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Antifungal Activity of Moringa (*Moringa Oleifera* Lamk.) Seed Oil from East Nusa Tenggara, Indonesia, against *Malassezia Furfur* Causes Seborrheic Dermatitis

Yulius Baki Korassa^{1,2}, Nyi Mekar Saptarini^{1*}, Resmi Mustarichie¹, Rini Hendriani³, Elisma², Fatmawati Blegur², Priska Ernestina Tenda², Yulita Iryani Mamulak⁴

¹ Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Padjadjaran University, Raya Bandung-Sumedang Street, Km. 21, Jatinangor, 45363, Indonesia

² Pharmacy Study Program, Kupang Health Polytechnic of Ministry of Health, Piet A. Tallo Street, Liliba, Kupang, East Nusa Tenggara, Indonesia

³ Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Padjadjaran University, Raya Bandung-Sumedang Street, Km. 21, Jatinangor, 45363, Indonesia

⁴ Biology Study Program, Mathematics and Natural Science Faculty, Widya Mandira Catholic University, Jl. Jenderal Ahmad Yani No. 50-52, Kupang, East Nusa Tenggara, Indonesia

* Corresponding author: nyi.mekar@unpad.ac.id

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Abstract: Hair is skin adnexa in the form of a dermis layer that functions to protect the scalp from environmental influences such as ultraviolet rays, temperature, and humidity. Oily hair can trigger the growth of *Malassezia furfur* (*M. furfur*), which causes seborrheic dermatitis. Moringa seed oil contains phytosterol compounds and fatty acids, which can inhibit the growth of fungi. This study aimed to determine the antifungal activity of moringa seed oil from East Nusa Tenggara, Indonesia, against *M. furfur*. The study was conducted in vitro using the well diffusion method with inhibition zone parameters, minimum inhibitory concentration test by liquid dilution, and minimum fungicidal concentration test by counting the number of colonies. Moringa seed oil at concentrations of 7.5, 10, and 12.5% had antifungal activity against *M. furfur* with inhibition zones of 14.5 ± 1.32 mm (strong), 18.3 ± 3.51 mm (strong), and 26.3 ± 2.36 mm (very strong) with a minimum inhibitory concentration of 2.50% and a minimum fungicidal concentration of 5.00%. Moringa seed oil can be developed as an antifungal, where the greater the concentration, the greater the inhibitory and killing power against *M. furfur*. The novelty of this study is the antifungal activity test of moringa seed oil from East Nusa Tenggara that was obtained by a cold pressed method targeting the fungus *M. furfur* ATCC 14521 using the well diffusion method.

Keywords: *Moringa oleifera* Lamk., fatty acids, phytosterols, *Malassezia furfur*.

来自印度尼西亚东努沙登加拉省的辣木(辣木.)籽油对马拉色菌引起的脂溢性皮炎的抗真菌活性

摘要: 头发是真皮层形式的皮肤附件,其功能是保护头皮免受紫外线、温度和湿度等环境影响。油性头发会引发糠秕马拉色菌(糠秕马拉色菌)的生长,从而导致脂溢性皮炎。辣木籽油含有植物甾醇化合物和脂肪酸,可以抑制真菌的生长。本研究旨在确定来自印度尼西亚

亚东努沙登加拉省的辣木籽油对糠秕马拉色菌的抗真菌活性。该研究在体外进行，采用抑菌圈参数的孔扩散法、液体稀释法的最低抑菌浓度测试、菌落计数的最低杀菌浓度测试。浓度为7.5%、10%和12.5%的辣木籽油对糠秕马拉色菌具有抗真菌活性，抑制区域为14.5 ± 1.32毫米（强）、18.3 ± 3.51毫米（强）和26.3 ± 2.36毫米（非常强）。最低抑菌浓度为2.50%，最低杀菌浓度为5.00%。辣木籽油可开发为抗真菌剂，浓度越高，对糠秕马拉色菌的抑制和杀灭能力越强。本研究的新颖之处在于对来自东努沙登加拉省的辣木籽油进行了抗真菌活性测试，该辣木籽油是通过冷压方法通过井扩散法针对真菌糠秕马拉色菌航空航天中心14521获得的。

关键词：辣木、脂肪酸、植物甾醇、糠秕马拉色菌。

1. Introduction

Hair is a skin adnexa in the form of a dermis layer or skin glands [1]. Hair has a function as scalp protection from environmental influences such as ultraviolet rays, temperature, and humidity [2]. Indonesia is a tropical country with high humidity, so it easily affects the scalp to become oily and triggers the growth of microorganisms in the hair, which results in scalp irritation, itching, flaking of dead skin cells, and dandruff [3].

