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# Antifungal Activities of Virgin Coconut Oil on *Candida albicans, Aspergillus niger* and *Mould* Species

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**Abstract**

The emergence of antimicrobial resistance, coupled with the availability of fewer antifungal agents with fungicidal actions, prompted this study to determine the effectiveness of virgin coconut oil as an antifungal agent on these species (*Candida albicans, Aspergillus niger and Mould* species). Their susceptibilities to virgin coconut oil and Griseofulvin were studied by using the well in agar diffusion technique. *Candida albicans* showed the highest susceptibility to coconut oil on the SDA plate, with a minimum inhibitory concentration (MIC) of 12.5mg/ml (1:8 dilution) in the broth, while Griseofulvin had 100% susceptibility on the *C. albicans* plate, with MIC of 14.29mg/ml (1:7 dilution) in the broth. *Mould species* showed high susceptibility (100%) to coconut oil on the *Mould* plate, with an MIC of 16.67mg/ml (1:6 dilution), while Griseofulvin had an MIC of 14.29mg/ml on the *Mouldspecies*. *Aspergillus niger* showed a high resistance to the virgin coconut oil both on the SDA plate and in the broth, while Griseofulvin showed activities on the *A. niger* plate with MIC of 20mg/ml in the broth. It is noteworthy that coconut oil was active against species of *Candida albicans* at 12.50mg/ml concentration compared to Griseofulvin. Coconut oil should be used in the treatment of fungal infections in view of emerging drug-resistant *Candida species*. Virgin coconut oil should be used for the treatment of fungal infections like Candidiasis and to prevent the spoilage of food substances by *Mould* species.

**Activités antifongiques de l'huile de noix de coco vierge sur les espèces *Candida albicans*, *Aspergillus niger* et Mold**

**Abstrait**

L'émergence d'une résistance aux antimicrobiens, associée à la disponibilité d'un nombre réduit d'agents antifongiques à action fongicide, a incité cette étude à déterminer l'efficacité de l'huile de noix de coco vierge en tant qu'agent antifongique sur ces espèces (espèces de Candida albicans, Aspergillus niger et Mold). Leurs susceptibilités à l'huile de noix de coco vierge et à la griséofulvine ont été étudiées à l'aide de la technique de diffusion puits dans gélose. Candida albicans présentait la sensibilité la plus élevée à l'huile de coco sur la plaque de SDA, avec une concentration inhibitrice minimale (CMI) de 12,5 mg / ml (dilution 1: 8) dans le bouillon, tandis

que la griséofulvine avait une sensibilité de 100% sur la plaque de C. albicans, avec CMI de 14,29 mg / ml (dilution 1: 7) dans le bouillon. Les espèces de moisissures présentaient une forte sensibilité (100%) à l'huile de coco sur la plaque de moisissure, avec une CMI de 16,67 mg / ml (dilution 1/6), tandis que la griséofulvine avait une CMI de 14,29 mg / ml pour l'espèce de moisissure. Aspergillus niger a montré une résistance élevée à l'huile de noix de coco vierge à la fois sur la plaque de SDA et dans le bouillon, tandis que la griséofulvine a présenté des activités sur la plaque d'A. Niger avec une CMI de 20 mg / ml dans le bouillon. Il est à noter que l'huile de coco était active contre les espèces de Candida albicans à une concentration de 12,50 mg / ml par rapport à la griséofulvine. L'huile de coco devrait être utilisée dans le traitement des infections fongiques compte tenu de l'émergence d'une nouvelle espèce de Candida résistante aux médicaments. L'huile de noix de coco vierge devrait être utilisée pour le traitement d'infections fongiques telles que la candidose et pour empêcher la détérioration des substances alimentaires par les espèces de moisissures.

