amphotericin B, fluconazole and griseofulvin.

## METHOD

### Study area

The study was carried out at Alex Ekwueme Federal Teaching Hospital Abakaliki, Ebonyi State. Ebonyi is one the states in the south eastern part of Nigeria. The state shares borders with Cross River State to the east, Enugu State to the west, Benue State to the north and Abia State in the south. Abakaliki is the capital of

Ebonyi state. According to 2006 population census, Abakaliki has 79,280 people. It is a cosmopolitan in nature, drawing and merging people of divergent national and international background. It is an educational and administrative settlement. The climate is characterized by a hot dry period which stretches from Novermber –April, while the rainy season is from May-October. The maximum temperature during dry season is $37.6^0$C while the minimum temperature is $27.1^0$C.

**Sample collections**

One hundred (100) clinical samples of urine was collected from patients attending Alex Ekwueme Federal Teaching Hospital Abakaliki, Ebonyi state using sterilized clinical urine container. The samples were transported to the Microbiology Laboratory, Ebonyi State University, Abakaliki for fungal analysis.

**Sterilization of Glassware`s**

The glassware that was used for this research was sterilized in hot air oven at $160^0$C for 1hour.

**Media Preparation**

The media used in this research were prepared according to manufacturer's specifications. These included, Sabouraud Dextrose agar, 32.5g was dissolved in 500ml volume of distilled water, nutrient agar, 14g of the media powder was dissolved in 500ml volume of distilled water, Muller Hinton agar, 19g was dissolved in 500ml volume of distilled water. All media was heated over flame to dissolved properly and then sterilized by autoclaving at $121^o$C for 15 minutes in well corked conical flasks. The sterilized media was allowed to cool to $45^o$C before being dispensed aseptically into sterile petri dish dishes in 20 ml volumes and then allowed to gel.

For nutrient broth, 1.3g was dissolved in 100ml volume of distilled water. This was aseptically dispensed in 5ml volume into test tubes, corked and autoclave for 15 minutes at $121^o$C at 15 psi to sterilize the broth and then allowed to cool at room temperature.

The sterilized media and broth were incubated at $37^o$C for 24 hours and then observed for turbidity.[5]

**Extraction of the *Cymbopogon citratus***

The leaves of the *Cymbopogon citratus* were collected, washed and dried under room temperature for 3 days. It was ground into fine powder. 20g was weighed and soaked in 100ml volume of distilled water for 48 hours. It was sieved with muslin cloth and then allowed to dry in a tray. The dried extract was scraped with a spatula onto a universal bottle for use.

**Antifungal Susceptibility Testing**

The antifungal susceptibility test was carried out using the Kirby-Bauer disc diffusion method as outlined in the current Clinical and Laboratory Standards Institute (CLSI) guidelines, (2018). The following antifungal drugs were used: Flucytosine (AFY 1µg), Amphotericin B (AMB 10µg), Fluconazole (FLU 100µg) and Griseofulvin (AGF 10µg) was aseptically placed onto the surfaces seeded solidified Muller-Hinton plates with a sterile forceps and gently pressed to ensure even contact. The plates were incubated at $37^0$C for 24 hours and the zones of inhibition observed after 24 hours of incubation. The inhibition zone diameter (IZD) around each disk was measured using a calibrated transparent meter ruler and recorded in millimeter.[6]

**Extracts of *Cymbopogon citratus***

The already prepared Muller-Hinton plates with the isolates of *Candida albicans* was perforated with a cork borer of 6mm deep. The extracts of *Cymbopogon citratus* of different concentrations of 100%, 50% and 25% were each placed on the holes made. The plates were incubated at $37^0$C for 24 hours and the zones of inhibition observed after 24 hours of incubation. The inhibition zone diameter (IZD) around each disk was measured using a calibrated transparent meter ruler and recorded in millimeter.

**Confirmatory test**

Germ Tube Test (Reynolds-Braude Phenomenon). A small portion of 72 hours old fungal culture was added to 0.5ml of human serum in a test tube. This procedure was repeated with a known culture of *Candida albicans*, the test tube was incubated at $37^0$C for 3 hours. A drop of yeast suspension was placed on a glass slide and covered with a cover glass. Viewed under a microscope, cluster of yeast cells were seen. *Candida albicans* produce germ tube**.**

**Preparation of 0.5 McFarland Equivalent Standards**

A 1% u/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water and 1% u/v solution of barium chloride by dissolving 0.5g of hydrated barium chloride in 50ml of distilled water. A 0.6ml of barium chloride was added to 99.4ml of the sulphuric acid solution and mixed. A small volume of the turbid solution was transferred to a screw capped bottle of the same type as used in preparing the test inoculums.[5]

**Standardization of Test Organism**

*Candida albicans* isolated was standardized before use by inoculating 5ml normal saline in sterile test tube with a loopful of a 24 hours culture of the test fungi from a culture plate. Then, 5ml of distilled water was put in a sterile test tube and autoclave for 15minutes at 15psi. Allowed to cool for 45 minutes. The test organism was inoculated using wire loop and shaked vigorously and adjusted to McFarland standard.

