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Short Communication

In Vitro Antimicrobial Properties of Coconut Oil on *Candida* Species in Ibadan, Nigeria

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ABSTRACT The emergence of antimicrobial resistance, coupled with the availability of fewer antifungal agents with fungicidal actions, prompted this present study to characterize *Candida* species in our environment and determine the effectiveness of virgin coconut oil as an antifungal agent on these species. In 2004, 52 recent isolates of *Candida* species were obtained from clinical specimens sent to the Medical Microbiology Laboratory, University College Hospital, Ibadan, Nigeria. Their susceptibilities to virgin coconut oil and fluconazole were studied by using the agar-well diffusion technique. *Candida albicans* was the most common isolate from clinical specimens (17); others were *Candida glabrata* (nine), *Candida tropicalis* (seven), *Candida parapsilosis* (seven), *Candida stellatoidea* (six), and *Candida krusei* (six). *C. albicans* had the highest susceptibility to coconut oil (100%), with a minimum inhibitory concentration (MIC) of 25% (1:4 dilution), while fluconazole had 100% susceptibility at an MIC of 64 μg/mL (1:2 dilution). *C. krusei* showed the highest resistance to coconut oil with an MIC of 100% (undiluted), while fluconazole had an MIC of >128 μg/mL. It is noteworthy that coconut oil was active against species of *Candida* at 100% concentration compared to fluconazole. Coconut oil should be used in the treatment of fungal infections in view of emerging drug-resistant *Candida* species.

KEY WORDS: • *coconut oil* • **Candida** *species* • *susceptibility*

INTRODUCTION

When antibiotics are used, normal bacterial commensals are often eliminated along with pathogenic ones. Consequently yeasts such as *Candida* species, which are not affected by antibiotics, overgrow unrestrained. The consequence is a yeast overgrowth implying infection.[1] The increase in immunocompromised hosts as a result of the spread of human immunodeficiency virus infection, the increased use of immunosuppressive agents in organ transplantation, aggressive anticancer chemotherapy, and improved life-saving medical techniques necessitating in-dwelling catheters have led to a substantial increase in the occurrence of serious infections.[2] Progress in the development of both topical and systemic antifungal agents has lagged behind that of antibacterial agents,[3] in part because of intensive research efforts in the area of antibacterial therapy that began in the 1940s following the large-scale production of penicillin[3] and also because of the relatively low incidence of serious fungal infections compared with that of bacterial infections.[4]

Of the two imidazole drugs currently in use for the treatment of systemic fungal infections, ketoconazole is administered orally, while miconazole is given intravenously. At concentrations of 10^{-5}–10^{-4} *M*, miconazole exerts a marked lethal effect against logarithmic-phase yeast cells of *Candida albicans*, but ketoconazole does not.[5] Vibunazole (Bay n 7133) and ICI 153,066, which are triazoles, have been found to possess insignificant fungicidal activity.[6] Fluconazole is the most recent of these promising oral triazoles. It has been reported to have better *in vivo* efficacy than ketoconazole for systemic candidiasis in immunocompromised as well as normal mice,[7,8] greater water solubility, and better oral bioavailability and produces higher plasma and extravascular concentrations. It possesses a longer plasma half-live, greater metabolic stability, and lower protein binding than ketoconazole.[9]

Fluconazole is well established as a first-line management option for the treatment and prophylaxis of localized and systemic *C. albicans* infections.[5] The increasing use of fluconazole for the long-term prophylaxis and treatment of recurrent oral candidiasis in acquired immunodeficiency syndrome patients has led to the emergence of *C. albicans* infections that

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are not responsive to conventional doses.[10] The emergence of this resistance, coupled with the availability of fewer antifungal agents with fungicidal actions centered on inhibition of ergosterol synthesis, has provided the need to explore nature in search of phytotherapeutic agents with novel targets and mode of action. The practice of complementary and alternative medicine is now on the increase[11] in developing countries in response to World Health Organization directives culminating in several preclinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections.[12]

