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COMPARATIVE ANALYSIS OF ANTIFUNGAL EFFECTS OF HOME MADE AND COMMERCIALLY AVAILABLE VIRGIN COCONUT OIL ON VULVOVAGINAL CANDIDA SPECIES IN ABAKALIKI

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ABSTRACT

This study was aimed at comparing the antifungal effects of homemade virgin coconut oil (VCO) and commercially available (VCO) on Vulvovaginal isolates of Candida species. Seventy-two isolates of various Candida species which included *C. albicans, C. tropicalis, C. glabrata, C. dubliniensis* and *C. krusei* were isolated from vaginal swab and endocervical swab specimens of women attending a tertiary hospital in Abakaliki. Agar well diffusion technique was used and the isolates were exposed to serially diluted VCO at dilutions ranging from 1:2, 1:4, 1:8 and 1:16. There were varying degrees of susceptibility and resistance to both homemade and commercially available VCO. Resistance was recorded more with the commercial VCO than the home made VCO. *C. albicans, C, glabrata* and *C, krusei* showed less susceptibility and high resistance than the other two species *C. tropicalis* and *C. dubliniensis*. This study shows a high prospect of Virgin coconut oil being a veritable alternative to contemporary antifungals.

KEYWORDS: Coconut oil, Candida, Abakaliki.

INTRODUCTION

Candida species are the most common cause of fungal infections leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases (Achkar and Fries, 2010). Of the disseminated mycoses, candidiasis remains the most prevalent, with Candida albicans causing more invasive infections than any other fungus.

Coconut oil is a type of tropical oil that has been used for centuries in traditional diets and remedies. There are two types of coconut oil available in the market, virgin coconut oil (VCO) and refined, bleached, and deodorized (RBD) coconut oil. They are differentiated by the process of making them. In the medical field, VCO is proved that can prevent occurrence of heart disease and infectious diseases. This oil is easily digested, stimulates metabolism, boosts energy, prevents deposition of fat and improves absorption of vitamins, minerals and amino acids. It also possesses anti-inflammatory, anti-microbial and antioxidant properties and can boost the immune system. (Tijn et al., 2016). At present, virgin coconut oil is said to have antibacterial, antiviral and antifungal properties.

The practice of complementary and alternative medicine is now on the increase 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 (Dilhuydy, 2003). Virgin coconut oil, a potent nondrug or natural yeast fighter, contains three medium chain fatty acids; lauric acid (50–53%), caprylic acid, and capric acid, all of which have antifungal effect against *Candida* and other fungi. Medium-chain free fatty acids have been found to have a broad spectrum of microbicidal activity (Isaac et al., 1995).

METHODOLOGY

Coconut oil preparation

Fresh coconut (*Cocos nucifera*) was obtained from the Institute of Agricultural Research and Training Centre, Apata, 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 (Meis et al., 2000). The oil was then filtered by passage through a filter to give an aqueous extract of coconut oil. This was collected in a sterile vial and stored at 4°C until use. 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.

Candida *isolates*

Seventy-two isolates of *Candida* species were obtained from various clinical specimens of women attending a tertiary health care centre in Abakaliki. The specimens were vaginal swabs and endocervical swabs. Five species of Candida were isolated. They include Candida albicans (25), Candida tropicalis (12), Candida dubliniensis (5), Candida glabrata (18) and Candida krusei (12).

Antifungal Susceptibility

Colonies of each isolate were picked up with a sterilized wire loop and emulsified in Nutrient broth and the turbidity adjusted to 0.5 McFarland standard. The Mcfarlands standard was prepared by adding 1ml of concentrated tetraoxosulphate (VI) acid (H_2SO_4) to 99 ml of distilled water and dissolving 0.5 g of dehydrated Barium Chloride ($Bacl_2\ 2H_2O$) in 50 ml of distilled water in separate reaction flasks respectively. Within 15 minutes of adjusting the turbidity, each isolate was plated onto a dried surface of a sterile Mueller-Hinton agar plate respectively using a sterile cotton swab. (CLSI, 2017).

The antifungal susceptibility was determined by using the agar-well diffusion technique. The doubling dilution technique was used for the coconut oil. 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 (Ogbolu et al., 2007; Tijn et al., 2016).

RESULT AND DISCUSSION

Table 1: Showing Antifungal Activity of Homemade Virgin Coconut oil on various species of *Candida.*

Names of Isolates	Susceptibility profile	100% undiluted	1:2 50%	1:4 25%	1:8 12.5%	1:16 6.25%
C. albicans	Susceptible Resistant	25(100) 0(0)	25(100) 0(0)	20(80) 5(20)	0(0) 25(100)	0(0) 25(100)
C. tropicalis	Susceptible Resistant	12(100) 0(0)	12(100) 0(0)	12(100) 0(0)	5(41. 67) 7(58.33)	0(0) 12(100)
C. dubliniensis	Susceptible Resistant	5(100) 0(0)	5(100) 0(0)	5(100) 0(0)	2(40) 3(60)	0(0) 5(100)
C. glabrata	Susceptible Resistant	18(100) 0(0)	18(100) 0(0)	15(83.33) 3(16. 67)	9(50) 9(50)	0(0) 18(100)
C. krusei	Susceptible Resistant	12(100) 0(0)	12(100) 0(0)	5(41. 67) 7(58.33)	0(0) 12(100)	0(0) 12(100)

Table 2: Showing Antifungal Activity of Commercially available virgin Coconut oil on various species of Candida.

