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Full Length Research Paper

In vitro susceptibility of clinical *Candida* species to some natural and synthetic antimycotic agents

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Abstract

Eighteen antifungal drugs, five mentholated balms, sulphur and crude extracts of garlic (*Allium sativum*), African basil (*Ocimum gratissimum*) and ginger (*Zingiber officinale*) were tested for *in vitro* inhibitory potentials on 107 *Candida* strains obtained from human oral, high vaginal and endocervical swabs. Between 47.1 and 92.0% (*C. albicans*); 50.0 and 100% (*C. glabrata*); 27.2 and 90.9% (*C. pseudotropicalis*) and 57.1 and 100% (*C. tropicalis*) respectively were resistant to the antifungal drugs. Only Candiderm cream (clotrimazole) was generally least resisted (27.3-57.1%) by the *Candida* species, while all the *Candida* strains were resistant to the mentholated balms and sulphur. Garlic and ginger were respectively inhibitory against 33.3-54.4% and 50.0-72.7% of the *Candida* strains but low (14.2-16.1%) inhibition was recorded in African basil. In conclusion, significant *in vitro* resistance among vulvovaginal *Candida* species to some classes of antifungal drugs was recorded, indicating a serious clinical challenge in treatment of oral and vulvovaginal candidasis. Meanwhile, this study also reported significant *in vitro* inhibitory potentials of crude extracts of garlic and ginger on azole- / griseofulvin-susceptible and resistant *Candida* species, and thereby, can serve as natural adjunct options for the treatment of oral and vulvovaginal candidasis, either as herbal toothpastes, topical anti-*Candida* agents, suppositories or as douches.

Keywords: *Allium sativum*, antifungal resistance, medicinal plants, mentholated balms, vulvovaginal candidasis, *Zingiber officinale*.

INTRODUCTION

Oral candidasis, which principally affect the oral cavities of males and females, as well as vulvovaginal candidiasis (VVC) that affect the vulva and vagina are caused by abnormal growth of *Candida* species that normally inhabit the oral and genital tracts. They are well-known infections of public health importance that affects millions of patients worldwide every year (Sobel, 1993; Shaheen and Taha, 2006; Sobel, 2007; Abraham, 2011; Dalben-Dota *et al*., 2011). Incidences of oral and VVC have been reportedly increasing in recent years (Shaheen and Taha, 2006; Ogunshe *et al*., 2009; Abraham, 2011; Dalben-Dota *et al*., 2011), while it is also recorded that approximately 75% of adult women have at least 1 episode of VVC in their lifetimes, and of these, 40 - 50% will undergo new outbreaks of which 5-8% result in recurrent vulvovaginal candidiasis (RVVC), with 4 or more episodes per year (Sobel, 2007). Also disturbing is the increasing resistance to commonly available antifungal agents (Gallè *et al*., 2011; Ogunshe *et al*., 2009, 2011a, b).

Certain *Candida* species are peculiar with oral and VVC, while some rare *Candida* spp. are also occasionally isolated from cases of oral and VVC, although the most-commonly implicated principal aetiological agents of candidasis include *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. lusitaniae* etc. (Holland *et al*., 2003;Ozcan *et al*., 2006; Rivera-Sánchez *et al*., 2006; Shaheen and Taha, 2006; Dota *et al*., 2008; Vermitsky *et al*., 2008; Ogunshe *et al*., 2009; Hetticarachchi *et al*., 2010; Kennedy and Sobel, 2010; Abraham, 2011; Gallè *et al*., 2011; Martins *et al*., 2011). Clinical experiences

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also indicated that induced virulence of these pathogens in the oral and lower genital tracts and their causal role in symptomatic patients require careful case-by-case determination rather than routine administration of antimycotic therapy (Kennedy and Sobel, 2010; Abraham, 2011; Gallè *et al*., 2011).

Symptoms in oral candidosis are mostly general (Abraham, 2011; Gallè *et al*., 2011) but overgrowth of *Candida* in the vagina produces localised symptoms of itching, burning and irritation, while abnormal discharge, characterised by cottage cheese-like consistency may also occur. VVC can cause serious discomforts in females and also affects sexual activities (Sojakova *et al*., 2004) and the increased number of oral (Gallè *et al*., 2011) and vaginal yeast infections in the past few years has however, been a disturbing trend, with several theories being put forth to explain the apparent increase by opportunistic fungal infections. Some women first attempt to cure vulvovaginal yeast infections with do-it-yourself treatments and natural remedies before turning to over-the-counter antifungal preparations (Dun, 1999); whereas, misuse of antifungal agents can also lead to colonisation with less susceptible species and to resistance among normally susceptible strains (Sobel *et al*., 2001).