## RESULT

**Morphology and confirmatory tests of *Candida albicans* from urine samples of patients attending Alex Ekwueme Federal Teaching Hospital Abakaliki, Ebonyi State.**

The morphology, microscopy and confirmatory test of the fungi isolates are shown in Table 1.

**Table 1: Morphology, Microscopy and confirmatory tests.**

| Morphology Shape Color | Microscopy Germ tube motility test | Suspected organism |
|---|---|---|
| Cocci green | + - | *Candida albicans* |

**Key: + = positive**
**- = negative**

**The percentage distribution of *Candida albicans* in urine samples of patients attending clinics AE-FUTHA.**

A total of 100 samples of urine were used for the research. Out of which 20 male were tested resulting to 5 (16.13%) positive whereas and 80 samples of tested female, 26 (83.17%) were positive. From the distribution, the female had more number of *Candida albicans* than male as observed**.**

**Table 2: Distribution of *Candida albicans* isolated from male and female patients in AE-FUTHA.**

| Sample | No. of samples collected | No. with positive cases | Percantage total |
|---|---|---|---|
| **Male** | **20** | **5** | **5(16.13)** |
| **Female** | **80** | **26** | **26(83.87)** |
| **Total** | **100** | **31** | **31(100)** |

**The aqueous extracts of *Cymbopogon citratus* zones of inhibition**

The extracts of *Cymbopogon citratus* showed various inhibition zone diameter at different concentration ranging from 100, 50, 25mg/ml. The 100mg/ml extract showed more inhibition than the 50 and 25mg/ml.

**Table 3: Inhibition Zone Diameter of Aqueous Extract of Lemon Grass (*Cymbopogon citratus*)**

| S/N | Isolates | Concentration (mm) | | |
|---|---|---|---|---|
| | *Candida albicans* | 100 | 50 | 25 |
| 1 | A | 17 | 09 | 08 |
| 2 | B | 17 | 13 | 09 |
| 3 | C | 14 | 13 | 04 |
| 4 | D | 12 | 09 | - |
| 5 | E | 18 | 10 | 05 |
| 6 | F | 18 | 10 | 06 |
| 7 | G | 17 | 09 | 06 |
| 8 | H | 14 | 10 | 07 |
| 9 | I | 12 | 06 | 05 |
| 10 | J | 11 | 04 | - |
| 11 | K | 14 | 07 | 03 |
| 12 | L | 09 | 05 | - |
| 13 | M | 08 | - | - |
| 14 | N | 10 | 06 | - |
| 15 | O | 15 | 10 | 04 |
| 16 | P | 14 | 09 | 03 |
| 17 | Q | 14 | 08 | 05 |
| 18 | R | 10 | 06 | 02 |
| 19 | S | 08 | 04 | - |
| 20 | T | 12 | 08 | 04 |
| 21 | U | 11 | 06 | 03 |
| 22 | V | 12 | 06 | 04 |
| 23 | W | 11 | 08 | 05 |
| 24 | X | 10 | 07 | 04 |
| 25 | Y | 14 | 09 | 03 |
| 26 | Z | 12 | 05 | 01 |

| | | | | |
|---|---|---|---|---|
| 27 | A1 | 14 | 08 | 06 |
| 28 | B1 | 13 | 08 | 04 |
| 29 | C1 | 09 | 04 | - |
| 30 | D1 | 08 | 05 | - |
| 31 | E1 | 14 | 10 | 08 |

**KEY: A-Z & A1-E1 = isolates, - = resistance.**

**The minimum inhibitory concentration (MIC) of *Cymbopopgon citratus***

The MIC of *Cymbopogon citratus* after testing various concentration ranges of (2mg/ml to 25mg/ml) of the extract showed inhibition on 21 isolates of the test organism at 25mg/ml. The MIC is presented in the table below

**Table 4: Inhibition Zone Diameter of MIC of the Extract (*Cymbopogon citratus*).**

| S/N | Isolates | MIC (mm) |
|---|---|---|
| 1 | A | 08 |
| 2 | B | 09 |
| 3 | C | 04 |
| 4 | E | 05 |
| 5 | F | 06 |
| 6 | G | 06 |
| 7 | H | 07 |
| 8 | I | 05 |
| 9 | K | 03 |
| 10 | O | 04 |
| 11 | P | 03 |
| 12 | Q | 05 |
| 13 | R | 02 |
| 14 | T | 04 |
| 15 | U | 03 |
| 16 | V | 04 |
| 17 | W | 05 |
| 18 | X | 04 |
| 19 | Y | 03 |
| 20 | Z | 01 |
| 21 | B1 | 04 |
| 22 | E1 | 08 |

**Key: MIC = minimum inhibitory concentration.**
**A-$E_1$= isolates from patient sample**

**Results of antifungal susceptibility pattern of *Candida albicans* isolated from patients attending AE-FTHA**

The table below shows the antifungal activities against the test organism (*Candida albicans*) collected from patients attending AE-FTHA. The organism has high susceptibility rate to flucytosine, accounting 77.42 % susceptibility rate, 9.68 % resistance and 12.90 % no inhibition. The organism also showed susceptibility to amphotericin B and griseofulvin, accounting for 38.71% and 3.23% respectively, the resistant rate of 22.58% and 16.13% respectively and the rate of no inhibition is 38.71% and 80.65% respectively. Fluconazole showed the highest rate of susceptibility at 74.19% and 25.81% resistance.