Dandruff is an anomaly on the scalp that is characterized by the exfoliation of the horny layer and the formation of fine scales called seborrheic dermatitis (DS) [4]. DS on the scalp is caused by *Malassezia restricta*, *M. globosa*, and *M. furfur* in the form of erythematous plaques and scales [5]. *M. furfur*, known as *pityrosporum ovale*, is a normal flora that exists on the scalp and thrives if there are excess oil glands in the hair or scalp [6].

Cases of DS reach 50% of the total population in the world [7]. The prevalence of DS in the general population is 1-5% and in the young adult group is 3-5% [6], where cases in males are higher than females [8]. The prevalence of DS in men is 20.7%, while that in women is 12.8% [9], this is because men have higher androgen hormones than women, thereby increasing the risk of DS [10].

Handling the DS problem requires an active substance that is useful as an antiseborrheic such as dipyrithion, pyroctone olamine, zinc pyrithione, selenium sulfide, salicylic acid, coal tar, hydrocortisone, and ketoconazole [11], but these substances can cause side effects such as dermatitis and hair discoloration, loss, and breakage [12]. The use of natural materials, both traditional and semi-traditional, can be a solution in overcoming DS problems without side effects [13, 14]. One of the materials is moringa seed oil, which is widely used as an antiseborrheic and antifungal [15]. The people of East Nusa Tenggara (NTT) have empirically used moringa seed oil for

health and beauty products in the form of hair oil as an antifungal and hair nourisher [16, 17].

Moringa seed oil has antifungal activity because it contains phytosterol compounds namely β -sitosterol, stigmasterol, and compasterol [18, 19]. Moringa seed oil also contains fatty acids, namely lauric acid, palmitoleic acid, palmitic acid, oleic acid, stearic acid, arachidic acid, palmitoleic acid, palmitic acid, oleic acid, stearic acid, eicosenic acid, arachidic acid, behenic acid, lignoceric acid and other compounds. methyl esters in the form of methyl oleate [20, 21].

Phytosterol compounds have lipophilic properties that can work as antifungal agents by inhibiting the growth and development of fungal spores through the cytoplasm [3]. Fatty acids have antifungal activity through the mechanism of damaging fungal cell walls, inhibiting topoisomerase enzymes that play a role in fungal DNA synthesis and inhibition of fungal sphingolipid biosynthesis [22].

This study aimed to determine the antifungal activity of moringa seed oil from NTT, Indonesia, against *M. furfur* ATCC 14521 in vitro using the well diffusion method with inhibition zone parameters, minimum inhibitory concentration test by liquid dilution, and minimum fungicidal concentration test by colony count. NTT moringa plants have the best quality in Indonesia with oil content in moringa seeds reaching 35-40% [23]. This is due to the habitat of the moringa plant that thrives in tropical areas in the NTT region, which has a climate with low rain intensity on all types of soil and is resistant to dry season with drought tolerance of up to 6 months [17].

2. Materials and Methods

Dried moringa seeds aged ± 3 months, obtained from Baumata village, NTT, Indonesia, taken from August to September 2022 (Determination Number: 03/LBM/IT/11/2021), chloroform (Merck), anhydrous acetic acid (Merck), concentrated H_2SO_4 (Merck), $KHSO_4$ (Merck), n-hexane (Merck), ethyl acetate

(Merck), Sabouraud dextrose agar media (Merck), Sabouraud dextrose broth media (Merck), *Malassezia furfur* fungus ATCC 14521 (Kwik Stik™ brand), 70% alcohol (Medika), distilled water (Onelab), NaCl 0.9% (Widatra Bhakti), ketoconazole (Kalbe Farma), chloramphenicol (Afi Farma), and Tween 80 (Merck).