Management of patients with VVC is often difficult because most of the commonly available antifungal agents of choice for the treatment have been resisted, leading to the need for new therapeutic options for VVC (Damke *et al*., 2011). Unlike in some countries, (Bergner, 2001), there is current sparsity of scientific data on bacteriostatic or bactericidal effects of medicinal plants and other non-antifungal drugs on clinical *Candida* spp. in Nigeria, especially with regards to isolates recovered from VVC. The aim of this study therefore, was to determine and compare *in vitro* inhibitory potentials of three suggested plant extracts and non-antifungal drug products (mentholated balms and sulphur) on clinical *Candida* species. *In vitro* susceptibility / resistance of the *Candida* species to various antifungal drugs that were commonly available in Nigeria was also carried out.

MATERIALS AND METHODS

Candida cultures

A total of 107 identified Candida strains used in the present study were C. albicans 87 (81.3%), C. glabrata 2 (1.97%), C. pseudotropicalis 11 (10.3%) and C. tropicalis 7 (6.54%). They were originally isolated from human oral swabs, high vaginal swabs (HVS) and endocervical swabs (ECS) of symptomatic patients presenting at the Department of Microbiology, Faculty of Science, University of Ibadan, Nigeria. The *Candida* strains were assayed for their *in vitro* susceptibility and resistance rates to antifungal drugs, mentholated balms, sulphur and crude extracts of three medicinal plants- garlic (*Allium sativum*), African basil (*Ocimum gratissimum*) and ginger (*Zingiber officinale*).

Antifungal drugs

Antifungal creams, caplets and powders were purchased from registered pharmacies, which obtained their products from the manufacturers. Presentations and active ingredients of the antifungal agents used in this study are as shown in Table 1. Ten gram each of the test antifungal creams, tablets / capsules and powders were separately homogenised in 10 ml (1:1 wt/vol) of sterile distilled water to make pasty suspensions.

Preparation of plant samples

Freshly prepared crude plant extracts of garlic (*Allium sativum*) [*áayú*: Yoruba], ginger (*Zingiber officinale*) [*ata'lè*: Yoruba] and African basil (*Ocimum gratissimum*) [*efinrin*: Yoruba] with no preservatives or chemicals were aseptically used for bioassay studies. The extraction method used was the simulation of the suggested methods prescribed by herbal sellers. 100 g of each fresh garlic cloves, ginger and African basil leaves were separately marcerated with sterile pestle in sterile stainless cups and the crude extracts (pastes) separately dispensed directly into the agar wells (Ekanola *et al*., 2013).

Mentholated balms

Mentholated balms (Robb, Aboliki, Heatol, Vilicin, Essentials) were purchased from registered pharmacies, which obtained their products from the manufacturers. The mentholated balms were separately dissolved at warm (45^0C) temperature, while crude sulphur were separately powdered and dissolved in warm (45^0C) sterile distilled water (10g/20ml wt/v) to prepare pasty suspensions.

Antimicrobial assay for detection of *in vitro* inhibitory activities

Detection of *in vitro* antagonistic activities of each antifungal agent, crude plant extract, sulphur and mentholated balm was determined using a modification of agar well-diffusion method of Tagg *et al*. (1976). Wells, 6.0 mm in diameter were bored into sterile Sabouraud dextrose agar (SDA) plates and each plate was later seeded with 500µl of 10^3 cfu ml^{-1} each of the *Candida*

Table 1. Presentations, active ingredients and overall resistance rates of antifungal agents

Lab codes	Antifungal agents	active ingredients	overall resistance rates
Drugs			
F1	Medcan capsule 50mg	fluconazole	36.4-85.7%
F2	Vulcan-50 capsule 50mg	fluconazole	54.5-100%
F3	Diflucan tablet	fluconazole	50.0-90.9%
F4	Lucon tablet	fluconazole	72.7-100%
G1	Fesovin tablet 500mg	griseofulvin	50.0-100%
G2	Grufin tablet 500mg	griseofulvin	54.5-100%
K4	Ketoconazole tablet 200mg	ketoconazole	0.0-92.0%
T2	Gyno-Tiocosid vaginal tablet	tioconazole	0.0-71.4%
Creams			
C1	Candiderm cream	clotrimazole	27.3-57.1%
C2	Canexcream	clotrimazole	71.4-100%
C3	Funbact A cream	clotrimazole	71.4-100%
C4	Mycoten cream	clotrimazole	45.5-82.8%
K3	Ketofung cream	ketoconazole	72.7-100%
K1	Nizoral cream	ketoconazole	36.4-71.4%
K2	Skineal cream	ketoconazole	50.0-86.2%
M1	Darktarin cream	miconazole	45.5-100%
Powders			
M2	Fungusol powder	miconazole	50.0-100%
T1	Trosyd powder	tioconazole	54.5-100%