**Table 5: Antifungal susceptibility result of *Candida albicans.***

| Isolate | Antifungal used | | | |
|---|---|---|---|---|
| ***Candida albicans*** | **AFY (1µg)** | **AMB (10µg)** | **FLU (100µg)** | **AGF (10µg)** |
| | - | - | - | - |
| | - | - | - | - |
| | S | - | - | - |
| | S | - | - | - |
| | S | R | - | - |
| | S | R | - | - |
| | S | R | - | - |
| | S | R | R | R |

| | | | | |
|---|---|---|---|---|
| | S | R | R | R |
| | S | R | R | R |
| | S | S | R | R |
| | - | - | - | - |
| | R | S | R | S |
| | S | S | - | - |
| | S | R | - | - |
| | S | S | - | - |
| | S | S | - | - |
| | S | - | - | - |
| | S | S | - | - |
| | S | S | R | R |
| | S | - | R | - |
| | S | S | - | - |
| | S | - | R | - |
| | S | - | - | - |
| | S | S | - | - |
| | S | S | - | - |
| | S | S | - | - |
| | S | - | - | - |
| | R | S | - | - |
| | R | - | - | - |
| | - | - | - | - |
| | | | | |

**KEY: AFY (1µg) = Flucytosine (≤ 12 = R, ≥ 20 = S), AMB (10µg) = Amphotericin B (≤ 10 = R, ≥ 15 = S), FLU (100µg) = Fluconazole (≤ 14 = R, ≥ 19 = S), AGF(10µg) = Griseofulvin (≤ 8 = R, ≥ 15 = S), R = Resistance, S = Susceptible, - = No inhibition.**

4.6 Table 6 summarizes the susceptibility, resistance and no inhibition pattern of the antifungal drugs used in this study.

**Table 6: Susceptibility, resistance and no inhibition pattern of antifungal drug against *Candida albicans.***

| **Isii Isolate** | **Antifungal used** | | | |
|---|---|---|---|---|
| **ccccandida albicans** | **AFY (1µg)** | **AMB (10µg)** | **FLU (100µg)** | **AGF (10µg)** |
| | 24(77.42) | 12(38.71) | 0(0) | 1(3.23) |
| | 3(9.68) | 7(22.58) | 8(25.81) | 5(16.13) |
| | 4(12.90) | 12(38.71) | 23(74.19) | 25(80.65) |
| **TOTAL** | 31(100) | 31(100) | 31(100) | 31(100) |

**KEY: S= susceptible. R=Resistance and - = no inhibition**

## DISCUSSION

A total of 100 samples of urine were used for the research. Out of which 20 male were tested resulting to 5 (16.13%) positive whereas and 80 samples of tested female, 26 (83.17%) were positive. From the distribution, the female had more number of *Candida albicans* than male as observed**.** This observation might be due to the anatomical structure of the females.

*Candida albicans* is a common commensal fungus that colonizes the oropharyngeal cavity, gastrointestinal and vaginal tract, and skin of healthy individuals. They are known to be opportunistic in nature. High prevalence of candidiasis has been reported among pregnant women and among immunocompromised individuals, coupled with antimicrobial resistance of this isolate and its underlying side effects hence the need for a natural alternative with little or no side effects.[9,10] *Cymbopogon citratus* known to have no side effects on users as it can be used to prepare herbal tea, used as spice to mention but a few.[23,8] The antifungal susceptibility test for the extracts of *Cymbopogon citratus* was carried out. The zone of inhibition of the extracts of *Cymbopogon citratus* showed that the 100 mg/ml extract had more inhibitory effects on *Candida albicans* than 50mg/ml and 25mg/ml with the highest IZD being 18mm and lowest 2mm, this finding is in agreement with.[21] The antifungal susceptibility test for the conventional antifungal drugs was carried out using the Kirby-Bauer disc diffusion method. The following antifungal drugs were used: Flucytosine (1µg), Amphotericin B (10µg), Fluconazole (100µg) and Griseofulvin (10µg) The organism showed high susceptibility rate to flucytosine, accounting 77.42% susceptibility rate, 9.68% resistance and 12.90% no inhibition. The organism also showed varying percentage of susceptibility to amphotericin B and griseofulvin, at 38.71% and 3.23% respectively, the resistance rate of 22.58% and 16.13% respectively and the percentage rate of no inhibition is 38.71% and 80.65% respectively[18,20] Fluconazole showed the highest

rate of susceptibity at 74.19%, 25.81% were resistance. This shows that comparatively the extracts of *Cymbopogon citratus* holds a promising note on microbial remedies using natural products.

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