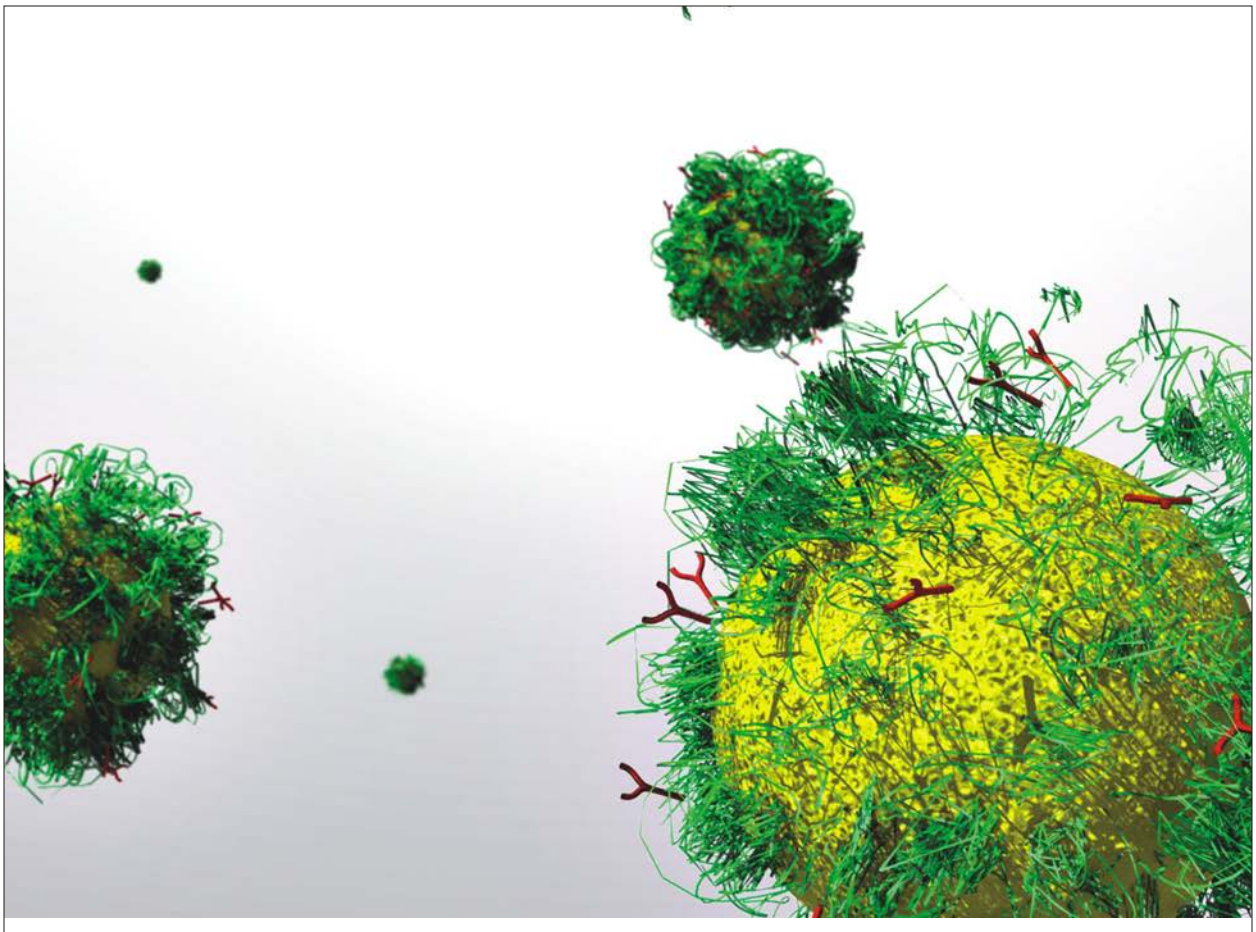

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Screening of Some Nigerian Medicinal Plants for Anti-candida Activity

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ABSTRACT

Medicinal plants used in oral medicine in Delta state South of Nigeria were investigated for anti-candida activity. The study addresses the high rate of candida infections and resistance to antibiotics in Nigeria. Twenty-one medicinal plants used in oral medicine for treating infectious diseases were tested for anti-candida activity using *Candida albicans* and *Candida krusei*. Ethanol, methanol, n-hexane, chloroform and water extracts of the plants were screened for antimicrobial activity using the paper disc diffusion method. Phytochemical screening of the medicinal plants showed they had significant concentrations of alkaloids, phlobatannins, terpenoids, flavonoids and cardiac glycosides. Tannins, steroids and tannins were not present in significant concentrations in all the plants tested. Nineteen (i.e., 90.48%) ethanol plant extracts were significantly active against *Candida albicans* and 80.95% of the plants were active with methanol extracts. The number of medicinal plants in percentage that showed significant inhibition with n-hexane, chloroform and aqueous extracts for this test organism was 71.43% (i.e., 15 plants), 61.90% (i.e., 13 plants) and 33.33% (i.e., 7 plants), respectively. The effect of the plant extracts on *Candida krusei* was comparable to their effect on *C. albicans* as 85.71% of the plants showed significant activity for ethanol and 80.95, 66.67 and 14.29% for methanol, n-hexane, chloroform, respectively and little or no activity with the aqueous extract. Five of the medicinal plants screened recorded inhibition zones ≥19.00 mm, significantly higher than the standard antibiotics tested. These plants and those that showed significant activity should be investigated as potential sources of new antimicrobials.