## Introduction

*Cocos nucifera* (Coconut)belongs to the family Arecaceae. It is commonly called Coconut and the "Tree of life" because all parts of the tree are utilised directly or as secondary products by humans and animals (Peedikayil, Sreenivasan & Narayanan, 2015; Kabara, 2011; Nasir, Jalaludin, Abllah, Shahdan & Manan, 2017). Medicinal plants as remedies for infections are effective in reducing the problems of multiple resistances and long term health issues from synthetic drugs or antimicrobial use (Anzaku, Asikong, Akeh, Upla & Tuluma, 2017; Taiwo, Omobola, David & Anthony, 2011). According to Dayrit, Buenafe, Chainani & Vera (2008), oil from coconut is usually obtainable in three major forms namely; Refined Coconut Oil (RCO), Copra Oil (CO) and Virgin Coconut Oil (VCO). VCO is extracted from coconut milk derived from mature coconut meat and processed at low temperature through mechanical and natural means without refining the oil (Dia, Garcia, Mabesa, Tecson-Mendoza &Tecson-Menddonza, 2005; Nasir *et al.*, 2017). It is composed of Short Chain Fatty Acids (SCFAs) and Medium Chain Fatty Acids (MCFAs) grouped as medium chain triglyceride (MCT) unlike common oils constituting of long chain fatty acids (Macallan, Noble, Baldwin, Foskett, McManus & Griffin, 2013; Mann, 2015; Nasir *et al.*, 2017).

MCTs are a better option than other saturated oils because they characteristically have shorter chain length (6–12 carbons chain) and molecules for faster absorption and metabolism in the body especially in malabsorption ailments treatment (Che Man & Marina, 2006; Nagao &Yanagita, 2010). The MCFAs of VCO are Caproic acid (C6), Caprylic acid (C8), Capric acid (C10) and Lauric acid (C12), constituting approximately 50% of the fatty acid composition (DebMandal & Mandal, 2011; Dayrit, 2014; Nasir *et al.*, 2017). These fatty acids and their corresponding monoglyceride derivatives formed inside the body (monolaurin, monocaprin, monocaproin and monocaprylin) exert antifungal effects and antimicrobial activities against pathogenic microorganisms including fungi, bacteria, viruses, protozoa and yeasts (Isaacs, Litov & Thormar, 2005). Lauric acid and its derivatives have been demonstrated to be the most active antimicrobial agent and inactivating fatty acids in VCO (Yeap *et al.,* 2015). These fatty acids and their monoglycerides inhibit fungi by interrupting the lipid bilayer of the plasma or cell membranes and affecting chitin formulation (Hashim, Clancy, Hegsted & Stare, 2007; Ogbolu, Oni, Daini & Oloko, 2007; Yuniwarti, Asmara, Artama & Tabbu, 2012). At present, Virgin Coconut Oil and its medium fatty acid components are constantly being used in researches against fungi such as *Candida albicans*, the most frequently isolated fungus from the human body (Ogbolu *et al*., 2007).

Coconut milk which is the source of VCOs protects against bacteria and fungi when utilized clinically and for treating skin infections (Carpo,

Verallo-Rowell &Kabara, 2007). Virgin Coconut Oil does not require enzymes to be easily absorbed, thus aiding the provision of cell energy. Insulin and blood glucose metabolism are also enhanced resulting in lower heart attacks and strokes rates (Harries & Clement, 2013; Huttunen *et al*., 2007). Virgin Coconut Oil exhibits a neutral effect body cholesterol levels and makes the skin soft and smooth when incorporated into soaps and other health products that promote hair growth and protection from bacterial and viral infections (Chan, Edward & Craig 2006; Naik, Raghavendra and Raghavarao, 2012). Importantly, VCO is stable in high heat as a result of its low oxidation point unlike other oils transformed during heating making them unhealthy (Anzaku *et al.*, 2017; Parfene, Horincar, Kumar, Malik &Bahrim, 2013).

