Antifungal Activity Test of Virgin Coconut Oil (VCO) Against *Microsporum canis*

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Abstract

Virgin Coconut Oil (VCO) is a coconut oil that is clearly colored and smells like pure coconut that is produced from the flesh of coconut fruit that is in the process of production, does not use warming up. owners of dogs and cats, this oil is used to heal the ringworm on the skin with smearing. The objective of this study was to test the antifungal activity of virgin coconut oil against Microsporum canis. The test methods used in this research were disk diffusion and well methods. In this study, we used commercially available VCO without any treatment. Antifungal activity tests using the well method showed that VCO can inhibit the growth of Microsporum canis.

Keywords: *Antifungal activity test*, *Microsporum canis*, *virgin coconut oil* (VCO).

1. Introduction

The increasing contact between humans and animals has led to an increase in the incidence of zoonoses. According to [1] zoonoses are diseases and infections that are naturally transmitted between vertebrates and humans. Organisms that cause zoonoses include Arthropods, Helminths, Protozoa, Fungi, Prions, Bacteria, Rickettsia, and Viruses. The incidence of zoonotic diseases will increase with an increase in the number of pets.

Mycosis is the infection or invasion of fungi into animal or human body tissues. The mycosis category, based on the location of the lesion, consists of superficial mycoses, such as dermatophytosis, subcutaneous mycoses, and systemic mycoses. The fungal disease that is most frequently found and has the potential to be transmitted to humans is dermatophytosis [2] According to the results of [3] the prevalence of superficial dermatophytosis in humans from January 2006 to December 2006 was 32.19%, or 547 cases out of the total 5627 cases of patients in the skin and genital clinic at Mataram Hospital. This number may increase owing to increasingly close interactions between pet owners and their beloved pets.

Treatment of dermatophytosis using antifungals has several disadvantages, including prolonged duration, drug resistance, and side effects such as decreased libido in men and fetal defects in pregnant women.

Dermatophytosis is treated with topical antifungals takes 2-3 weeks. The excessive use of antifungals causes resistance in some mold strains. Apart from the lack of efficiency of the drug, antifungals have side effects such as infertility, decreased libido in men, and fetal defects in pregnant women. In addition, the antifungals administered are sometimes licked by animals, which can cause poisoning. This has encouraged research into antimicrobial agents from herbal sources, including essential oils. The antifungal effect of essential oils is a very promising solution and can overcome the therapeutic shortcomings of antimycotic drugs [4].

Pet owners prefer to use alternative medicines over commercial antifungal medicines because of their safety and efficacy. Virgin coconut oil (VCO) is used as an alternative medicine. VCO is the oil produced from fresh coconut flesh that is not heated during the production process. VCO mostly consists of saturated fatty acids (92%), monounsaturated fatty acids (6%), and polyunsaturated fatty acids (2%); therefore, VCO does not contain any trans fatty acids, which are fats that are dangerous to the body [5]. The health benefits of VCO are associated with its lauric acid content, which is thought to have anti-inflammatory, antibacterial, antifungal, and antiviral properties, especially in the skin [6]. The aim of this study was to determine the antifungal effect of VCO on dermatophyte infections in vitro, so that it could be used as an effective alternative medicine to treat dermatophytosis.

2. Materials and Methods

2.1 Place and Materials

This research was conducted at the Mycology Laboratory, Medical Microbiology Section, Department of Animal Disease and Veterinary Public Health, Faculty of Veterinary Medicine, Bogor Agricultural Institute. The ingredients used in this study are VCO (Virgin Coconut Oil (VCO) produced by CV Flora and Fauna Herab Indonesia, which is registered with the Indonesian Ministry of Health. 448.3/10481, *Microsporum canis* culture was obtained from the Mycology Laboratory, Faculty of Veterinary Medicine, IPB, Dermatophyte Selective Agar (DSA) media, disinfectant, 70% alcohol, distilled water, 10% KOH, lactophenol cotton blue (LCB), 2% ketoconazole, 10% clotrimazole, and 2% miconazole. The composition of DSA agar (Dermatophyte Selective Agar) consisted of 1 g peptone, 2 g dextrose, 1.5 grams of agar, and 1 ampoule of cycloheximide for 500 ml of agar.