Key words: Inhibition, plant extracts, candidiasis, medicinal plants, antibiotics

INTRODUCTION

Nigeria is a developing country with quite a lot of people living below average conditions and as such expensive drugs (antibiotics) are generally not affordable. The use of locally sourced plants and visits to herbal practitioners is a common practice. The traditional healers use indigenous medicine plants and with high rates of success the locals claim. These plants are worthy of investigation and as such this research. Yeast infections are among the ailments treated by these practitioners. The most common yeast infection encountered in Nigeria is vaginal candidiasis. It has been documented that the incidence of this disease is quite high in places like Nicaragua and other parts of the world too. The yeasts commonly encountered are *Candida albicans*, *Candida tropicalis* and *Candida krusei* (Bello *et al*., 2002). The incidence of *Candida albicans* is higher than that for

other *candida species*. In places like Malaysia apart from the *Candida albicans* other yeast species like *C. glabatra*, *C. lusitaniae*, *C. faImata*, *C. krusei* and *C. parapsilosis* have been isolated from patients (Chong *et al*., 2003). The incidence of vaginal candidiasis varies from city to city in Nigeria ranging from 29-65% particularly among sexually active women (Bello *et al*., 2002; Jombo *et al*., 2010; Bukbuk and Chuku, 2011; Akpan *et al*., 2011; Akerele *et al*., 2002). Records also show candidiasis is more prevalent among HIV positive patients particularly in some cities in Northern Nigeria (Umeh and Umeakanne, 2010). There are other predisposing factors like hormonal imbalance that can occur in pregnancy, menstrual cycles and with the use of oral contraceptives (Jombo *et al*., 2010).

The use of plant decoctions is quite popular among Nigerians. The herbal practitioners are popularly patronized and they lay claims to effective methods for treating a lot of ailments and Candidiasis is not left out. Oral candidiasis is usually treated with drugs like clotrimazole, nystatin, miconazole, fluconazole, itraconazole and amphotericin B. The most popular drug used for treating vaginal candidiasis in Nigeria is canesten (clotrimazole). The problems faced by both patients and health practitioners include drug resistance, limited number of effective antifungal agents, relapse of Candida infections and the high cost of antifungal agents (Runyoro *et al*., 2006). There is the need for development of new drugs that are safer and more affordable. Medicinal plants already used in folklore medicine should be investigated for possible remedies and the claims of local herbal practitioners substantiated through research. Medicinal plant derived compounds therefore have potentials for new drugs. These drugs could potentially be used for the treatment of fungal diseases like Candidiasis.The objective of this survey is to identify plants potentials for active components that could serve as cheaper and more effective remedy for yeast infections.

MATERIALS AND METHODS

Plant collection: Traditional practitioners were consulted for the names of plants used for treatment of skin infections and other infections caused by microorganisms. Traditional practitioners were assured that if there were any monetary benefits from the research, they would be informed. Fresh plants used by traditional medical practitioners in Ughelli North Local Government Area of Delta State, Nigeria were collected randomly from gardens and bushes in the locality. The staff of the University of Lagos herbarium using plant presses and pictures of the plants presented to them confirmed the taxonomic identities of the plants. Voucher specimens of the plants were also submitted to them.

The collected plants were washed under running tap water, air dried, homogenized to fine powder and stored in air tight bottles at 4°C.

Preparation of crude plant extracts

Solvent extraction: Dried powdered plant materials about 100 g were extracted with 200 mL of ethanol, methanol, n-hexane, chloroform kept in a rotary shaker for 24 h. The extraction process was repeated three times and the filtrates were added together and centrifuged at 5000 rmp for 15 min. The supernatant was collected and the procedure was repeated twice and these were added together and concentrated in a rotary evaporated. The filtrate was evaporated to dryness and weighed. One gram of each extract was weighed and 10 mL of the solvents added to create a solution with a concentration of 100 mg mL^{-1}. One millilitre of extracts was pipetted into already cut paper discs (6.3 mm in diameter), known antibiotic discs were also prepared; these include amphotericin B, fluconazole, butoconazole, clotrimazole, tioconazole and miconazole. The drugs were dissolved in distilled water to obtain stock solution of 128 mg mL^{-1}.

Aqueous extraction: One hundred grams of dried plant material was extracted in distilled water for 6 h at slow heat. The solution was filtered every 2 h through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the procedure repeated twice and after 6 h it was concentrated in a rotary evaporated and eventually evaporated to dryness. The final weight of the filtrate was got and 10 mL of solvent added to 1 g of the extract to give a solution with a concentration of 100 mg mL^{-1}.

Inhibition studies: Sabouraud dextrose agar was used to prepare the culture medium according to manufacturer's directions. Clinical isolates of *Candida albicans* and *C. krusei* were got from the Medical Microbiology Department of Delta State, University Teaching Hospital Oghara, Delta, Nigeria. They were ssaseptically inoculated into petri dishes containing sterilized, cooled and settled medium. The Petri dishes were incubated at 35°C for 24 h to give white round colonies against a creamy background. The paper discs containing the extracts and antibiotics were placed on the medium after 24 h incubation. The inhibition zones for the extracts and the antibiotics were taken after another 24 h and then 48 h.