This study examined the fungicidal potency of virgin coconut oil (VCO) on fungal species *Candida albicans, Aspergillus niger* and *Mould species* which are known to cause common diseases and conditions that influence humans. *C. albicans* causes candidiasis of the mouth, throat and genitals while *Mould spp* cause Asthma, allergies and act as an irritant to the eyes, nose and airways (Parrott, 2009; Singh, Raksha & Urhekar, 2013). *A. niger* leads to food spoilage, ear infections and other digestive and respiratory system infections (Schuster, Dunn-Coleman, Frisvad & Van Dijck, 2002).

## Materials and Methods

### *Sample Collection*

Twenty (20) fully matured coconuts were procured from the Ihiagwa market in Owerri, Imo State (South East), Nigeria.

### *Production of Virgin Coconut Oil (VCO)*

The mesocarp of the coconuts were manually cracked with hammer. The edible part was removed and sliced into pieces using a kitchen knife, for easy passage through the grinder blade. The sliced coconuts were washed with clean water to remove dirt and afterwards the coconut meat was ground three times in a mechanical industrial grinder for complete extraction of the coconut milk. Warm water was mixed with the ground coconut to elevate the optimum temperature for *Lactobacillus sp* (range of 35-40$^0$C) which would carry out the consequent fermentation. The coconut milk was then squeezed out of the chaff with an industrial sieve.

The coconut milk was poured into fermentation bowels and wrapped with black nylons, to maintain a constant temperature and the bowls taken into a fermentation chamber for 48hours of anaerobic fermentation by *Lactobacillum sp.* After the fermentation process, five layers were observed in each of the fermentation bowl; the fermented curd layer, the virgin coconut oil layer, the fermented skim milk layer and the gummy layer. The first fermented layer was scooped out and the first grade coconut oil collected with an industrial spoon. The fermented curd was allowed to stand for another 24hours and the premium grade coconut oil collected (Anzaku *et al*., 2017, Ogbolu *et al*., 2007).

### *Purification of Virgin Coconut Oil*

The first grade and the premium grade virgin coconut oil were mixed and allowed to pass through an ultra-filtration machine to remove all the fermented curd.

### *Dilution of the sample into ten sequential concentrations*

About 100mg each of the sample Virgin Coconut Oil was measured with the aid of an electronic weighing balance into ten (10) sterile sample collection bottles. A sterile pipette was used to pipette 1ml of distilled water into the first sample collection bottle, to make 100mg/ml concentration of the sample. Also, the pipette was used to pipette 2ml of distilled water into the second sample collection bottle, to make 50mg/ml concentration of the sample. The dilution continued with an increase of 1ml of the diluent distilled water until it got to the 10th sample collection bottle, which gave 10mg/ml concentration of the sample Virgin Coconut Oil.

### *Media preparation*

*Nutrient agar*

About 14 g of nutrient agar powder was weighed in a weighing balance into 750ml sterile conical flask. Then 0.5 litres of distilled water was measured with a sterile measuring cylinder into the 750ml sterile conical flask containing the nutrient agar powder and stirred gently. The pH of the mixture was checked and recorded (pH: 7.1±1

at 25$^0$C). The mixture was heated over a burning Bunsen burner to fully dissolve all the components. The dissolved mixture was autoclaved at 121$^0$C for 15 minutes. The sterile nutrient medium was allowed to cool to about 47$^0$C and poured into 20 petri dishes (each containing about 20ml).

*Sabouraud Dextrose Agar (SDA) medium*
About 32.5 g of the agar powder was weighed into a 750ml conical flask containing 500ml of distilled water. The mixture was heated with frequent agitation and boiled for one minute to completely dissolve the medium. The dissolved medium was autoclaved at 121° C for 15 minutes. The sterile medium was allowed to cool and 0.5ml of chloramphenicol added. The medium was then poured into petri dishes and tubes for slants. The sterile sabouraud dextrose agar medium was allowed to cool to about 47$^0$C and poured into 20 petri dishes (each containing about 20ml).

*Normal saline*
A solution of 9g of sodium chloride (NaCl) was dissolved in water to a total volume of 1000 ml (weight per unit volume (w/v)).