Names of Isolates	Susceptibility Profile	100% undiluted	1:2 50%	1:4 25%	1:8 12.5%	1:16 6.25%
C. albicans	Susceptible Resistant	15(60) 10(40)	15(60) 10(40)	0(0) 25(100)	0(0) 25(100)	0(0) 25(100)
C. tropicalis	Susceptible Resistant	12(100) 0(0)	12(100) 0(0)	8(66. 67) 4(33. 33)	0(0) 12(100)	0(0) 12(100)
C. dubliniensis	Susceptible Resistant	5(100) 0(0)	5(100) 0(0)	3(60) 2(40)	1(20) 4(80)	0(0) 5(100)
C. glabrata	Susceptible Resistant	16(88. 89) 2(11. 11)	7(38. 89) 11(61. 11)	0(0) 18(100)	0(0) 18(100)	0(0) 18(100)
C. krusei	Susceptible Resistant	10(83. 33) 2(16.67)	3(25) 9(75)	0(0) 12(100)	0(0) 12(100)	0(0) 12(100)

Findings from our study have shown that there are varying degrees of both resistance and susceptibility to homemade and commercially available VCO.

For home made VCO *C. albicans* were completely susceptible to the undiluted oil and the first two dilutions (1:2 and 1:4) and resisted the last three dilutions (1:4, 1:8 and 1:16). *C. tropicalis* were completely susceptible to the undiluted oil and the first three dilutions (1:2, 1:4 and 1:8) and resisted the last two dilutions (1:8 and 1:16). *C. dubliniensis* were completely susceptible to the undiluted oil and the first three dilutions (1:2, 1:4 and 1:8) and resisted the last two dilutions (1:8 and 1:16). *C. glabrata* were completely susceptible to the undiluted oil and the first three dilutions (1:2, 1:4 and 1:8) and resisted the last three dilutions (1:4, 1:8 and 1:16). *C. krusei* were completely susceptible to the undiluted oil and the first two dilutions (1:2 and 1:4) and resisted the last three dilutions (1:4, 1:8 and 1:16).

For commercially available VCO *C. albicans* were completely susceptible to the undiluted oil and the first

dilution (1:2) and resisted all dilutions (1:2, 1:4, 1:8 and 1:16). *C. tropicalis* were completely susceptible to the undiluted oil and the first two dilutions (1:2 and 1:4) and resisted the last three dilutions (1:4, 1:8 and 1:16). *C. dubliniensis* were completely susceptible to the undiluted oil and the first three dilutions (1:2, 1:4 and 1:8) and resisted the last three dilutions (1:4, 1:8 and 1:16). *C. glabrata* were completely susceptible to the undiluted oil and the first dilution (1:2) and resisted all dilutions (1:2, 1:4, 1:8 and 1:16). *C. krusei* were completely susceptible to the undiluted oil and the first dilution (1:2) and resisted all dilutions (1:2, 1:4, 1:8 and 1:16).

Our results show that resistance was recorded more with the commercial VCO than the home made VCO. *C. albicans, C, glabrata* and *C, krusei* showed the less susceptibility and high resistance than the other two species (*C. tropicalis* and *C. dubliniensis*). These could be attributed factors such as various exposures of the commercial VCO especially in storage. Homemade VCO are relatively more nutritive than commercially produced VCO. Preservation measures and refining techniques could still play a part in neutralizing the phytochemicals.

Our findings agree with that of Ogbolu et al (2007) and Sheehan et al (1999) where *C. krusei* was noted to have recorded the highest form of resistance. In our study, *C. dubliniensis* and *C. tropicalis* recorded the highest level of susceptibility and this disagrees with studies of Tijn *et al* (2016) and Ogbolu *et al* (2007) where *C. albicans* recorded the highest form of susceptibility. While our studies looked at Vulvovaginal isolates of Candida some other studies also looked at Oral isolates of Candida. Ogbolu et al (2007) have shown *C. albicans* to be more susceptible to Coconut oil than Fluconazole.

VCO is made out of various types of fatty acids such as caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid. These fatty acids have been proven of providing fungicidal effects toward *C. albicans* with diverse mechanism of action (Bwalan and Chaplan, 2006). Fatty acid naturally inserts themselves into the lipid bilayer of the fungal membranes and physically disturbs the fungal cell membrane resulting in increased fluidity of the membrane which causes a generalized disorganization of the cell membrane that leads to conformational changes in membrane proteins, the release of intracellular components, cytoplasmic disorder and eventually cell disintegration. Studies have shown that if lipids are toxic in cell cultures, they are not toxic to skin and mucosa at much higher concentrations (Bergesson et al., 2001).

Our study has shown that coconut oil can serve as a very veritable alternative to contemporary antifungals especially as it has lesser side effects.

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