Statistical analysis: The experiment was replicated thrice and the mean was calculated for statistically analysis. Least significant difference and Duncan's multiple range tests was used to determine areas where there was significant difference.

PHYTOCHEMICAL SCREENING OF TEST PLANTS

Chemical test were carried out on aqueous extracts and dried powdered specimen using standard procedures to identify the constituents following methods used by Edeoga *et al.* (2005) and Sofowora (1993).

Tannins: Dried powder samples of test plants (0.5 g) were boiled in 20 mL of water in a test tube and then filtered. Few drops of 0.1% Ferric chloride was added and observed for brownish green or a blue-black colouration.

Phlobatannins: Deposition of a red precipitate when an aqueous extract of each plant sample was boiled in 1% hydrochloric acid was taken as evidence for the presence of phlobatannins.

Alkaloids: The ethanolic extract of 2.5 g of plant material was evaporated to dryness and the residue was heated on a boiling water bath with 2 N HCL (5 mL). After cooling the mixture was filtered and the filtrate divided into two equal portions. One portion was treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity on precipitation. A (+) score was recorded if the reagent produced only a slight opaqueness; a (++) score was recorded if a definite turbidity, but no flocculation was observed and a (+++) score was recorded if a definite heavy precipitate or flocculation was produced.

Saponins: The powdered plant sample (2 g) was boiled in 20 mL of distilled water in a water bath and then filtered. The filtrate (10 mL) was mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The froth was mixed with three drops of olive oil and then shaken vigorously and then observation was done for the formation of emulsion.

Flavonoids: A portion of the powdered sample was in each case heated with 1 mL of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Steroids: Acetic anhydride was added to 0.5 g of ethanolic extract of each sample with 2 mL H_2SO_4, the colour changed from violet to blue or green in some samples indicating the presence of steroids.

Terpenoids (Salkowski test): The plant extracts (2 mL) were mixed with 2 mL of chloroform and 3 mL of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration at the interface was formed to show the presence of terpenoids.

Cardiac glycosides: Plant extracts were treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was under played with 1 mL of concentrated sulphuric acid. A brown ring at the interface indicates deoxy sugar characteristics of cardiac glycosides. A violet ring may appear below the brown ring while in the acetic acid layer and a greenish ring may form just gradually throughout the thin layer.

RESULTS

The plants selected were mostly those that have history of been active or used for treatment of diseases that are caused by microorganisms. They were collected from the wild following information given above from traditional healers in Delta State (Table 1).

The plants-*Senna alata*, *Cymbopogon citratus*, *Aspilia africana* and *Azadirachtha indica* had significant concentration of alkaloids (Table 2). Tannins were slightly present in most plants but,

Table 1: Medicinal plants used and their local uses in oral traditional medicine in Delta State, South Nigeria

Scientific names	Family	Traditional medical uses
Senna alata	Zingiberaceae	Measles, venereal disease and skin diseases
Carica papaya	Caricaceae	Gonorrhoea, ringworm, roundworm, convulsion
Etlingera elatoir	Zingiberaceae	Arthritis of the kneel, rheumatism, skin disease
Canna indica	Cannaceae	Malaria and asthma
Veronia amygdalina	Asteraceae	Itching, stomach ache, ringworm, eczema, diabetes
Acalypha wilkesiana	Euphorbiaceae	Skin rashes, flatulence and constipation
Cympobogon citratus	Poaceae	Cough, ringworm, stomach tonic
Ocimum gratissimum	Labiatae	Fever, insect repellent, diarrhoea
Aspilia africana	Asteraceae	Skin rashes, cleaning sores, stomach disorders, guinea worm and nerve disorder
Momordica charantia	Cucurbitaceae	Pile, jaundice, night blindness
Gossypium barbadense	Malvaceae	Asthma, ulcers, dysentery
Blighia sapinda	Sapindaceae	Malaria, hypoglycaemic agent and antimicrobial
Carpologia lutea	Polygalaceae	Rheumatism, toothache and antimicrobial
Luffa cyllindrica	Cucurbitaceae	Blood tonic, malaria and antimicrobial
Phyllanthus amarus	Euphorbiaceae	Gonorrhoea, tuberculosis, antimicrobial
Acalypha, godseffiana	Euphorbiaceae	Skin infection, syphilis, antimicrobial
Alstonia congensis	Apocynaceae	Toothache, antimicrobial and malaria
Nauclea latifolia	Rutaceae	Pile, measles, sores and antimicrobial
Diodia scandens	Rutaceae	Pile, gonorrhoea and antimicrobial
Sphenocentrum jollyanum	Menispermaceae	Wounds, jaundice, cough and antimicrobial
Azadirachta indica	Meliaceae	Syphilis, eczema, sore throat, malaria thyphoid, antimicrobial, abortificant

Table 2: Phytochemical screening of medicinal plants used in oral medical practice in Delta State, South Nigeria