strains by surface streaking. Prepared suspensions (500µl) of each of the antifungal drugs, mentholated balms, sulphur and crude plant extracts, were separately dispensed into the agar wells. The plates were incubated at 30^0C for 24-48 hours, after which zones of inhibition were measured and recorded in mm diameter, while zones of inhibition less than 10.0 mm in diameter or absence of inhibition zones were recorded as resistant (negative).

RESULTS

Out of a total of 18 antifungal drugs tested for their *in vitro* inhibitory activities against the 107 *Candida* strains, significantly high resistance rates were recorded, while only few of the *Candida* strains were susceptible to the antifungal drugs overall (Table 1). Among the clotrimazoles, recorded resistance rates were between 27.3 and 100%, while 36.4-100% resistance rates were recorded among the fluconazoles. Griseofulvin drugs were also generally resisted by the *Candida* strains, exhibiting resistance rates of 50.0-100%. Some of the *Candida* strains were more susceptible to the test ketoconazole tablet (0.0-92.0%) but higher overall resistance were exhibited by the *Candida* strains towards the ketoconazole creams (36.4-100%). The miconazoles (45.5-100%) and tioconazole powder (54.5-100%) were similarly moderately or highly resisted but some of the *Candida* strains were more susceptible to the test tioconazole tablet (0.0-71.4%).

Low to significantly high percentage multiple antifungal resistance (%MAR) of 16.7-100%, 44.4-88.9%, 22.2-88.9% and 44.4-94.4% were recorded among the *C. albicans*, *C. glabrata*, *C. pseudotropicalis* and *C. tropicalis* respectively (Table 2). Based on zones of inhibitions, apart from the significantly wider zones of inhibition (25.0 mm) recorded among some strains of *C. albicans*, similar range of zones of inhibition (10.0-15.0 / 10.0-20.0 mm) were generally recorded among the remaining *Candida* strains (Table 2).

Crude extract of *Ocimum gratissimum* (African basil) was the least inhibitory plant extract *in vitro*, to which 14.3% of the *C. tropicalis* and 16.1% *C. albicans* strains were susceptible, while moderate and high *in vitro* inhibition rates were exhibited by ginger (*Zingiber officinale*) and garlic (*Allium sativum*), to which 33.3-54.5% and 50.0-72.7% of the *Candida* strains were susceptible respectively. Although none of the *C. glabrata* and *C. pseudotropicalis* was inhibited but based on zones of inhibition, similar *in vitro* inhibitory profiles were exhibited by the *Candida* strains towards the plant extracts, *Ocimum gratissimum* (10.0-20.0 mm diameter), *Zingiber officinale* (10.0-16.0 mm diameter) and *Allium sativum* (10.0-20.0 mm diameter). All the five mentholated balms and sulphur were however; totally resisted *in vitro* by all the 107 *Candida* strains (Table 3).

DISCUSSION

Many antimycotics are available as therapeutic agents for treatment of VVC, usually in topical and oral forms, as

Table 2. *In vitro* percentage resistance rates of clinical *Candida* strains against antifungal drugs