***Collection of the test organisms***
The pure culture of the test organisms, *Candida albicans*, *Aspergillus niger* and *Mould species* were procured from the Laboratory of Microbiology Department, University of Nigeria, Nsukka (UNN).

***Dilution of the fungal species to match with the Mcfarland Standards***
Sterile wire-loop was used to transfer the fungal colonies from each of the stocked culture into three (3) separate test tubes containing 5ml sterile normal saline each. The turbidity of the cells in each test tube was compared with a standard (McFarland Standards) under a bright light source.

***Inoculation of the test organisms into the Sabouraud Dextrose Agar plates***
In an aseptic environment, a sterile cotton board was used to smear the *Candida albicans, Aspergillus niger* and the *Mould species* from the diluted cells in duplicates on six SDAplates. Using a sterile cork borer of 5mm in diameter, five wells were bored on each of the fungal culture plates for the different concentrations of VCO (one for 100mg/ml of VCO, one for 50mg/ml, 33.33mg/ml,25mg/ml and one for the 20mg/ml of VCO).

***Introduction of the Virgin Coconut Oil (VCO) into the wells***
Using a sterile pipette, two (2) ml of each of the five different concentrations of the Virgin Coconut Oil were placed into each of the bored wells and labelled accurately. A Griseofulvin tablet of eight (8) mm in diameter was placed on the centre of each SDA plate, as the positive control.

***Minimal Inhibition Concentration of the test organisms by the sample and the positive control***

*Minimal Inhibition Concentration of the test organisms by the sample*
About 9ml each of the broth was pipetted into 30 sterile bijou-bottles and placed in three sets of 10 bijou-bottles each. 0.5ml each of the three diluted fungal species was poured into each of the 10 sets of bijou-bottles. Then 1ml each of the 10 different concentrations of the sample VCO was pipetted into the three sets of 10 bijou-bottles. The bottles were allowed to stand at room temperature for four days. After the incubation period, the bijou-bottles were examined for growth and the result recorded.

*Minimal Inhibition Concentration of the test organisms by the positive control*
About 9ml each prepared broth was pipetted into 30 sterile bijou-bottles and placed in three sets of 10 bijou-bottles each. 0.5ml each the diluted fungal species was pipetted into each of the sets of bijou-bottles. 1g of the Griseofulvin was divided into ten sample collection bottles and diluted sequentially. From each of the 10 different concentrations of the synthetic antifungal drug concentrations, 1ml was pipette into the three sets of 10 bijou-bottles and the bottles allowed to stand at room temperature four days. Growth was examined and recorded after incubation period.

## Results

**Virgin Coconut Oil Yield**
The virgin coconut oil was colourless and odourless above 30$^0$C and it was white when in its solid form and the percentage yield was 9.34% (w/w).

The yield value was derived using the formula; YIELD= $\frac{\text{Mass of VCO (g) x 100}}{\text{Mass of the coconuts(g) 1}}$

Table 1: The Yield Obtained from Cocos nucifera nuts

| **Plant** | ***Cocosnucifera*** |
|---|---|
| Number of nuts used | 20 |
| Weight of the 20 coconuts (g) | 2900 |
| Weight of the edible part(g) | 1500 |
| Weight of the coconut milk (g) | 825 |
| Volume of the coconut milk (ml) | 830 |
| Weight of the virgin coconut oil (g) | 271 |
| Volume of the virgin coconut oil (ml) | 255 |

Table 2: Inhibition Zone Diameter (IZD) of Virgin Coconut Oil on Fungal isolates (mm)

| **S/N** | **Coconut oil on the fungal species** | **100mg/lm (1:1)** | **50mg/ml (1:2)** | **33.33mg/ml (1:3)** | **25mg/ml (1:4)** | **20mg/ml(1:5)** |
|---|---|---|---|---|---|---|
| 1i | *Candida albicans* (plate 1) | 17.5 | 13 | 11 | 9.5 | 9 |
| 1ii | *Candida albicans* (plate 2) | 18 | 13.5 | 11 | 10 | 8 |
| 2i | *Mould species* (plate 1) | 13 | 10 | 9 | 7 | 6 |
| 2ii | *Mould species* (plate 2) | 14 | 11 | 8.5 | 7 | 6.5 |
| 3i | *Aspergillus niger* (plate 1) | R | R | R | R | R |
| 3ii | *Aspergillus niger* (plate 2) | R | R | R | R | R |