Scientific name	Al	Ta	Sp	Pht	Tp	Fl	Cg	St	Part used
Senna alata	+++	+	+	+	++	++	-	+	Leaves
Carica papaya	+	+	+	+	++	+	+	+	Leaves
Etlingera elatoir	++	+	+	+	+	++	+	+	Rhizome and leaves
Canna indica	+	+	+	+	+	+	+++	-	Leaves
Vernonia amygdalina	++	+	-	+	++	+++	+	-	Leaves
Cymbopogon citratus	+++	+	+	-	+++	++	+	+	Leaves
Ocimum gratissimum	+	-	-	+++	++	++	+	+	Stems, leaves and flowers
Aspilia Africana	+++	+	+	++	++	++	+	+	Stems, leaves and flowers
Momordica charantia	+++	+	+	+	+++	+	+	+	Stems, leaves and flowers
Gossypium ba rbadense	++	-	++	+	+++	+	-	+	Leaves and stems
Blighia sapinda	+	+	+	++	+++	++	+	++	Leaves and flowers
Carpolobia lutea	-	+	+	-	+	+++	+	+	Leaves
Luffa cyllindrica	++	+	+	++	+++	+	+	+	Leaves and stems
Phyllanthus amarus	+	+	+	++	+++	+	+	+	Whole parts
Alstonia congensis	+	+	+	+	+++	+++	+	+	Leaves
Nauclea latifolia	++	+	+	+	++	++	+	+	Leaves
Diodia scandens	+	+	+	-	++	+	-	+	Leaves
Sphenocentrum jollyanum	++	+	+	-	++	+++	+	++	Leaves
Azadirachta indica	+++	-	+	+	+++	++	+	+	Leaves and stems
Acalypha wilkesiana	+	+	+	+++	+	-	+	+	Leaves
Acalypha godseffiana	+	-	+	++	++	++	+	+	Leaves

+: Constituent slightly present, ++: Constituent present, +++: Absolute presence of constituent, Absence of constituent, Al: Alkaloids, Pht: Phlobatannins, Cg: Cardiac glycosides, Ta: Tannins, Tp: Terpenoids, Sp: Saponins, Fl: Flavanoids, St: Steroids

Azadirachtha indica, *Acalypha godseffiana*, *Ocimum gratissimum* and *Gossypium barbadense* (Table 2). Saponins were also slightly present in most plants but *Vernonia amygdalina* and *Ocimum gratissimum*. Phlobatannins were significantly present in *Ocimum gratissimum* and *Acalypha wilkesiana* and not found in *Cymbopogon citratus*, *Carpolobia lutea*, *Diodia scandens* and *Sphenocentrum jollyanum* (Table 2). Terpenoids were found in all plants assessed. Flavonoids were found in all but one plant analysed (*Acalypha wilkesiana*) in varying concentrations. Cardiac glycosides were slightly present in most of the medicinal plants analysed but not in the following plants; *Senna alata*, *Gossypium barbadense* and *Diodia scandens*. Steroids were also slightly present in varying concentrations in most of the plants used but not in *Canna indica* and *Vernonia amygdalina* (Table 2).

The plant extracts caused varying degrees of inhibition with the two yeasts used (*Candida albicans* and *Candida krusei*) depending on the plant species and the type of solvent used for extraction. Plants that recorded inhibition ≥10 mm are considered significant (Edeoga *et al.*, 2005). Some of the plants showed strong anti-candida activity, while others showed none. The type of solvent used for extraction also affected the degree of inhibition. Some plants inhibited the growth of *Candida albicans* but not that of *Candida krusei* (Table 3, 4).

***Candida albicans*:** Over 71% of the medicinal plants screened showed significant anti-candida activity with the ethanol and methanol extracts when tested against *Candida albicans* (Table 3). The number of medicinal plants that showed significant inhibition with n-hexane, chloroform and aqueous extract for this test organism was 9, 6 and 1, respectively out of a total of 21 plants (Table 3). Plants that recorded inhibition zones higher than 10 mm were considered significant.