***Candida* species**		**Antifungal drugs**																
C1	**C2**	**C3**	**C4**	**F1**	**F2**	**F3**	**F4**	**G1**	**G2**	**K1**	**K2**	**K3**	**K4**	**M1**	**M2**	**T1**	**T2**	**%MAR**
% Resistance rates / Zones of inhibition (mm diameter)																		
***C. albicans* [87]**		47.1	90.8	82.8	82.8	65.5	69.0	87.4	92.0	75.9	74.7	67.8	86.2	81.6	92.0	63.2	94.3	75.9	58.6
	16.7-100	(10.0-25.0)	(10.0-15.0)	(10.0-20.0)	(10.0-20.0)	(10.0-25.0)	(10.0-25.0)	(10.0-20.0)	(15.0-20.0)	(10.0-25.0)	(10.0-25.0)	(10.0-25.0)	(10.0-20.0)	(10.0-25.0)	(10.0-25.0)	(10.0-25.0)	(10.0-20.0)	(10.0-30.0)	(10.0-30.0)
***C. glabrata* [2]**		50.0	100	100	50.0	50.0	100	50.0	100	50.0	100	50.0	50.0	100	0.0	100	50.0	100	0.0
	44.4-88.9	(20.0)	(-)	(-)	(15.0)	(20.0)	(-)	(10.0)	(-)	(20.0)	(-)	(20.0)	(10.0)	(-)	(10.0)	(-)	(15.0)	(-)	(10.0)
***C. pseudo-tropicalis* [11]**		27.3	81.8	90.9	45.5	36.4	54.5	90.9	72.7	90.9	54.5	36.4	72.7	72.7	81.8	45.5	63.5	54.5	36.4
	22.8-88.9	(10.0-20.0)	(10.0-20.0)	(10.0)	(10.0-15.0)	(10.0-20.0)	(10.0-20.0)	(15.0)	(10.0)	(14.0)	(10.0-20.0)	(10.0-20.0)	(10.0-20.0)	(10.0-20.0)	(10.0-20.0)	(10.0-20.0)	(10.0-20.0)	(10.0-20.0)	(10.0-15.0)
***C. tropicalis* [7]**		57.1	71.4	71.4	57.1	85.7	71.4	85.7	100	100	85.7	71.4	85.7	100	85.7	71.4	100	85.7	71.4
	44.4-94.4	(15.0 25.0	(10.0-20.0)	(10.0-20.0)	(10.0)	(15.0)	(15.0)	(20.0)	(-)	(-)	(20.0)	(10.0-20.0)	(10.0-20.0)	(-)	(10.0-20.0)	(15.0)	(-)	(20.0)	(20.0)

Keys: C1 = candiderm cream [clotrimazole]; C2 = canexcream [clotrimazole]; C3 = Funbact A cream [clotrimazole]; C4 = Mycoten cream [clotrimazole]; F1 = Medican capsule 50mg [fluconazole]; F2 = Vulcan-50 capsule 50mg [fluconazole]; F3 = Diflucan [fluconazole]; F4 = Lucon tablet [fluconazole]; G1 = Fesovin tablet 500mg [griseofulvin]; G2 = Grufin tablet 500mg [griseofulvin]; K1 = Nizoral cream [ketoconazole]; K2 = Skineal cream [ketoconazole]; K3 = Ketofung cream [ketoconazole]; K4 = Ketoconazole tablet 200mg [ketoconazole]; M1 = Darktarin cream [miconazole]; M2 = Fungusol powder [miconazole]; T1 = Trosyd powder [tioconazole]; T2 = Gyno-Tiocosid vaginal tablet [tioconazole].
Values in parenthesis are zones of inhibition in mm diameter
*** = least resisted; ** = less resisted; * = moderately resisted; ± = most resisted; % MAR= % multiple antibiotic resistance.

well as some vaginal formulations (Sobel, 1993; Watson and Calabretto, 2007), and among them are fluconazoles, miconazoles, itraconazoles, ketoconazoles, griseofulvins and polyenes, such as nystatin. However, certain factors tend to reduce the availability of the antifungal agents below that of the effective therapeutic concentrations, and the yeasts thereby, undergo only a limited exposure to the antifungals during therapy (Ellepola and Samaranayake, 1998). In Nigeria, azoles and other few available classes of antifungal agents are used by a larger population of females as treatment of choice for VVC, most times indiscriminately. Whereas, in the current study, apart from the two *C. glabrata* strains, which were susceptible to the azoles, and also the moderate 36.4% resistance recorded among the *C. pseudotropicalis* strains, as high as 50.0-100% resistance rates were recorded towards the test antifungal agents, while even as high as 44.4-100% multiple antifungal resistance rates were recorded. These findings therefore, highlighted that antifungal resistance to azoles and griseofulvin was apparent in the study population,

Table 3. *In vitro* anti-candidal activities of crude plant extracts, mentholated balms and sulphur on clinical *Candida* strains