2.2 Microsporum canis culture.

The M. canis mold isolate was obtained from the Mycology Laboratory, Medical Microbiology Section, Department of Animal Disease and Veterinary Public Health, Faculty of Veterinary Medicine, IPB. M. canis mold was propagated by culturing in DSA media then incubating at room temperature and waiting for 7 days. Macroscopic and microscopic observations were performed to confirm that the mold growth was M. canis. Macroscopic observations focused on the growth patterns, colony shape, color of the top and bottom colonies, and aspects of the mold colony. Microscopic observations were performed using lactophenol cotton blue (LCB).

The culture results were confirmed using the riddle-slide culture method. Microscopic observation focused on the shape of the conidia, both macroconidia and microconidia, mycelium, and hyphae.

M. canis suspension was made by adding 10 ml of distilled water to the culture dish. The M. canis suspension was then poured into a sterile test tube. The suspension was then centrifuged at 2500 rpm for 15 min. The precipitate and supernatant that had been obtained were separated and inoculated onto DSA agar plates. Wait for this culture for one week, and then observe the nature of the growth. The best mold growth was then used as a suspension for testing [7].

2.3 Microorganism Counting

The sediment suspension was separated from the supernatant to count macroconidia and microconidia. The precipitate was then counted using a counting chamber. The numbers of macroconidia and microconidia in 7 the old mold were 1,650 macroconidia/ml and 16,650 microconidia/ml, respectively.

The suspension was diluted 10 ×, 100 ×, and 1000 ×. A 10 × dilution was carried out by adding 1 ml of the macroconidia solution with a pipette, 9 ml of distilled water, and then homogenizing. For 100x dilution, 1 ml of macroconidia solution was 10x, place it in a test tube, and 9 ml of distilled water was added and homogenized. For 1000 × dilution, 1 ml of the solution was diluted 100 × and poured into 9 ml of distilled water.

The suspension was grown on DSA agar to observe mold growth. The number of microorganisms that grew was counted using the cup-counting method. The number of colonies obtained was expressed in colony-forming units (cfu) per ml. The number of microorganisms was 7.7 x 104 CFU/ml. The suspension with 100x dilution has a colony number of 100 – 150 colonies per cup. Furthermore, the mold suspension was used for further testing [8].

2.4 Determining the Size of Test Repetitions

The size of the repetition was determined using the Federer formula [9].

$$
\begin{aligned}
(n-1)\,(t-1) &\geq 15 \\
(n-1)\,(3-1) &\geq 15 \\
(n-1)\,2 &\geq 15 \\
2n-2 &\geq 15 \\
2n &\geq 17 \\
N &\geq 8
\end{aligned}
$$

Information

N = number of repetitions

T = number of treatment groups

Because this study uses 1 treatment group

2.5 Testing with the Well Method

Mold suspensions (0.1 ml of mold suspension were placed in streaks on DSA agar. The way to make a well is by pressing the culture medium using a tool in the form of a glass tube with a depth of ± 5 mm. The three wells were used to store the substances to be tested. Each culture medium contained 5 wells. Each well was filled with 0.06 ml of antifungal fluid, namely clotrimazole 1%, miconazole 2%, and ketoconazole 2%. This method was performed three times to determine the activity of each antifungal tested using the well diffusion method.

2.5 VCO Testing with the Well Method

0.1 ml of the mold suspension solution was removed, and the mold was placed in scratches. A circle of filter paper with a diameter of 1 cm was dipped into each disc of the solution to be tested, namely VCO oil, 1% clotrimazole as a positive control, and distilled water as a negative control. These discs were placed on DSA agar with equilateral triangle spacing. This method was repeated nine times.

2.6 Observation of Results

The results were obtained by observing colony growth and inhibition zones around the well holes. The distance was measured from the side of the hole to the outside of the zone (mm). The activity index was determined by measuring the inhibition zone of the substance being tested using a standard inhibitory zone size. The clear area visible around the hole indicates activity in the sample [8].

