The antigungal effect of Moringa

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The Antifungal Effect of Moringa Oleifera on the Growth and Cell Surface Changes of Candida albicans in Oral Mucose Infection

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Abstract

Candida albicans (C. albicans) were reported as a causative pathological agent that triggers oral candidiasis infection. Moringa leaves (Moringa oleifera) have antifungal properties and contain antioxidant compounds that can potentially prevent interactions between C. albicans receptors and protein ligands of epithelial cells and fibroblast cells.

This study aimed to evaluate changes in C. albicans cell surface on hydrophobicity activity and hydrocarbon response from the effect of C. albicans growth associated with healing in a mucosal infection model by C. albicans. Changes in C. albicans cell surface (hydrocarbon and hydrophobicity) using xylene and Crystal violet staining. Assessment of C. albicans growth by spectrophotometry and mucosal healing by H and E staining. M. oleifera has hydrocarbon properties at 24 hours (13%), 48 hours (12%), and 72 hours (12%). At 48 hours, all concentrations of the test material experienced increased hydrocarbon activity against C. albicans. At 72 hours, the movement of hydrocarbons tends to stabilize on the surface of C. albicans cells. M. oleifera maintained the quantity of C. albicans colonies <300 CFU/mL (OD:<0.01). M. oleifera had a relationship with inhibiting the growth of C. albicans at the incubation time of 24 hours and 48 hours, respectively.

The M. oleifera also increased the healing of wounds infected by C. albicans based on increased fibroblast cells. M. oleifera can increase hydrocarbons and reduce the hydrophobicity of the cell surface of C. albicans and reduce growth. In addition, M. oleifera can increase the healing of oral mucosal infections after being infected with C. albicans, which is indicated by an increase in fibroblast cells.

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Introduction

Candida albicans (C. albicans) is a causative pathological agent that triggers candidiasis infection. This infection can have implications for a decrease in the mucosal defense system. In addition, C. albicans has the characteristic of spreading more quickly if the biological conditions of the oral cavity are not balanced. Changes in temperature, salivary pH, and hormonal disturbances can trigger the development of these fungi, thereby exacerbating the infection.[1]

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Several factors can increase the risk of C. albicans growth in the oral cavity. One of the most common causes is the overuse of antibiotics, co-morbidities such as cancer, AIDS, diabetes, and denture users, which cause chronic irritation. Candidiasis can be fatal if it reaches the bloodstream or vital organs such as the heart, but it is rare, except in chronic cases. Decreased immunity further exacerbates this infection.[2]

The fungus C. albican has several virulence factors involved in the pathogenesis of the infection. Hydrophilic and hydrophilic properties are two virulence factors that often act when involved in pathogenesis. In addition, adhesion factors and biofilm formation are reported to contribute together with hydrophilic properties when penetrating and infecting the host mucosa.[3] Increasing the hydrophilic properties of the cell surface is a strategy for

adhesion or binding to the ligands present in the host cell. This bond helps C. albicans to spread and form new communal as a phase to expand the infection. [4] In candidiasis caused by cigarette smoke, C. albicans tendBonds between nicotine receptors and nicotine receptor ligands can cause changes in cell properties that support the development of C. albicans.[5]

Albert (2002) reported that each cell type has almost the same membrane structure, where the cell membrane consists of a phospholipid bilayer. This phospholipid bilayer is two layers of fat that bind to phosphates. So it can be explained that each cell maintains its hydrophobicity, especially in the inner layer of the cell.[6] Even so, the hydrophilic nature of the cell surface is also supported to balance the active transport process. Especially when there is a bond between antioxidant receptors and cell ligands to maintain a hydrophobic atmosphere when there is an interaction between pathogens and host cell ligands.[7] This mechanism is focused on supporting the balance of response between drug and antigen and between drugs with cell protection properties as antioxidants.[8]

Several studies on antifungal drugs refer to hydrophilic and hydrophobic systems. One side of the drug must have hydrophilic properties with the cell surface, and it must also have hydrophobic receptors to prevent interactions between pathogens, including C. albicans. Host cells influence the cells to become malignant.[9] In candidiasis caused by cigarette smoke, C. albicans tend to develop because nicotine and tar can increase the expression of nicotinic acetylcholine receptors.[10] The bond between nicotine receptors and nicotine ligands can cause changes in cell properties that support the development of C. albicans.[11]