Table 3: Mean zone of inhibition for *Candida albicans* with plant extracts

Test plants	Ethanol	Methanol	n-Hexane	Chloroform	Water
Senna alata	20.00±2.00^{a}	20.33±1.53^{a}	10.33±0.58^{c}	00.00±0.00^{d}	00.00±0.00^{d}
Carica papaya	19.00±1.00^{a}	14.00±3.61^{b}	8.67±1.15^{c}	00.00±0.00^{d}	5.67±0.58^{d}
Etlingera elatoir	20.00±2.00^{a}	22.33±2.51^{a}	12.33±2.52^{b}	10.67±1.15^{c}	11.33±1.53^{c}
Canna indica	10.00±2.00^{c}	20.33±1.53^{a}	6.67±1.15^{d}	00.00±0.00^{d}	00.00±0.00^{d}
Vernonia amygdalina	10.67±1.15^{c}	10.00±2.00^{c}	00.00±0.00^{d}	00.00±0.00^{d}	00.00±0.00^{d}
Cymbopogon citratus	15.00±3.61^{b}	15.33±3.51^{b}	13.00±2.00^{c}	8.67±1.53^{c}	8.00±1.00^{c}
Ocimum gratissimum	10.67±1.15^{c}	8.67±1.15^{c}	8.67±1.15^{c}	8.67±1.15^{c}	5.67±0.58^{d}
Aspilia Africana	8.67±1.52^{c}	11.33±3.05^{c}	10.67±1.15^{c}	10.67±1.15^{c}	00.00±0.00^{d}
Momordica charantia	11.00±3.00^{c}	18.00±2.00^{a}	13.00±2.65^{c}	8.67±1.15^{c}	8.33±1.53^{c}
Gossypium barbadense	15.67±2.08^{b}	00.00±0.00^{d}	8.67±1.15^{c}	10.67±1.15^{c}	6.67±1.15^{d}
Blighia sapinda	00.00±0.00^{d}	00.00±0.00^{d}	00.00±0.00^{d}	00.00±0.00^{d}	00.00±0.00^{d}
Carpolobia lutea	10.67±1.15^{c}	00.00±0.00^{d}	6.67±1.15^{d}	8.67±1.15^{c}	00.00±0.00^{d}
Luffa cylindrical	8.67±1.53^{c}	8.33±0.58^{c}	8.67±1.15^{c}	6.67±1.15^{d}	00.00±0.00^{d}
Phyllantus amarus	13.67±1.53^{c}	00.00±0.00^{d}	00.00±0.00^{d}	6.67±1.15^{d}	8.67±1.15^{c}
Alstonia congensis	8.67±1.53^{c}	12.00±2.65^{c}	10.67±1.15^{c}	08.33±0.58	5.33±0.58
Nauclea latifolia	00.00±0.00^{d}	10.33±2.08^{c}	12.33±2.52^{c}	8.67±1.15^{c}	6.67±1.15^{d}
Diodia scandens	10.00±2.00^{c}	10.67±1.15^{c}	00.00±0.00^{d}	00.00±0.00^{d}	8.00±2.00
Sphenocentrum jollyanum	13.33±1.53^{c}	12.33±2.08^{c}	8.67±1.15^{c}	8.33±0.58^{c}	6.67±1.15^{d}
Azadirachta indica	13.33±1.53^{c}	20.33±1.53^{a}	14.33±1.15^{b}	13.33±1.53^{c}	00.00±0.00^{d}
Acalypha wilkesiana	13.67±1.53^{c}	17.00±2.00^{a}	9.33±1.15^{c}	10.67±1.15^{c}	9.00±1.73^{c}
Acalypha godselffiana	9.67±1.53^{c}	17.67±1.53^{a}	13.67±1.53^{c}	16.33±1.53^{a}	8.00±1.00^{c}

Superscripts with same different letters are significantly different (p≥0.05) using Duncan's multiple range tests

Table 4: Mean zone of inhibition for *Candida krusei* with plant extracts

Test plants	Ethanol	Methanol	n-Hexane	Chloroform	Water
Senna alata	20.00±2.00^{a}	20.33±1.53^{a}	13.67±1.53^{b}	8.67±1.15^{c}	9.00±1.73^{c}
Carica papaya	19.33±2.30^{a}	16.67±1.15^{a}	13.67±4.73^{b}	00.00±0.00^{d}	8.67±1.15^{c}
Etlingera elatoir	20.33±1.52^{a}	22.67±2.52^{a}	16.00±2.00^{a}	13.00±2.64^{c}	8.33±0.58^{c}
Canna indica	19.67±2.08^{a}	11.33±2.31^{c}	13.67±1.53^{b}	8.67±1.15^{c}	00.00±0.00^{d}
Vernonia amygdalina	00.00±0.00^{d}	00.00±0.00^{d}	00.00±0.00^{d}	00.00±0.00^{d}	6.67±1.15^{d}
Cymbopogon citratus	17.00±1.00^{a}	10.00±1.73^{c}	11.33±0.58^{c}	8.67±1.15^{c}	00.00±0.00^{d}
Ocimum gratissimum	13.00±2.00^{c}	14.67±1.53^{b}	00.00±0.00^{d}	6.67±1.15^{d}	6.67±1.15^{d}
Aspilia Africana	16.00±1.73^{a}	17.67±2.52^{a}	11.33±1.15^{c}	8.67±1.53^{c}	5.67±0.58^{d}
Momordica charantia	17.00±1.00^{a}	12.33±1.53^{b}	13.00±2.65^{c}	8.67±1.15^{c}	5.67±0.58^{d}
Gossypium barbadense	16.67±1.15^{a}	8.67±1.15^{c}	6.67±1.15^{d}	8.33±0.58^{c}	00.00±0.00^{d}
Blighia sapinda	10.00±2.00^{c}	16.04±1.18^{a}	6.67±1.15^{d}	00.00±0.00^{d}	7.33±1.15^{d}
Carpolobia lutea	13.33±1.52^{c}	15.00±3.00^{b}	00.00±0.00^{d}	8.67±1.15^{c}	7.33±1.15^{d}
Luffa cylindrical	8.67±1.53^{c}	00.00±0.00^{d}	13.00±2.00^{c}	8.67±1.15^{c}	6.67±1.15^{d}
Phyllantus amarus	16.33±1.53^{a}	12.33±1.53^{c}	13.00±2.00^{c}	6.67±1.15^{d}	8.67±1.15^{c}
Alstonia congensis	14.67±1.53^{b}	10.33±2.08^{c}	13.00±2.00^{c}	6.67±1.15^{d}	8.67±1.15^{c}
Nauclea latifolia	12.00±3.00^{c}	16.67±1.15^{a}	11.33±0.58	8.33±0.58^{c}	6.67±1.15^{d}
Diodia scandens	19.00±1.00^{a}	10.00±3.00^{c}	13.00±3.00^{c}	00.00±0.00^{d}	5.67±0.57^{d}
Sphenocentrum	00.00±0.00^{d}	15.33±2.52^{b}	00.00±0.00^{d}	8.33±0.58^{c}	6.67±1.15^{d}
Azadirachta indica	13.33±1.53^{c}	00.00±0.00^{d}	16.00±2.00^{a}	14.33±1.15^{b}	00.00±0.00^{d}
Acalypha wilkesiana	19.33±1.15^{a}	12.33±1.53^{c}	8.67±1.15^{c}	00.00±0.00^{d}	5.67±0.58^{d}
Acalypha godselffiana	16.33±1.53^{a}	10.67±1.54^{c}	13.67±1.53^{c}	10.67±1.15^{c}	6.67±1.15^{d}