3. Results

3.1 Microsporum canis culture

The Microsporum canis used in this study was a mold culture from the Lab. Mycology, Medical Microbiology, Faculty of Veterinary Medicine, IPB. Macroscopic and microscopic observations were performed to confirm this. M. canis on DSA media had a colony growth phase of–5-10 days, with a macroscopic appearance in the form of flat white colonies and yellow to orange on the reverse side. When the culture was more than 14 days old, the colonies appeared sandy because they contained numerous macroconidia and microconidia. The macroscopic morphology of the microsporum canis mold can be seen in Figure 1. This mold is spindle-shaped, forms on special conidiospores, and has

Figure 1 *Microsporum canis* on DSA media in top and bottom views.

mycelium like cotton or wool (Ellis 2013). The microscopic morphology of the microsporum canis mold can be seen in Figure 2

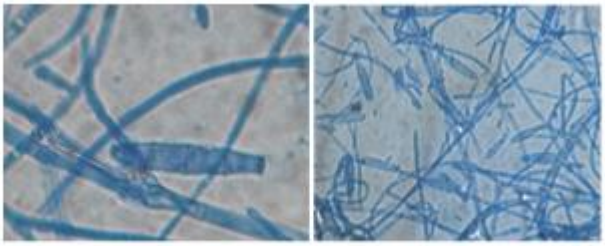

Figure 2 *Microsporum canis*. (a) 400x, (b) 100x.

3.2 Control Antifungal Activity Test

The antifungals tested were clotrimazole (CLT), mikonazole (MK) and ketoconazole (KLT). These three control antifungals were tested using disc diffusion and well hole diffusion methods. Using the disc diffusion method, varying results were obtained regarding the growth *of Microsporum canis*. Clortrimazole (CLT) formed the longest clear zone, namely 13.5 mm. Miconazole (MK) forms a clear zone 4.2 mm long. Ketoconazole forms a clear zone 1.3 mm long. Diameter of the clear zone (mm) around the antifungal disc for the control of *Microsporum canis* can be seen in Tables 1 and Figures 3.

Table 1 Diameter of the clear zone (mm) around the antifungal disc for the control of *Microsporum canis*

Antifungal	Diameter of clear zones (mm)			Mean
	1	2	3	
CLT	14.8	15.5	17.3	15.9
MK	10.7	5.1	9.3	8.4
KT	9.3	6.3	10.6	8.7

Information: CLT : Clotrimazole, MK : Mikonazole, KT : Ketokonazole

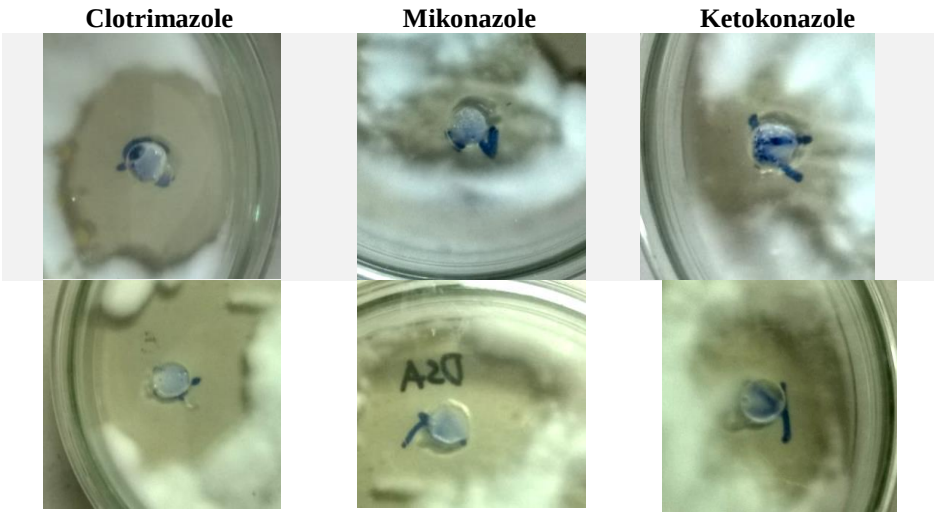

Figure 3 Zones formed around the Clortrimazole (CLT), Miconazole (MK), and

3.3 VCO Antifungal Activity Test

Testing the antifungal activity of virgin coconut oil using the disc diffusion method showed that an inhibition zone 3.2 mm of the tests. The positive control was clotrimazole, which formed an inhibition zone with a length of 13.9 mm. However, the negative control, distilled water (AQ), did not form an inhibition zone. Diameter of the clear zone (mm) around the antifungal disc for the control of *Microsporum canis* can be seen in tables 1 and figures 3. Diameter of the inhibition zone (mm) around the antifungal disc for the control of *Microsporum canis* can be seen in Tables 2 and Figures 4.

Table 2 Inhibition zone diameter (mm) around VCO wells and control against Microsporum canis.