The use of several natural ingredients, such as Moringa leaves (Moringa oleifera), has been reported to have antifungal properties [12] and contains high antioxidants.[13] Our previous research found that the ethanol extract of Moringa leaves had good cytotoxic properties against bacterial cells, which correlated with antioxidant content. Research on the hydrophobicity of Moringa leaves related to antioxidants against epithelial cells and fibroblasts has not been widely reported. This research is expected to obtain a correlation between the hydrophobicity of the ethanol extract of Moringa leaves and its antioxidant power to support the protection of epithelial cells and fibroblast cells affected by C. albicans infection to prevent oral candidiasis infection.

Candidiasis of the oral cavity caused by C. albicans is still a threat. Ligands from fibroblast cells and mucosal epithelial cells are targets for the adhesion receptors of C. albicans. The use of antifungal drugs increases the hydrophobicity of the cell surface and affects the hydrophobic properties of the C. albicans cell surface.[14] This concept applies to all antifungal and other pathogenic drug systems. Moringa leaf extract has been reported to have antifungal and antioxidant properties in cell lines. The study of hydrophobic properties related to the protective response of cells due to C albicans infection has not been reported before, so this study is expected to find a relationship between the hydrophobicity index of the ethanol extract of Moringa leaves and its ability to prevent damage to fibroblast cells and oral mucosal epithelial cells after being infected with C. albicans.

Materials and methods

The research has approved ethical clearance No 324/KE/FKG/2021 from the Faculty of Dentistry, Syiah Kuala University, Darussalam, Banda Aceh, Indonesia. The research material consisted of ethanol extract of M. oleifera and C. albicans isolate ATCC 10231. Evaluation of the antifungal effect on changes in the response of C. albicans cells using time and concentration bases

Extraction of Moringa oleifera

Moringa leaves (Moringa oleifera) separated from the stalks were collected as much as 1 kg and then washed with water. Drying was carried out for two days until wilted, then dried in an oven at 50 □C for ±48 hours. Moringa leaves are crushed with a blender to obtain Moringa leaf powder. Moringa leaf powder obtained is then stored in an airtight container. The powder was placed in a clean flat bottomed glass container, closed, and soaked in 100 mL of 96% ethanol. The residues and filtrates were separated for three days and interspersed with replacing the same solvent. The filtrates were collected and concentrated with a rotary vacuum evaporator at a temperature of 50c°C and a pressure of 75 mm Hg to obtain the extract.

Culture of Candida albicans

Candida albicans isolate ATCC 10231

was revived by growing on SDA media for 48 h at 37 °C, and one colony was cultured in liquid peptone medium for 48 h at 37 °C. Then equalized Mc Farlan 0.5 or equivalent to 0.08-0.1 nm, then applied to experimental animals.

Assessment of Cell surface changes of Candida albicans

The C. albicans cell surface assessment is based on changes to the hydrocarbon and hydrophobicity of C. albicans cells after interaction with M. oleifera. The C. albicans suspension was added with 5 mL of M. oleifera extract at concentrations of 3.125%, 6.25%, 12.5%, 25% and 50%. Then incubated for one h at 37 ^{0}C and centrifuged at 2500 rpm for 20 minutes. Then the supernatant was removed, 1 mL xylene (sigma) was added, and placed in a water bath at 37 ^{0}C for 10 min. After that, it was vortexed for 30 seconds to mix the suspension with xylene, then stored in a water bath with a temperature of 37 ^{0}C for 30 min to separate the break from xylene. Then the residue was carefully transferred to another sterile tube, and the remaining xylene in the pipette was resuspended with 2 ml of PBS pH 7.0. Then 1 mL of 1% crystal violet was added to the tube. Then let it stand for 5 min. The hydrophobic rings and the hydrocarbon areas formed are then measured with a caliper (mm) (Fig 1). The value of the effect of M. oleifera in inhibiting the formation of hydrophobicity and hydrocarbons on C. albicans cells was calculated using the tube area formula. The formula for the area of a cylinder V= π.r2.t, where V= volume of the tube (mm³) π= phi (3.14 or 22/7) r= radius of the tube (mm) t= height of the tube (mm).[15]