2.1. Preparation of Moringa Seed Oil Test Materials

The dried moringa seeds were peeled from the skin, cleaned, and then weighed as much as 200 g. The moringa seeds were put in an oil press maksindo (cold press). The oil obtained was then filtered from the dregs using flannel cloth and stored in a closed container and then the yield value was calculated using the following formula [24]:

$$\text{Yield} = \frac{\text{Weight of oil produced (g)}}{\text{Moringa seed weight (g)}} \times 100\%$$

2.2. Phytochemical Test

2.2.1. The Liebermann-Burchard Reaction

1 ml of moringa seed oil was taken, put in a test tube, dissolved in 0.5 ml of chloroform, and added with 0.5 ml of anhydrous acetic acid. The mixture was then added with 1-2 ml of concentrated H₂SO₄ through the tube wall, a positive result containing steroids was indicated by the formation of a bluish green color [25].

2.2.2. The Salkowski Test

1 ml of moringa seed oil was taken and put in a test tube, then 1-2 ml of concentrated H₂SO₄ was added through the wall of the tube; the presence of unsaturated steroids was indicated by the appearance of a red ring [26].

2.2.3. Identification of Steroids Using TLC

Moringa seed oil was spotted on the stationary phase of GF₂₅₄ gel and the mobile phase n-hexane:ethyl acetate (4:1). The results were indicated by the presence of a sulfuric acid anisaldehyde spotter, the presence of steroids was indicated by the occurrence of purple color on the plate spots, and then the R_f value of the steroid was calculated [26].

2.2.4. Acrolein Test

1 ml of moringa seed oil was taken, and 3 drops of KHSO₄ were added. The mixture was heated slowly on a fire. The presence of fatty acids was indicated by the pungent acrolein smell, which was distinguished from the smell of SO₄ [27].

2.2.5. Translucent Test

The translucency test was carried out by taking 2-3 drops of moringa seed oil and then dripping it on filter paper, the presence of stains on the filter paper indicated the presence of fat [28].

2.3. Antifungal Activity Test

2.3.1. Inoculum Preparation and Characterization of *M. Furfur*

Fungus inoculums were prepared by taking a stick of *M. furfur* (Kwik Stik™) and then smearing it evenly on SDA media; after that, it was incubated at a temperature of 37°C for 24-48 h while observing the growth of the fungus. After the fungus was declared to grow, it was characterized macroscopically, which was stated positive if a shiny beige fungus colony formed, and microscopically using a light microscope with a magnification of 20 times on the preparation with lactophenol cotton blue staining; positive results were obtained if we saw small oval, round, and cylindrical fungus shapes with short and unbranched hyphae [29].

1 ose of the *M. furfur* fungus stock was taken, suspended in 5 ml of 0.9% NaCl solution, and compared with 0.5 McFarland Equivalence Turbidity Standard [30]. Preparation of *M. furfur* inoculum was carried out using the pour method by pouring the fungus suspension into liquid SDA media with a ratio of 1:9. 5 ml of the fungus suspension was taken and put into 45 ml of warm SDA, then mixed evenly and poured into a Petri dish with a diameter of 15 cm [31].

2.3.2. Antifungal Testing Using the Well Diffusion Method

The SDA media in the Petri dish was left until it solidified, and then, the media was perforated using a 6-mm diameter perforator. In each well, 50 µL of moringa seed oil test preparations were dripped with concentrations of 7.5%, 10% and 12.5%, 1% tween 80 (as negative control) and 2% ketoconazole (as positive control). The Petri dishes were then incubated for 24 h at 37°C. The inhibition zone formed was measured using a caliper [30].

2.3.3. Testing Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The MIC test was conducted using liquid dilution method by planting *M. furfur* in Sabouraud Dextrose Broth (SDB) media in test tubes. The test used 12 test tubes for samples including ketoconazole 2% (positive control), 1% tween 80 (negative control), moringa seed oil concentration were 0.25, 0.50, 0.75, 1.00, 1.25, 2.50, 3.75, 5.00, 6.25, and 7.50%. Each sample was made in 3 tubes, namely for three repetitions by taking 1 ml aseptically to read the absorbance at a wavelength of 600 nm (0 hour), then incubated at 37°C for 24 hours, vortexed and measured the absorbance value again. KHM value was calculated by MIC = OD after the incubation - OD before the incubation [32], where OD = optical density.