Virgin coconut oil, a potent nondrug or natural yeast-fighter, contains three medium chain fatty acids, *i.e*., lauric acid (50–53%), caprylic acid, and capric acid, all of which have antifungal effect against *Candida* and other fungi.[13] Medium-chain free fatty acids have been found to have a broad spectrum of microbicidal activity.[14] The mechanism by which the lipids kill bacteria is not known, but electron microscope studies indicate that they disrupt cell membranes.[15] Variations in composition, plant, and genetic disparity among bacteria and fungi of the same or different species have been found to be responsible for the few inconsistencies in the antibacterial and antifungal properties of plant extract.[16] Apparently, little or no work has been done on coconut oil in this country to ascertain its antimicrobial effect in view of the emergence of multi–drug-resistant strains of *Candida* species.

We therefore set out to characterize *Candida* species in our environment and determine the effectiveness of virgin coconut oil as an antifungal agent and to compare this with the activity of fluconazole.

MATERIALS AND METHODS

Coconut oil preparation

Fresh coconut (*Cocos nucifera*) was obtained from the Institute of Agricultural Research and Training Centre, Apata,

TABLE 1. COMPARATIVE SUSCEPTIBILITY PATTERN OF ISOLATES

	Susceptibility at given dilution (concentration)															
	Neat (100%)		*1:2 (50%)*		*1:4 (25%)*		*1:8 (12.5%)*		*1:16 (6.25%)*		*1:32 (3.13%)*		*1:64 (1.57%)*		*1:128 (0.79%)*	
Agent, species (number of strains)	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*
Coconut oil																
C. albicans (17)	17 (100)	0 (-)	17 (100)	0 (-)	17 (100)	0 (-)	15 (88)	2 (12)	15 (88)	2 (12)	13 (76)	4 (24)	11 (65)	6 (35)	8 (47)	9 (53)
C. glabrata (9)	9 (100)	0 (-)	9 (100)	0 (-)	8 (89)	1 (11)	8 (89)	1 (11)	6 (67)	3 (33)	5 (56)	4 (44)	5 (56)	4 (44)	2 (29)	7 (71)
C. stellatoidea (6)	6 (100)	0 (-)	5 (83)	1 (17)	5 (83)	1 (17)	5 (83)	1 (17)	4 (67)	2 (33)	4 (67)	2 (33)	2 (33)	4 (67)	0 (-)	6 (100)
C. tropicalis (7)	7 (100)	0 (-)	7 (100)	0 (-)	6 (86)	1 (14)	6 (86)	1 (14)	5 (71)	2 (29)	4 (57)	3 (43)	4 (57)	3 (43)	3 (43)	4 (57)
C. krusei (6)	6 (100)	0 (-)	3 (50)	3 (50)	3 (50)	3 (50)	2 (33)	4 (67)	2 (33)	4 (67)	2 (33)	4 (67)	1 (17)	5 (83)	0 (-)	6 (100)
C. parapsilosis (7)	7 (100)	0 (-)	6 (86)	1 (14)	6 (86)	1 (14)	6 (86)	1 (14)	5 (71)	2 (29)	5 (71)	2 (29)	4 (57)	3 (43)	3 (43)	4 (57)
	Susceptibility at given concentration															
	128 μg/mL		*64 μg/mL*		*32 μg/mL*		*16 μg/mL*		*8 μg/mL*		*4 μg/mL*		*2 μg/mL*		*1 μg/mL*	
Fluconazole																
C. albicans (17)	17 (100)	0 (-)	17 (100)	0 (-)	15 (88)	2 (12)	11 (65)	6 (35)	11 (65)	6 (35)	10 (59)	7 (41)	9 (53)	8 (47)	5 (29)	12 (71)
C. glabrata (9)	9 (100)	0 (-)	7 (78)	2 (22)	6 (67)	3 (33)	6 (67)	3 (33)	6 (67)	3 (33)	5 (56)	4 (44)	4 (44)	5 (56)	1 (11)	8 (89)
C. stellatoidea (6)	5 (83)	1 (17)	5 (83)	1 (17)	5 (83)	1 (17)	4 (67)	2 (33)	3 (50)	3 (50)	2 (33)	4 (67)	1 (17)	5 (83)	0 (-)	6 (100)
C. tropicalis (7)	7 (100)	0 (-)	6 (86)	1 (14)	6 (86)	1 (14)	5 (71)	2 (29)	4 (57)	3 (43)	4 (57)	3 (43)	2 (29)	5 (71)	1 (14)	6 (86)
C. krusei (6)	4 (67)	2 (33)	3 (50)	3 (50)	3 (50)	3 (50)	2 (33)	4 (67)	1 (17)	5 (83)	1 (17)	4 (83)	0 (-)	6 (100)	0 (-)	6 (100)
C. parapsilosis (7)	6 (86)	1 (14)	6 (86)	1 (14)	5 (71)	2 (29)	4 (57)	3 (43)	4 (57)	3 (43)	2 (29)	4 (71)	1 (14)	6 (86)	0 (-)	7 (100)