Superscripts with same different letters are significantly different (p≥0.05) using Duncan's multiple range tests

The plant *Blighia sapinda* did not inhibit the test organism at all with all the extracts (Table 3). The highest zones of inhibition against *Candida albicans* was recorded in the plants *Senna alata, Etlingera elatoir, Canna indica, Sphenocentrum jollyanum* and *Azadirachta indica.* These recorded inhibition zones of 20 mm and above (Table 3).

Inhibition zones within a plant varied with the type of solvent used for extraction. In the plant *Senna alata* inhibition was strongest with ethanol and methanol extracts (inhibition zone 20 mm and above), although, n-hexane also recorded significant inhibition (10.33 mm zone of inhibtion). There was however no inhibition with the chloroform and aqueous extracts for this plant (Table 3).

All extracts of the plant *Etlingera elatoir* with the various solvents used showed significant to strong inhibition against *C. albicans* (Table 3).

In the plant *Canna indica*, only the methanol extract showed strong inhibition (20.33 mm), while the ethanol extract had significant inhibition (10.00 mm). There was no inhibition with the aqueous extract and chloroform extract did not give any significant inhibition (Table 3).

Only the methanol extract of the plants *Sphenocentrum jollyanum* and *Azdirachta indica* showed strong inhibitory activities while ethanol, chloroform and n-hexane had significant inhibition zones. There was however no inhibition with the aqueous extracts (Table 3). The plant *Vernonia amygdalina* showed significant inhibition with the methanol and ethanol extract, while the chloroform, n-hexane and aqueous extract did not show any inhibition (Table 3). The ethanol, methanol and n-hexane extracts of the plant *Cymbopogon citratus* showed significant inhibition zones, while the chloroform and aqueous extracts showed above average inhibition (Table 3). Chloroform extracts of *Gossypium barbense* performed better than other extracts as it showed significant inhibition while others did not, aqueous and ethanol extracts showed no sign of inhibition (Table 3).

All extracts of *Acalypha wilkesiana* showed inhibition with the highest been the methanol extract that recorded strong activity. The inhibition zones recorded for this plant with methanol and chloroform extracts were also considered significant (Table 3). *Acalypha godselffiana* had chloroform extract showing a higher inhibition zone than the ethanol extract (Table 3). The methanol and aqueous extracts of *Carpolobia lutea* did not record inhibition while the ethanol extract recorded significant inhibition (Table 3).

The best antibiotics for *Candida albicans* were amphotericin B, Clotrimazole and miconazole as these recorded the highest inhibition zones (16.00-16.57) (Table 5). The plants that recorded inhibition zones of 16 mm and above are *Senna alata, Carica papaya, Etlingera elatoir* with ethanol and methanol extracts. *Azdirachtha indica, Acalypha wilkesiana, Acalypha godselffiana* with the methanol extracts. The chloroform extract of *Acalypha godselffiana* showed inhibition above 16 mm (Table 3 and 5). Other plants showed inhibition zones that were comparable to other antibiotics like fluconazole, butoconazole, tioconazole and miconazole with inhibitions zone between 13-14 mm. The plants are *Cymbopogon citratus* (ethanol, methanol and n-hexane), *Momordica charantia* (methanol and n-hexane extracts), *Gossypium barbadense, Sphenocentrum jollyanum, Azdirachtha indica* (ethanol, n-hexane and chloroform extracts), *Acalypha wilkesiana* (ethanol extract) and *Acalypha godselffiana* (n-hexane extract) (Table 3, 5).

Candida krusei: The effect of the plants extracts on *Candida krusei* was slightly different as the plants that showed significant inhibition were up 15 out of 21 plants for ethanol extracts (Table 4).