The MFC test was carried out by counting the number of colonies by planting fungus on media (SDA) in Petri dishes. The test was carried out by growing the culture from each MIC test tube in a pour plate on sterile SDA media by taking 1 mL of the sample, growing it on SDA media and incubating at

temperature of 37°C for 24 hours, then the growth of the fungus was observed by counting the number of colonies using a colony counter. MIC was indicated by the absence of fungal colonies growing on the media [33].

2.4. Data Analysis

The research data were analyzed by Analysis of Variation (ANOVA) using SPSS 15 version. The data analysis was carried out by testing the data normality using the Kolmogorov-Smirnov test [30]. Fig. 1 presents the study flowchart.

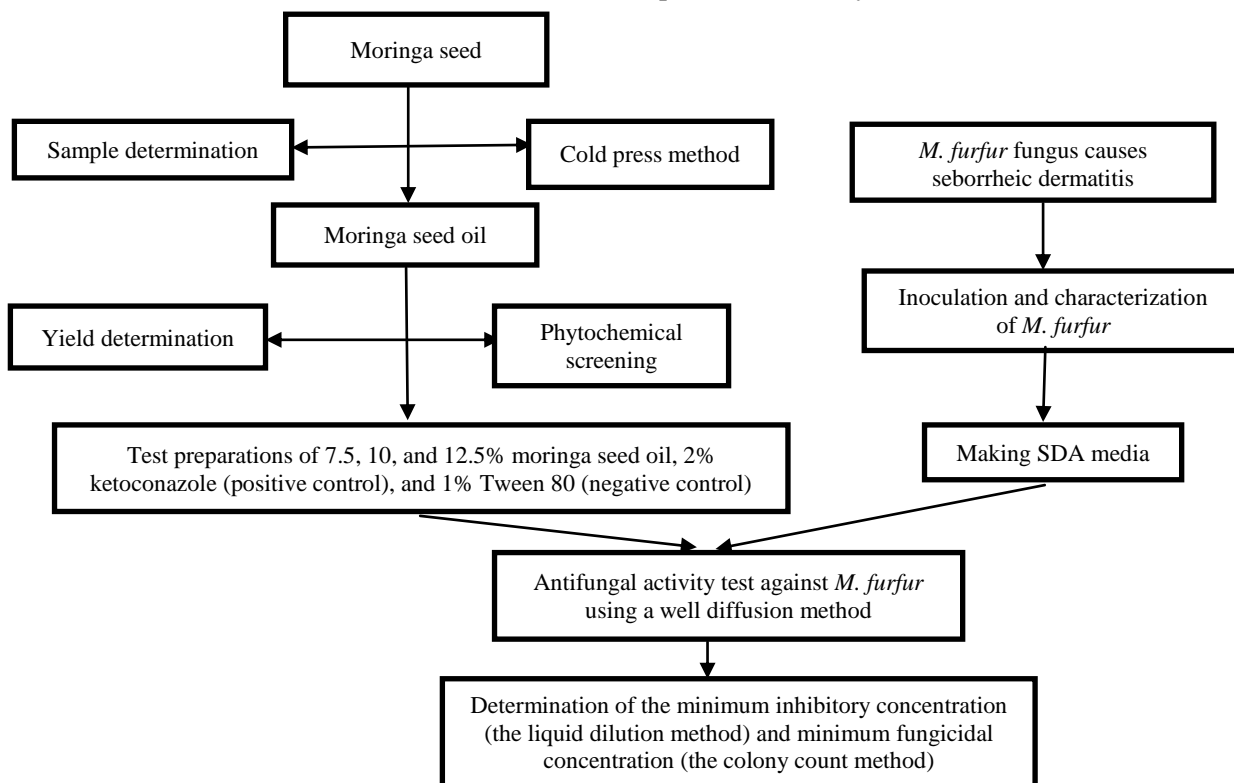


Fig. 1 Flowchart of the study

3. Results and Discussion

3.1. Moringa Seed Oil Preparation Results

The results of pressing 200 g of dried moringa seeds obtained a yield of 8.23%. The yield obtained was in accordance with the range of cold pressed moringa seed oil, namely 4-10% [34]. The color of moringa seed oil ranges from clear light to dark yellow with a distinctive aroma and slightly bitter taste.