S, sensitive; R, resistant. Numbers in parentheses are percentages.

Ibadan, Nigeria. The fresh coconut meat was grated and pressed using a sterilized sieve to produce coconut milk, which was allowed to ferment for 48 hours, after which the solids and water content were separated from the oil. The oil was then heated slightly to remove remaining moisture.[17] The oil was then filtered by passage through a 25-μm-pore size filter (Millipore, St. Quentin, France) to give an aqueous extract of coconut oil. This was collected in a sterile vial and stored at 4°C until use.

Sterility test

Extracted coconut oil was cultured on chocolate and MacConkey's agar plates and incubated overnight at 37°C, the MacConkey's agar aerobically and the chocolate agar in a candle extinction jar. This was done to ensure that the extract was completely sterile. All media prepared were picked at random and incubated overnight at 37°C for the same purpose.

Candida *isolates*

Fifty-two recent isolates of *Candida* species were obtained from various clinical specimens sent to the Medical Microbiology Laboratory, University College Hospital, Ibadan. The specimens were high vaginal swabs, endocervical swabs, urine, ear swab/discharge, and wound swabs.

Antifungal testing

The antifungal susceptibility was determined by using the agar-well diffusion technique. The inoculum was standardized to 0.5 McFarland units using a densitometer. This was flooded onto two sets of Mueller-Hinton agar supplemented with 0.2% glucose and 0.5 μg/mL methylene blue dye medium, drained, and allowed to stand for 1 hour. The doubling dilution technique was used for the coconut oil: the neat solution was diluted with 1% ethanol. Using sterile distilled water as diluent, 128 μg/mL fluconazole was also diluted down to 64 μg/mL, 32 μg/mL, etc., down to 0.5 μg/mL. One hundred microliters of each fluconazole dilution was put in the precut wells of the second plate. Wells filled with 100 μL of phosphate-buffered saline served as positive control. The solutions were allowed to diffuse for 30 minutes in the agar medium, after which the plates were then incubated at 35°C for 24 hours. The zones of inhibition were measured in millimeters.