Table 5: Mean zone of inhibition *Candida albicans* and *Candida krusei* with antibiotics

Antibiotics	*Candida albicans*	*Candida krusei*
Amphotericin B	16.57±1.15[a]	13.67±1.72[a]
Fluconazole	14.33±1.15[a]	15.67±1.15[a]
Butoconazole	13.67±1.53[a]	8.67±1.15[b]
Clotrimazole	16.00±2.00[a]	12.67±1.11[a]
Tioconazole	13.67±1.53[a]	13.00±2.65[a]
Miconazole	16.00±2.00[a]	10.00±2.00[b]

Superscripts with same different letters are significantly different ($p \geq 0.05$) using Duncan's multiple range tests

The number of plants that showed significant inhibition for methanol, n-hexane and chloroform extracts were 17, 14 and 3, respectively against *C. krusei* out of a total of 21 plants tested (Table 4). Plants that recorded inhibition zones higher than 10 mm were considered significant. There was no significant inhibition with the aqueous extracts of the medicinal plants used with this organism (Table 4).

The plants that showed strong inhibitory activities were *Senna alata*, *Etlingera elatoir*, *Canna indica*, *Diodia scandens* and *Acalypha wilkesiana* against *Candida krusei*. These plants recorded inhibition zones of 19 mm and above (Table 4). All extracts in *Senna alata* recorded significant inhibition with the strongest been the ethanol and methanol extracts that recorded over 20 mm inhibition zones (Table 4). The plant *Etlingera elatoir* had all extracts been significant too, with ethanol and methanol showing the strongest activity (Table 4). *Blighia sapinda* that did not show any inhibition for *Candida albicans* did for *Candida krusei*. The ethanol and methanol extracts of this plant showed significant inhibition (Table 4). The ethanol, methanol and n-hexane extracts of *Aspilia Africana*, *Phyllanthus amarus*, *Alstonia congensis*, *Diodia scandens*, *Mimordica charantia* and *Acalypha godselffiana* showed significant inhibition (Table 4). There was no inhibition with the n-hexane extract in the following plants; *Vernonia amygdalina*, *Ocimum gratissimum*, *Carpolobia lutea* and *Sphenocentrum jollyanum* (Table 4). The chloroform extracts of the following plants did not also show any inhibition; *Carica papaya*, *Vernonia amygdalina*, *Blighia sapinda*, *Diodia scandens* and *Acalypha wilkesiana* (Table 4).

The best antibiotic for *Candida krusei* was fluconazole as it recorded the highest zone of inhibition 15.67 mm. The plants that recorded inhibition zones above 15 mm are considered as good as this antibiotic (Table 5).

The ethanol and methanol extracts of these plants are comparable or better than fluconazole the best antibiotic for *Candida krusei* as recorded by this research; *Senna alata*, *Carica papaya*, *Etlingera elatoir*, *Aspilia Africana* (Table 4 and 5). Only the ethanol extracts of the following plants were comparable or better than fluconazole; *Cymbopogon citratus*, *Gossypium barbadense*, *Phyllanthus amarus*, *Diodia scandens*, *Canna indica* and *Acalypha godselffiana* (Table 5). The methanol extracts of the following plants were comparable to the antibiotic fluconazole; *Blighia sapinda*, *Carpolobia lutea*, *Nauclea latifolia* and *Sphenocentrum jollyannum* (Table 4, 5). The n-hexane extracts of the plants *Etlingera elatoir* and *Azadirachtha indica* were also comparable to fluconazole (Table 5).

The mean inhibition zones varied for the various antimicrobial agents or drugs used for the treatment of Candida infections. The highly efficient ones were Amphotercin B, Clotrimazole and Miconale foe *Candida albicans* and Fluconazole for *Candida krusei* (Table 5). There were some plant extracts that recorded inhibition zones higher than those recorded for even the most efficacious antibiotics (Table 3, 4).

Table 6: Minimum inhibitory concentration of plant extracts with *Candida albicans* and *Candida krusei*

Scientific name	MIC *Candida albicans* (mg mL^{-1})	RI *Candida albicans*	MIC *Candida krusei* (mg mL^{-1})	RI *Candida krusei*
Senna alata	3.3	5-18	5	5-18
Carica papaya	5	6-18	10	5-17
Etlingera elatoir	2.5	4-19	3.3	6-19
Canna indica	5	5-18	20	6-9
Vernonia amygdalina	-	-	20	5-10
Cymbopogon citratus	5	6-15	10	6-14
Ocimum gratissimum	10	5-8	20	8-12
Aspilia Africana	10	6-15	20	6-10
Momordica charantia	3.3	6-15	20	6-10
Gossypium barbadense	10	7-14	20	8-14
Blighia sapinda	100	8	-	-
Carpolobia lutea	100	10	40	8-10
Luffa cyllindrica	20	6-8	100	8
Phyllanthus amarus	6.7	5-15	10	5-14
Acalypha wilkesiana	6.7	7-16	20	7-13
Alstonia congensis	10	5-10	100	7
Nauclea latifolia	-	6-12	-	-
Diodia scandens	-	-	100	8
Sphenocentrum jollyanum	10	6-8	20	6-10
Azadirachta indica	3	6-12	10	6-14
Acalypha godseffiana	10	6-15	20	6-10

MIC: Minimum inhibitory concentration, RI: Ranged of inhibition

The minimum inhibitory concentrations of the plant extracts varied. Some plants were more efficacious than others as their minimum inhibitory concentrations varied. Plants like *Blighia sapinda* and *Carpolobia lutea* did not inhibit Candida growth at concentrations less than 100 mg mL^{-1}. Other plants had minimum inhibitory concentrations that were between 3-10 mg mL^{-1} and these were considered very efficacious (Table 6). No minimum inhibitory concentration could be established for *Diodia scandens*, *Nauclea latifolia* and *Vernonia amygdalina* (Table 6).