3.2. Phytochemical Test Results

Phytochemical screening was a qualitative preliminary test to determine the content of phytosterols and fatty acids in moringa seed oil. Phytosterol content in moringa seed oil was identified using the Liebermann-Burchard, Salkowski reagents, and TLC. The content of fatty acids in moringa seed oil was identified using the acrolein and translucent tests. The results of the phytochemical screening are presented in Table 1.

Table 1 Phytochemical test results for moringa seed oil

Compounds	Method/Reagent	Result	Reference
Phytosterol	Liebermann-Burchard	Bluish green color was formed	[40]
	Salkowski	Red colored ring was formed	[41]
	TLC mobile phase n-hexane : ethylacetate (4:1)	Appearance of purple anisaldehyde sulfuric acid spots	[41]
Fatty acids	Acrolein test	Specific pungent smell of acrolein was smelled	[42]
	Translucent test	Transparent stain was formed	[43]

Table 1 shows that moringa seed oil contained components of phytosterols and fatty acids where both components belong to the lipid group [35]. Moringa seeds have a phytosterol content of 27.47% [36]. Phytosterol components in moringa seed oil include brassicasterol, ergostadienol, methylene cholesterol, campasterol, campestanol, stigmasterol, ergostadienol, clerosterol, β -sitosterol, stigmastanol, avenasterol,

stigmastadienol, isoavenasterol and stigmastenol [37].

3.3. Characterization of *M. Furfur*

The results of the *M. furfur* characterization were obtained by direct observation and using a microscope. The results of direct observations showed that the color was yellowish cream which would turn into brown, soft in texture and would become dry and wrinkled (Fig.

2A). Observation with a microscope with lactophenol blue staining showed that it was small oval, round,

cylindrical, as well as has short and unbranched hyphae (Fig. 2B and 2C) [29].

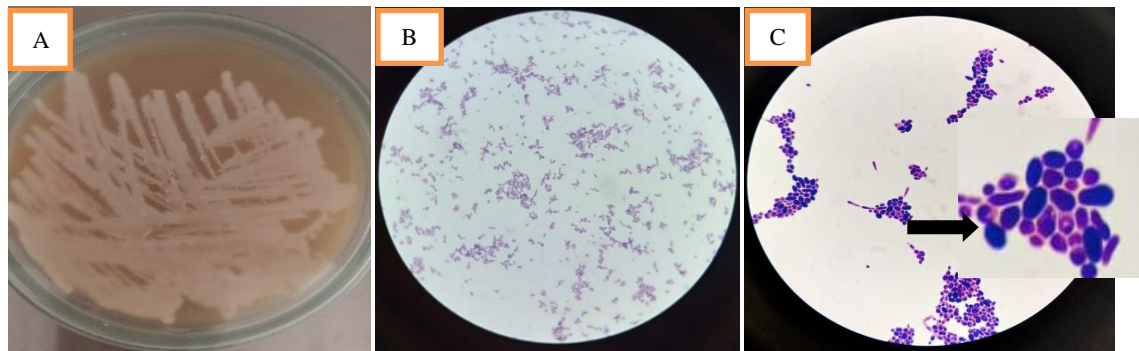


Fig. 2 Observation of *M. furfur* characterization

3.4. Results of Antifungal Activity Test of Moringa Seed Oil

The results of antifungal test of moringa seed oil in Table 2 shows an inhibition of the *M. furfur* growth; this could be seen from the formation of an inhibition zone around the well area. According to Hutasoit, the criteria for antimicrobial power with an inhibition zone diameter of less than 5 mm are categorized as weak, an inhibition zone of 5-10 mm is categorized as medium, an inhibition zone of 10-20 mm is categorized as strong and an inhibition zone greater than 20 mm is

categorized as very strong [38]. Based on these criteria, the strength of the antifungal power of moringa seed oil against the *M. furfur* at a concentration of 7.5%, 10% was included in the strong category while the 12.5% concentration is included in the very strong category. Ketoconazole as a positive control was also included in the very strong category while the negative control 1% tween 80 did not show an inhibition zone, which means it had no inhibitory activity so that as a solvent it did not affect the activity of the active substance that being dissolved.