**Key:**
R= resistant
1i = The first plate of the *Candida albicans* duplicated culture plates.
1ii = The second plate of the *Candida albicans* duplicated culture plates.

Table 3: Minimal Inhibition Concentration (MIC) of the VCO on the Fungal species

| **Sample** | **VCO** | **(1:1)** | **(1:2)** | **(1:3)** | **(1:4)** | **(1:5)** | **(1:6)** | **(1:7)** | **(1:8)** | **(1:9)** | **(1:10)** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1i | *C. albicans* | + | + | + | + | + | + | + | + | - | - |
| 1ii | *C. albicans* | + | + | + | + | + | + | + | + | - | - |
| 2i | *A. niger* | - | - | - | - | - | - | - | - | - | - |
| 2ii | *A. niger* | - | - | - | - | - | - | - | - | - | - |
| 3i | *Mould* | + | + | + | + | + | + | - | - | - | - |
| 3ii | *Mould* | + | + | + | + | + | + | + | - | - | - |

**Keys:**
+ = Susceptible - = Resistant VCO = Virgin Coconut Oil.
1:1 = 100mg of VCO in 1ml of distilled water.
2i = The first plate of the *Aspergillus niger* duplicated culture plates.
2ii = The second plate of the *Aspergillus niger* duplicated culture plates.

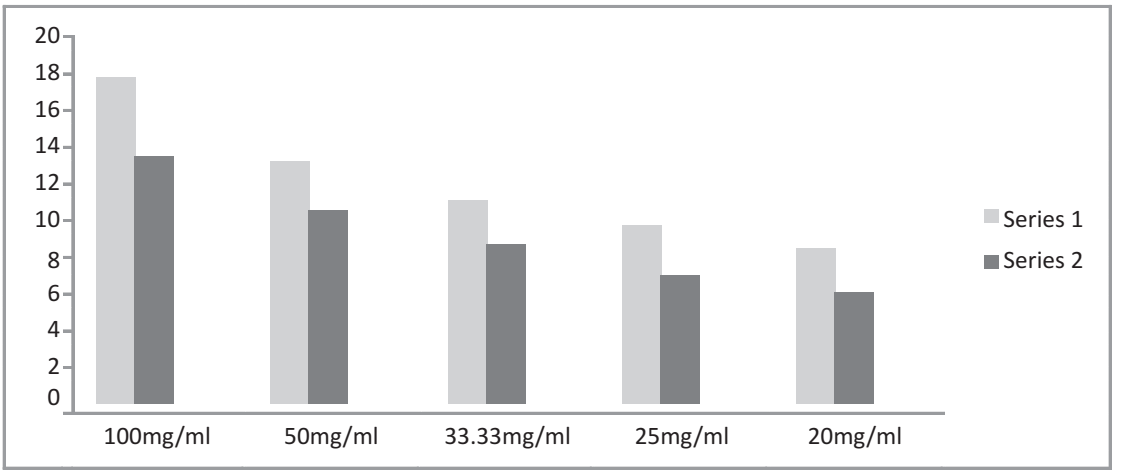


Figure 1: The Inhibition Zone Diameters of Virgin Coconut Oil on *Candida albicans* and Mould species

**Key:**
Series 1 =The diameter of zone of inhibition of *Candida albicans* by virgin coconut oil.
Series 2 = The diameter of zone of inhibition of *Mould species* by virgin coconut oil.