Antifungal	Diameter of clear zones (mm)								Mean
	1	2	3	4	5	6	7	8	
VCO	7.3	4.75	3.8	2	3.4	1	1.2	2.3	3.3
+ (CLT)	12.2	15.0	15.4	14.4	13.6	11.4	15.9	13.1	13.9
- (AQ)	-	-	-	-	-	-	-	-	-

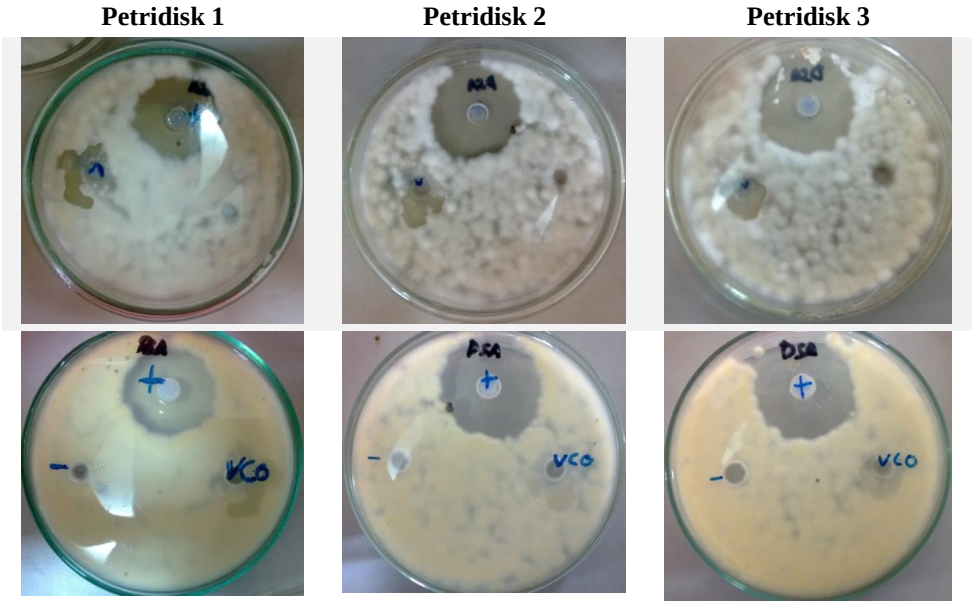

Figure 4 Diameter of the inhibition zone (mm) around the VCO disc and control of *Microsporum canis.*

4. Discussion

Microsporum canis on DSA media had a colony growth phase of–5-10 days, with a macroscopic appearance in the form of flat white colonies and yellow to orange on the reverse side. When the culture was more than 14 days old, the colonies appeared sandy because they contained numerous macroconidia and microconidia. This is in accordance with the statement by [11] that on Saboraud agar media, *Microsporum canis* has a rapid colony growth phase with a macroscopic appearance in the form of flat white colonies like cotton colonies and yellow-orange on the reverse side. *Microsporum canis* colonies will be clearly visible during the incubation period of 5-10 days. If a large number of macroconidia are formed, the surface of the mold will appear sandy and have colonies with a hairy aspect and mold growth pattern that spreads radially. The pigment in the lower colony was yellow – orange. *Microsporum canis* was microscopically observed using the riddle slide culture method, focusing on the shapes of the macroconidia and microconidia. Based on observations, this mold has straight hyphae and septa, many macroconidia, and even more microconidia. Macroconidia have rough, spiny walls and have a coil at the end like a plug. The microconidia were oval and round in shape. The septa in macrokinidia number 4-6 septa. . These results are in accordance with research by [10] and [11] that M. canis has straight hyphae and septa. Microscopically, it has many multicellular macroconidia with a size–10-150 µm consisting of 8-15 cells with thick walls and a tip. curved or barbed hook. Macroconidia have rough, fusiform walls while microconidia are round, pear-shaped, sometimes oval [12].

The activity tests of three types of antifungals, clotrimazole, miconazole, and ketoconazole, were carried out using two methods: the disc diffusion method and the well diffusion method. In both testing methods, clotrimazole had the largest zone of inhibition, namely 13.5 mm using disc diffusion and 15.9 mm using well diffusion. These results are in accordance with research by [13] that in testing various antifungals using the agar disk diffusion method, it was shown that clotrimazole was the antifungal agent that produced the largest zone of inhibition compared to ketoconazole and miconazole. Clotrimazole acts by inhibiting ergosterol synthesis, which increases fungal cell membrane permeability. Thus, clotrimazole inhibits ergosterol biosynthesis in a concentration-dependent manner by inhibiting 14 alpha demethylation of lanosterol. When ergosterol synthesis is inhibited, cells can no longer build intact and functional membranes. Ergosterol also directly promotes fungal cell growth like a hormone; therefore, the rapid onset of these events causes dose-dependent inhibition of fungal growth [14].