***Candida* species**	**Plant extracts**			**Mentholated balms**					
Sulphur									
	Garlic	***Ocimum***	**Ginger**	***Aboliki***	**Essential**	**Heat-on**	**Robb**	**Vilcin**	
C. albicans [87]	72.4 (10.0-20.0)	16.1 (10.0-20.0)	33.3 (10.0-16.0)	0.0 (-)	0.0 (-)	0.0 (-)	0.0 (-)	0.0 (-)	0.0 (-)
C. glabrata [2]	50.0 (15.0)	0.0 (-)	50.0 (15.0)	0.0 (-)	0.0 (-)	0.0 (-)	0.0 (-)	0.0 (-)	0.0 (-)
C. pseudotropicalis [11]	72.7 (10.0-15.0)	0.0 (10.0)	54.5 (10.0-15.0)	0.0 (-)	0.0 (-)	0.0 (-)	0.0 (-)	0.0 (-)	0.0 (-)
C. tropicalis [7]	71.4 (10.0-15.0)	14.3 (10.0)	42.9 (10.0-15.0)	0.0 (-)	0.0 (-)	0.0 (-)	0.0 (-)	0.0 (-)	0.0 (-)

Keys: garlic (*Allium sativum*), ginger (*Zingiber officinale*), *efinrin* (*Ocimum gratissimum*)
Values in parenthesis are zones of inhibition in mm diameter

just as previously documented in other similar studies (Ribeiro *et al*., 2001; Richter *et al*., 2005; Fan *et al*., 2007; Asticcioli *et al*., 2009; Ogunshe *et al*., 2011).

Long-term and intense azole-based and other antifungal treatments have been linked to increase in resistant *Candida* and non-*Candida* fungal species (Dun, 1999), as well as adverse side effects like toxicity and kidney / renal problems. Meanwhile, in spite that higher rates of antifungal resistance is being considered a serious concern in several developed countries, current studies on pathogenic fungi in Nigeria are quite limited, and are also not attracting required attention and redress. The future of limiting such clinical disadvantages thus, lies in identifying the factors that promote resistance, and implementing policies to prevent them, most especially since there is noticeable and significant increasing resistance by *Candida* species to commonly available antifungal drugs in Nigeria. by *Candida* species. A major alternative has been development of natural agents as therapeutic agents for treatment of oral and VVC, and in a study conducted through the use of questionnaire administration, it was reported that a large number of Nigerian females across various age ranges used mentholated balms (with menthol being the active ingredient) as topical *relief* therapy in cases of vulvovaginal itching (Pers. Comm.).

Menthol ($C_{10}H_{20}O$) is a waxy, crystalline, clear or white in colour organic terpenoid compound that is extracted from peppermint and other mints, although it can be synthetically prepared. It is solid at room temperature but melts slightly above room temperature and its antibacterial property makes it to be used in topical preparations to assist their permeation through the skin surface (http://www.assistpainrelief.com/dyn/303/ Menthol.html).However, all the five brands of commonly available mentholated balms in the country used in the current study, which were with a menthol smell, were totally resisted *in vitro* by all the *Candida* strains, indicating the non-inhibitory potentials of the mentholated balms *in vitro*. In spite of all the information available in literature, no studies on synthetic antifungal potentials of menthol were obtained; rather, inhibitory potentials of natural menthol from essential oils and medicinal plants were reported. Therefore, the industrial mentholated balms, which were used in the current study probably assisted in temporary relief due to the menthol effect during itching or after-cool effects of menthol on the skin. Menthol is an ingredient known for its cooling properties, by reacting with thermoreceptors in the skin, thereby, replacing the pain message with a cooling sensation in order to provide temporary relief from localised pain, and also provides relief from itching by engendering a thermal sensation that replaces the irritation (http://www .assistpainrelief .com/dyn/303/Menthol.html).

Elemental sulphur is a bright yellow crystalline

solid at room temperature, and being abundant in native form; sulphur was known and used in ancient times as fumigants, while sulphur-containing medicinal mixtures were used as balms and antiparasitics (Wikipedia, 2013). Sulphur was documented to have antifungal, antibacterial and keratolytic activities (Weld and Gunther, 1947), while 10% topical sulphur was even found comparable to the performance of oral tetracycline (Gupta and Nicol, 2004). However, crude sulphur samples, which were assayed for in this study were totally resisted *in vitro* by all the *Candida* strains, indicating their non-inhibitory potentials on *Candida in vitro*, in spite of the fact that sulphur is commonly used as crude suphur powder added to some body creams or local shear butter, and also sometimes used for treating fungal infections as industrial suphur ointments. Sulphur ointment, with generic name of precipitated sulfur (5% - 10 %), is available over the counter under many brand names and has been reported to have mild antifungal and antibacterial qualities and also useful for treating some skin disorders, like scabies seborrheic dermatitis (a flaky skin condition known as cradle cap in infant), pimples, acne in adults and children (Green, 2013). Therefore, it is likely that the potency of sulphur may be enhanced in combined form with other agents as in the cases of sulphur formulations for treatment of candidosis.