Table 4: Minimal Inhibition Concentration (MIC) of the Positive control, Griseofulvin on the Fungal species

| S/N | Griseofulvin | (1:1) | (1:2) | (1:3) | (1:4) | (1:5) | (1:6) | (1:7) | (1:8) | (1:9) | (1:10) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1i | *C.albicans* | + | + | + | + | + | + | - | - | - | - |
| 1ii | *C. albicans* | + | + | + | + | + | + | + | - | - | - |
| 2i | *A. niger* | + | + | + | + | + | - | - | - | - | - |
| 2ii | *A. niger* | + | + | + | + | - | - | - | - | - | - |
| 3i | *Mould* | + | + | + | + | + | + | + | - | - | - |
| 3ii | *Mould* | + | + | + | + | + | + | + | - | - | - |

**Keys:**
+= Susceptible - = Resistant
1:10 = 100mg of the positive control, Griseofulvin in 10ml of distilled water.
3i = The first plate of the *Mould species* duplicated culture plates.
3ii = The second plate of the *Mould species* duplicated culture plates.

## Discussion

In this study of antifungal potency of virgin coconut oil on *Candida albicans*, *Aspergillus niger* and *Mould species*, it was found that *Candida abicans* were highly susceptible to the virgin coconut oil as shown in Table 2. The inhibition zone diameter *C. albicans* ranged from 8mm – 18mm in diameter, while the *Mould species* showed a relatively high susceptibility to *Cocos nucifera* oil with inhibition zone diameter values ranging from 6mm–14mm as shownin table 3. The *Aspergillus niger* exhibited a high resistance to the virgin coconut oil as seen in both Tables 2 and 3. This could be attributed to the secretion of mycotoxin by the genus, *Aspergillus* (Anzaku *et al*., 2017).

The MIC examination of the sampled virgin coconut oil on the *Candida albicans* revealed that the susceptibility of the *Candida species* to the sample is maintained until the concentration dropped below 12.50mg/ml (that is 1:8).

Therefore, the Minimal Inhibition Concentration (MIC) of virgin coconut oil(VCO) on the *Candida albicans* was 12.50mg/ml as shown in Table 3. More so, the Minimal Inhibition Concentration (MIC) of the virgin coconut oil on *Mould species* was revealed in Table 3 to be 16.67mg/ml (that is, 1:6). Table 3 also revealed that the *Aspergillus niger* is highly resistance to the VCO sample from the lowest concentration (10mg/ml) to the highest (100%) concentration.

*Aspergillus niger* was slightly resistant to the purified antifungal drug Griseofulvin that was used as the positive control in the study as seen in Table 4, while for *C. albicans* this antifungal drug had a lower inhibition rate (MIC) than the VCO sample. Although for mould the inhibition rate for both the sample VCO and the control was approximately the same at 14.29 mg/ml (100mg of the sample in 7ml of distilled water).

The findings in this work corroborates with that of Ogbolu *et al*., (2007) which showed that coconut oil was active against species of *Candida* at 100% concentration and even low concentrations when compared to their control, fluconazole. Hence, VCO should be used in the treatment of fungal infections in view of emerging drug-resistant to *Candida* spp. It is obvious that the antimicrobial activities of virgin coconut oil against some tested fungal isolates are due to the presence of free fatty acids of medium chain and their monoglycerides such as lauric acid which has the higher composition in virgin coconut oil.

## Conclusion

Coconut oil was active against species of *Candida albicans* and *Mould species* especially at 100% concentration. More so, the virgin coconut oil was more effective than the positive control, Griseofulvin on the *Candida albicans.* Coconut oil can be recommended for use in the treatment of fungal infections like candidiasis and for the preservation of food from microbial spoilage. Lastly, virgin coconut oil may not be effective for the treatment of infection resulting from *Aspergillus niger*, since the result revealed that the organism showed some signs of resistance to the oil.

For effective, reliable and more accurate results in scientific research such as this, inter-disciplinary synergy should be encouraged and practised. Further research should be carried out on the virgin coconut oil to identify the active ingredients that are responsible for its antifungal activities.

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