The susceptibility pattern measured as zones of inhibition of the organisms to the coconut oil extract was compared with those of fluconazole. The susceptibility pattern was reported as either sensitive (zone diameter $\geq$28 mm) or resistant (zone diameter $\leq$27 mm).[3]

RESULTS

Of the 52 *Candida* species obtained from different clinical specimens, 17 were *C. albicans*, nine *Candida glabrata*, seven each of *Candida tropicalis* and *Candida parapsilosis*, and six each of *Candida stellatoidea* and *Candida krusei*. The dilution of coconut oil and fluconazole, their corresponding concentrations, and the comparative susceptibility pattern of the isolates to various dilutions of coconut oil and fluconazole, respectively, are shown in Table 1. *C. albicans* was most sensitive to both coconut oil with a minimum inhibitory concentration (MIC) of 25% concentration with 100% sensitivity, while fluconazole at 1:4 (μg/mL) had 88% sensitivity comparatively. Hence the MIC of fluconazole for *C. albicans* was 64 μg/mL. *C. krusei* showed the greatest resistance to coconut oil with an MIC of 100% (neat), whereas fluconazole had an MIC of $>$128 μg/mL. It is noteworthy that all of the species of *Candida* isolates were sensitive to coconut oil when neat (100% concentration) compared to fluconazole at $>$128 μg/mL.

A summary of the sensitivities of the various *Candida* species to various dilutions of coconut oil and fluconazole is shown in Table 2. All the *Candida* species were sensitive to coconut oil at 100% concentration, 90% of *Candida* species at 50% concentration, and 35% of *Candida* species at 0.79% concentration. To 128 μg/mL fluconazole, 92% of *Candida* species were sensitive, while 85% of *Candida* species were sensitive to 64 μg/mL, and 13% of *Candida* species were sensitive to 1 μg/mL.

DISCUSSION

In this study *C. albicans* was the most common isolate from the clinical specimens. This correlates with the work of Hiroshige *et al*.,[3] in which 24 of 72 isolates were *C. albicans*. It also had the highest susceptibility to coconut oil

Table 2. Sensitivity Patterns of *Candida* Strains

Agent dilution	*Number of sensitive strains*	*% of strains*	
		Sensitive	*Resistant*
Coconut oil			
Neat	52	100	0
1:2	47	90	10
1:4	45	87	13
1:8	42	81	19
1:16	37	71	29
1:32	33	63.5	36.5
1:64	27	52	48
1:128	18	35	66
Fluconazole			
Neat	48	92	8
1:2	44	85	15
1:4	40	77	23
1:8	32	62	38
1:16	29	56	44
1:32	24	46	54
1:64	17	33	67
1:128	7	13	87

with 100% sensitivity at an MIC of 25% concentration and to fluconazole at an MIC of 64 μg/mL. *C. krusei* had the highest resistance to coconut oil with an MIC of 100%, whereas fluconazole had an MIC of >128 μg/mL. *C. krusei* is intrinsically resistant to fluconazole.[18] Generally, coconut oil showed a remarkable activity against the *Candida* species. Bergsson *et al*.[19] tested the components of coconut oil (capric acid, lauric acid) for cytotoxicity, and it was stated that even if lipids are toxic in cell cultures, other studies have shown that they are not toxic to skin and mucosa at much higher concentrations. If these chemical constituents are extracted and made into cream, there might be better activity, and the MIC would be much reduced.[19]

Candida isolates had higher susceptibility to coconut oil than to fluconazole (Table 2). It has been reported that fluconazole even at higher concentrations is fungistatic rather than fungicidal.[20,21] Although it may not be clear in this study whether the zone of inhibition depicts a fungicidal or fungistatic activity, another study that tested the *in vitro* killing of *C. albicans* by capric acid and lauric acid (both components of coconut oil) counted calories and analyzed lipid–yeast mixtures by the Turkey-Kramer method of multiple comparison. The reduction in infectivity titers suggested some fungicidal activity by capric acid and lauric acid.[15]

The presence of monocaprin in hydrogels tested against the vaginal mucosa of the mice and rabbits has been shown not to cause irritation. It can thus be said, through this study and previous ones, that coconut oil is active in killing *C. albicans* and other *Candida* species. We suggest that coconut oil should be used for infection caused by *Candida* species.

In conclusion, the results obtained in this study have elucidated the use of coconut oil in complementary and alternative medicine, especially in this era of emerging drug-resistant *Candida* species.

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