Consistent reports of antifungal resistance has led to search for alternative bio-agents, such as, investigating the potentials of various medicinal plants as antifungal agents, which had been a longtime advocacy of possible treatment regimen for vulvovaginal candidasis. Few studies have reported the antimicrobial activities of ethanolic extracts of same medicinal plants / spices used (Ficker *et al*., 2003; Nweze and Eze, 2009; Oboh *et al*., 2009; Atai *et al*., 2009; Wikipedia, 2011a) but in this study, crude extract of *Ocimum gratissimum* was minimally inhibitory *in vitro*, while crude extracts of ginger and garlic were moderately and highly inhibitory *in vitro* respectively. Ginger (*Zingiber officinale*), which is a rhizome with a warm, sweet, strongly aromatic odour and sharp pungent flavour is popularly known for its culinary and medicinal properties (Wikipedia, 2011b). However, just as it was reported for its moderate inhibitory potentials against wound-borne Gram-positive and Gram-negative bacteria in the study of Ekanola *et al*. (2013), ginger also generally exhibited moderate inhibitory potentials towards human clinical *Candida* species, probably due to the known identified active agents, gingerol and shagelol (Ficker *et al*., 2003; Zahra *et al*., 2009; Supreetha *et al*., 2011).

Garlic (*Allium sativum*) has been used as a spice, food and folk medicine since ancient times (Block, 1985), and in addition to garlic and ginger being listed on the Food and Drug Administration (FDA) asgenerally recognised as safe GRAS (O'Hara *et al*., 1998; Weidner and Sigwart, 2000) for human consumption, numerous reports indicated that garlic extract has broad spectrum antimicrobial activities (Tynecka and Gos,1973; Appleton and Tansey, 1975; Barone and Tansey, 1977; Adetumbi *et al*., 1983; Wikipedia, 2011b, c; Ekanola *et al*., 2013). It also has been reported to have protective effect against *in vivo* experimental fungal infections (Prasad *et al*., 1982), including *Candida* vaginitis (Sandhu *et al*., 1980). Garlic and elephant garlic clove homogenates / supplement products have also been documented to demonstrate greater anti-candidal activities than a number of onion types, with the major growth inhibitory components recognised as diallyl thiosulphinate (allicin), methyl allyl thiosulphinate and allyl methyl thiosulphinate, found in aqueous garlic clove and powder homogenates (Cavallito and Bailey, 1944; Stoll and Seeback, 1951; Wills, 1956; Amonkar and Banerji, 1971; Appleton and Tansey, 1975; Barone and Tansey, 1977; Hughes and Lawson, 1991; Wikipedia, 2011c).

Kabelik (1970) also demonstrated that garlic extract was more effective against pathogenic yeasts, especially *C. albicans* than nystatin, gentian violet or methylene blue. In the study of Adetumbi *et al*. (1986), it was found that the anti-candidal effect of aqueous garlic extract on *C. albicans* was by complete inhibition of biosyntheses of macromolecules of lipid synthesis, i.e., blockage of lipid production by garlic extract is an important physiological mechanism for growth inhibition in *Candida*. Based on scanning electron microscopy and cell leakage studies on the mode of action of aqueous garlic extract in *C. albicans*, it was observed that garlic treatment affected the structure and integrity of the outer surface of the yeast cells (Ghannoum, 1988; Wikipedia, 2011c). All these mechanisms of action probably accounted for the significant *in vitro* inhibitory potentials recorded in garlic against pathogenic *Candida* species in this study. It therefore confirms that medicinal plants are not only inhibitory on indigenous oral bacterial pathogens (Ogunshe and Odumosu, 2010) but also on oral, as well as vulvovaginal *Candida*.

In conclusion, the current study indicated that oral and vulvovaginal *Candida* strains from a number of Nigerian patients were mostly azole- and griseofulvin-resistant but the moderate *in vitro* inhibitory potentials of crude extracts of garlic and ginger on the azole- / griseofulvin-susceptible and multi-resistant *Candida* species further suggested their potentials in serving as natural adjunct options for the treatment of oral and vulvovaginal candidasis, either as anti- Candida or as oral agents incorporated into toothpastes or as suppositories, douches. agents like herbal toothpastes.

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