Table 2 Results of the antifungal activity test of moringa seed oil against *M. furfur*

Sample Test	Range of Inhibition Zone (mm)	Statistic Analysis	
	Average \pm SD	Significant difference from negative control (p)	Significant difference from positive control (p)
7.5% moringa seed oil	14.5 \pm 1.32*	0.00 > 0.05*	0.00 < 0.05*
10% moringa seed oil	18.3 \pm 3.51*	0.00 > 0.05*	0.00 > 0.05*
12.5% moringa seed oil	26.3 \pm 1.04*	0.00 > 0.05*	0.00 > 0.05*
Positive control	51.6 \pm 2.36*	0.00 < 0.05*	-
Negative control	0 \pm 0	-	0.00 < 0.05*

* Significant difference between moringa seed oil concentrations and control

The formation of inhibition zone was caused by the metabolite compounds contained in the moringa seed oil that diffusing into the agar media, so that the growth of fungi that came into direct contact with the media would be inhibited [30]. Observations of the antifungal activity of moringa seed oil against *M. furfur* showed that there was a correlation between the concentration of moringa seed oil and the diameter of the inhibition zone, the higher the concentration, the larger the diameter of inhibition zone formed [39]. The difference in the diameter of inhibition zone indicated the difference in the ability of moringa seed oil to inhibit the growth of *M. furfur* based on concentration. The concentration of the antifungal test substance also affects its speed of diffusion with the fungus, where the greater the concentration, the faster it diffused to inhibited fungal growth [40].

Data analysis was carried out using the Kolmogorov-Smirnov test for data normality. The test results showed that the data were not normally distributed ($p < 0.05$). With the results of the one-way

ANOVA test on the area of inhibition with a value of $p = 0.021 < 0.05$ (α), it could be concluded that H_0 was rejected, which means there was a significant difference in inhibition zone of moringa seed oil (7.5, 10, and 12.5%), positive control (2% ketoconazole), and negative control (1% Tween 80).

3.5. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) Test Results

The MIC test results in Table 3 show that moringa seed oil at a concentration of 2.5, 3.75, 5.00, 6.25, and 7.50% has a decrease in the absorbance value, which means there was an inhibition of the *M. furfur* growth. It could be seen that the absorbance value before incubation was greater than the absorbance value after incubation, as well as the positive control (2% ketoconazole). This means that based on the results of UV-Vis spectrophotometer a concentration of 2.50% was the MIC of moringa seed oil against the *M. furfur* growth. This was in line with a research that conducted

by Riad, where moringa seed oil at concentration of 2% was able to reduced the growth of fungal spores *Fusarium sp.* and moringa seed oil at concentration of 2.50% were able to inhibited the growth of pathogenic fungi as well as moringa seed extract at a concentration of 5, 10, 15, 20 and 25% also showed linear antifungal activity as the concentration increased [41].

Table 3 Results of MIC test of moringa seed oil for the growth of *M. furfur* with spectrophotometer

Samples	Absorbance Average ± SD		MIC Value ± SD (After-Before)
	0 Hour	24 th Hour	
Negative Control	0.132 ± 0.05	0.325 ± 0.03	0.193 ± 0.03
Positive Control	0.251 ± 0.06	0.149 ± 0.04	-0.102 ± 0.02
7.50%	1.874 ± 0.13	1.153 ± 0.12	-0.721 ± 0.02
6.25%	1.441 ± 0.18	1.435 ± 0.05	-0.006 ± 0.14
5.00%	1.459 ± 0.21	1.325 ± 0.03	-0.134 ± 0.18
3.75%	1.458 ± 0.05	1.259 ± 0.03	-0.199 ± 0.04
2.50%	1.155 ± 0.13	1.059 ± 0.40	-0.096 ± 0.29
1.25%	0.718 ± 0.12	0.859 ± 0.06	0.141 ± 0.13
1.00%	0.366 ± 0.08	0.467 ± 0.13	0.101 ± 0.20
0.75%	0.409 ± 0.10	0.419 ± 0.07	0.011 ± 0.06
0.50%	0.368 ± 0.07	0.459 ± 0.07	0.091 ± 0.02
0.25%	0.227 ± 0.00	0.359 ± 0.08	0.132 ± 0.08

Moringa seed oil at a concentration of 0.25, 0.50, 0.75, 1.00 and 1.25%, negative control (1% Tween 80) could be seen that there was an increase in the absorbance value, which means that at this concentration there was no inhibition of the *M. furfur*. Data analysis was carried out by testing for data normality using the Kolmogorov-Smirnov test. The test results showed that the data were not normally distributed ($p < 0.05$) so that it was continued with the one-way ANOVA test. The results of ANOVA test showed a significant difference in the number of fungi at each concentration and incubation time ($p < 0.05$); post hoc test was carried out with the Tukey method.