Ketoconazole has approval for use in the treatment of fungal infections of the skin and systemic fungal infections. These include blastomycosis, histoplasmosis, paracoccidioidomycosis, coccidioidomycosis, and chromomycosis. The most common use of ketoconazole for skin infections is that of tinea versicolor. Due to the advent of more effective fungal infection treatment options, ketoconazole is typically not the first-line medication anymore. It should only be an option when other first-line treatments are not available or not tolerated by the patient. The use of this drug requires a careful risk-benefit analysis when selecting ketoconazole as the treatment of fungal infections. Clinicians should avoid using ketoconazole in the treatment of onychomycosis, cutaneous dermatophyte, and candida infections [15].

Inhibition zone formed by VCO in M. canis culture. The inhibition zone formed is 3.2 mm long, and this inhibition zone is smaller than the length of the inhibition zone by the positive control (CLT) of 13.9 mm. The clear zone formed in the control antifungal well generally had a larger area than the clear zone formed in the VCO well. This is caused by the antifungal substance contained in the control antifungal disc, which is an antifungal substance that has been produced commercially and has undergone a purification process (refining the active substance), while VCO is coconut oil that is still intact without undergoing purification of the active substance, so it is an antifungal substance. contained in VCO has not been separated and is still mixed with other active substances which are not necessarily antifungal

The antimicrobial activity of VCO is based on the action of lauric acid and capric acid [5]. Based on a study by [16] VCO can inhibit the growth of Candida better than fluconazole can. The lauric acid in VCO acts as a strong antimicrobial, based on research by Nakatsuji et al (2009), the lauric acid content in VCO can kill pathogenic bacteria that cause acne vulgaris, namely Propionibacterium acnes. Lauric acid and capric acid cause disintegration of the fungal cell plasma membrane and damage it, resulting in irregular and wrinkled fungal cytoplasm. The fungal plasma membrane consists of a double layer of phospholipids bound to proteins and ergosterol [18]. Ergosterol is the main sterol in the plasma membrane and plays a role in biosynthesis, taking up and releasing materials, producing a series of carbohydrates, transmitting signals from the environment, and as a storage site for cell wall enzymes, namely, chitin synthase and glucan synthase. Ergosterol

plays a vital role in fungal survival, making it a potential target for antifungal agents. If the plasma membrane is damaged, the components within it, including ergosterol, will be damaged such that its function is disrupted, which causes the processes in ergosterol to not run smoothly and interfere with mold growth [19].

Lauric acid and capric acid cause lysis of fungal cell walls. The fungal cell wall is a dynamic structure that defends itself from changes in osmotic pressure and external environmental influences, and allows the fungus to interact with the external environment. The structure and biosynthesis of fungal cell walls is a part that has a complex function in fungal growth; therefore, it is an excellent target for antifungal agents. Maintained the fungal shape [20]. Fungal cell walls contain components of chitin, glucosamine, mannose, glucuronic acid, glucose, other sugars and proteins. The cell walls of mold hyphae contain 80-90% polysaccharides, 1-15% protein and 2-10% lipids. The biosynthesis of various components of the fungal cell wall is important for the formation of a functional cell wall [20]. Damaged cell walls and lysis due to lauric acid contained in VCO disrupt all biosynthesis processes, disturb osmotic pressure, and prevent the mold from defending itself from external environmental influences. This series of processes causes the *Microsporum canis* mold to die and lyse, thus forming an inhibitory zone/clear zone on DSA agar media.

The On average, VCO sold commercially in shops have the same quality and substance content. This is in accordance with the research by [21] that the different methods used in making VCO do not affect the substance content and do not affect the quality of the VCO produced.

5. Conclusion

Based on this research, it can be concluded that virgin coconut oil (VCO) can inhibit the growth of Microsporum canis in vitro using the well hole diffusion method. VCO cannot inhibit the growth of Microsporum canis due to various factors. The wellbore diffusion method is a simple, easy, and inexpensive method that provides more accurate results for mold growth tests.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

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