DISCUSSION

The medicinal plants used for this study are those used traditionally among the urhobo tribe in Ughelli South Local Government Area of Delta State, Nigeria to treat different ailments. The results show that over 70% of these plants showed some degree of anticandida activity. The plants have also been used in literature with varying results. Runyoro *et al.* (2006) used the plant *Carica papaya* against Candida and reported that it had no effect but this result indicates that it does. This could be attributed to extraction methods as the aqueous and chloroform extracts in this study did not show any significant activity (Table 3 and 4). The type of solvent used affected the degree of inhibition for the various medicinal plants used. This indicates that extracting the right compounds from the plants with the right solvent is very important. The number of plants that were active in this study emphasizes the fact that the traditional herbal practitioners should not be ignored as they hold the key to discovery of new drugs from plant sources in Africa. Some of the plants reported here as active against Candidiasis have been reported before as either active against other fungi or Candida. Some have not been associated with antifungal activities before

now. Plants that were not active against *Candida albicans* were active against *C. krusei* (Table 3, 4). This indicates that sensitivity tests should be carried out before recommendation of antibiotics. Anti- candida albicans activity had been recorded for the plant *Cymbopogon martini* and *C. winterianus* (Duarte *et al.*, 2005). The ethanol extract for these plants were not effective but the water distillation method also indicating that the method of extraction affects the inhibitory activity of the extract, implying that different bioactive compounds are extracted with the different extracts. The compounds implicated for the inhibitory activity isolated from medicinal plants include 1, 8-cineole, geranial, germacrene-D, limonene, linalool and menthol (Duarte *et al.*, 2005). In this study a broad classification of chemical extracts from the plants was made into alkaloids, phlobatannins, cardiac glycosides, tannins, terpenoids, saponins, flavonoids which were present in varying concentrations and absent in some (Table 3). The presence of these compounds indicates that the plants have medicinal values and this varies with the compound and their concentration. The researchers here admit that there is need to extract, purify and identify the exact compounds responsible for the inhibitory properties in the plants that showed high or strong anti-candida activity and is an on-going research. The fruits and seeds of *Blighia sapinda* K. Konig (Sapindaceae) and the leaves had been previously reported to be poisonous by Ajibesin *et al.* (2002) did not inhibit the growth of *Candida albicans*. The methanol extract of the same plant however showed significant inhibition with *Candida krusei*.

Makinde *et al.* (2007) reported that *Senna* (*Cassia*) *alata* inhibited the growth of *Candida albicans*, but Palanichamy and Nagarajan (1990) reported that ethanolic extracts of *Senna alata* did not have any inhibitory effect on *Candida albicans*. This report however shows that the ethanolic extracts inhibited the growth of *Candida albicans* agreeing with Makinde *et al.* (2007). The chloroform and aqueous extracts of the plant however, did not inhibit the growth of *Candida krusei* (Table 3, 4).

Other researchers have also resulted to the use of medicinal plants when there were reports of drug resistance with *Candida albicans*. Bonjar (2004) reported that clotrimazole resistant *Candida albicans* responded to methanol extracts of nineteen plants species used in Iranian folklore medicine. Okigbo and Mmeka (2008) reported that the ethanol, cold and hot water extracts of the plants *Vernonia amygdalina* and *Cymbopogon citratus* inhibited the growth of *Candida albicans*. Other researchers have recorded similar results as shown here like that of Sule *et al.* (2010); they reported that ethanol extracts of *Senna alata* had antifungal activities.

Owoyale *et al.* (2005) recorded that the methanolic extracts of *Senna alata* leaves showed the highest antifungal activity when compared with ethanol and petroleum ether extracts. There was however no significant difference between the performance of ethanol and methanol extracts in this study, they however recorded the highest anti-candida activity (Table 3, 4).

The results of Lachumy *et al.* (2010) agrees with the current research with regards to the plant *Etlingera elatoir*. The plant was effective against yeast and other fungal species with methanol extract in their study. Santos *et al.* (2012) drew the same conclusion as this research with regards to *Momordica charantia* as it was able to significantly inhibit the growth of both *C. albicans* and *C. krusei* as recorded here. Agaraku and Nwokedi (2004) recorded anti-candida activity with *Luffa cylindrica* with ethanol extract but in this investigation, only n-hexane extract showed significant inhibition; the ethanol extract did not. There was also significant inhibition of *Candida albicans* by *Nauclea latifolia* and this agrees with the report of Tekwu *et al.* (2012).

CONCLUSION

The extraction of active compounds from medicinal plants hold the key to new antimicrobials and this research emphasize the need to get the right solvent for extraction of the various

compounds. The compounds extracted can be purified, properly identified and more investigations carried out on them before they can be recommended for use as new antimicrobials. The local people use herbs for the treatment of candidiasis and other ailments, this should not be discouraged but the practitioners should be helped to standardize their methods.

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