Fig. 3 shows that with moringa seed oil at a concentration of 5.00, 6.25, and 7.50%, there was no fungal growth, while a concentration of 0.25-3.75% showed the growth of *M. furfur*. These results indicated that the minimum concentration of moringa seed oil that kill the fungal growth was at concentration of 5.00%. Research conducted by Riad also showed that 5.00% moringa seed extract provided high effectiveness in inhibiting the growth of pathogenic fungi *in vitro* [41].

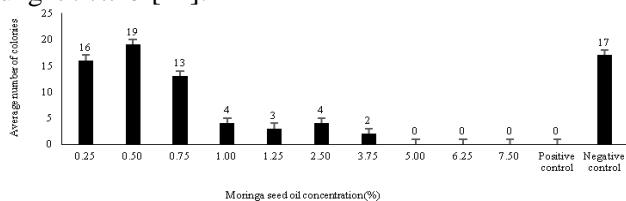


Fig. 3 Graph of MFC results for moringa seed oil against *M. furfur*

The positive control of 2% ketoconazole showed no fungal growth, which means that 2% ketoconazole had good abilities of inhibiting and destroying growth of *M. furfur*, the target mechanism of action on the ergosterol biosynthetic pathway by inhibiting the enzyme 14 α -sterol demethylase, which converts lanosterol to

ergosterol, thereby causing the fungal cell membrane to become vulnerable, easy to lysis, and leads to fungal cell death [42].

The novelty of this study is antifungal activity test of moringa seed oil from East Nusa Tenggara that obtained by cold pressed method that targeting the fungus *M. furfur* ATCC 14521 using the well diffusion method. Results of this study were that 12.5% moringa seed oil was the most effective concentration and has very strong antifungal activity. The ability of moringa seed oil to inhibit and destroy the growth of *M. furfur* was because it contained phytosterols and fatty acids. Phytosterols are a group of compounds that can inhibit or destroy growth of fungi by interacting with membrane sterols. The main effect of phytosterols on microbes is the release of proteins and enzymes from within the cell. Phytosterols contribute as an antifungal mechanism by reducing the surface tension of the sterol membrane of the cell wall so that its permeability increases. Increased permeability results in a more concentrated intracellular fluid being pulled out of the cell so that nutrients, metabolic substances, enzymes, proteins in the cell come out and the fungus has death while the mechanism of action of fatty acids as an antifungal occurs through interaction with the lipid bilayer of the fungal cell membrane, which results in the release of electrolytes and intracellular protein which is not controlled so that the disintegration of cytoplasm of fungal cell is occurs [43]. The limitations of this study are that the active compound of moringa seed oil which has an antifungal effect was not studied yet, an analysis of its relative antifungal potential and the mechanism of antifungal inhibition was not carried out yet, so that its activity as fungicide or fungistatic was not known certainly yet.

4. Conclusion

Moringa seed oil at concentrations of 7.5, 10 and 12.5% has antifungal activity against *M. furfur* with inhibition zone of 14.5 ± 1.32 mm (strong category), 18.3 ± 3.51 mm (strong category) and 26.3 ± 2.36 mm (very strong category). Moringa seed oil has the potential to be developed as an antifungal, where the greater the concentration the greater the inhibition and fungicidal power against *M. furfur*. The novelty of this study is antifungal activity test of moringa seed oil from East Nusa Tenggara that obtained by cold pressed method that targeting the fungus *M. furfur* ATCC 14521 using the well diffusion method. 12.5% moringa seed oil was the most effective concentration and has very strong antifungal activity with a minimum inhibitory concentration of 2.50% and a minimum fungicidal concentration of 5.00% The limitations of this study are that the active compound of moringa seed oil which has an antifungal effect was not studied yet, an analysis of its relative antifungal potential and the mechanism of antifungal inhibition was not carried out yet, so that its activity as fungicide or fungistatic was

not known certainly yet.

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