Candida Infection model on Mucosa

In this study, C. albicans infection was made by smearing C. albicans solution (Mc Farland 0.5) on rats' Mucosa and gum area using a disposable micro applicator (2 mm). C. albicans was applied for 2 to three days. Mice were acclimatized for one week before the experiment, placed in individual cages for a 12-hour light/dark cycle, and received food in the form of pellets and water. Mice in this study provided as many as 35 rats divided into five treatment groups, one positive control group, and one negative control group. Each group obtained five rats. The infection treatment for seven days and the use of M.Oleifera as an antifungal for seven days.

Growth of Candida albicans Assay

Swabs from the mucosa of mice infected with C. albicans were transferred in a peptone medium and then cultured on selective Cromagar media for 24 hours at incubation. One C. albicans colony was then cultured on peptone medium as isolate stock. It was next calibrated with Mc. Farlan 0.5 (1.5x108 CFU/mL). Furthermore, 100 µL of C. albicans suspension was included in 1 mL of an extract with different concentrations, homogenized at room temperature for 15 min. Then it was cultured in an aerobic atmosphere at 37 0C for 24 hours. Then it was homogenized at 5000 xg for 15 min, and 150 µL supernatant was added and read on the spectrophotometer at 600 nm.

Identification of fibroblast and Epithelial Cells

Mucosal retrieval was performed after the rats were euthanized with xylazine. Mucosa was excised with a thickness of 5 mm to the submucosa. After that, the mucosal preparation was fixed using a 10% Neutral Buffer Formalin solution, and then organ trimming was performed and put in a plastic cassette tissue. The next stage was a dehydration process using two stages of acetone for 1.5 hours, and then clarification was carried out using two stages of benzol for 1.5 hours. The printing or paraffinization process was done with benzol + paraffin (1:1 ratio) for 1.5 hours and paraffin for 1.5 hours. The preparation is put into a block/printer containing half the volume of paraffin, and the practice is placed vertically and horizontally so that the cross-section is attached to the paraffin base. After it starts to freeze, paraffin is added again until the block/mold is complete, and leave until the paraffin hardens. The paraffin block was then cut into 5 mm thin using a microtome.

The results of ribbon-shaped pieces (ribbon) are stretched over warm water at a temperature of 46 ^{0}C and immediately lifted, which helps extend the details, so they don't fold or be removed folds due to cutting. The preparation was then removed, placed on a glass object, and dried for 18 h in an incubator at 60 ^{0}C. Furthermore, staining was performed with Mayer's Hematoxylin and Eosin for eight min and then washed in running water for 30 sec. Then do the Eosin staining for 2-3 min, then rinse with running water for 30 seconds. The following process is dipping the preparations in 95% alcohol and absolute alcohol (I and II) 10 times. Then, the immersion was carried out gradually in

absolute alcohol and xylol I for 1 minute each and then in xylol II for 2 min. The last process is closing the tissue by flatly placing the object-glass on the tissue paper. Entellan adhesive dripped onto both object glasses and covered with a cover glass.

Statistical Analyses

Data on growth, cytotoxicity, and index hydrophobicity were analyzed by One Way ANOVA, while Kruskal Wallis analyzed data on the repair of epithelial cells and fibroblasts. Meanwhile, the correlation between the two uses Pearson and Spearman, with a significance limit of p<0.05 and a relationship (r = 1).

M. oleifera	N	Hydrocarbon area (mm^3)						** p-value
		24 jam (n=21)		48 jam (n=21)		72 jam (n=21)		
		Mean±SD	Freq	Mean±SD	Freq	Mean±SD	Freq	
50%	3	596.6±0.28	13%	694.73±0.64	14%	741.83±0.21	15%	
25%	3	753.6±0.85	17%	757.53±0.21	16%	663.33±1.20	14%	
12.50%	3	600.53±1.63	13%	737.9±0.42	15%	675.1±1.84	14%	
6.25%	3	702.58±0.49	16%	714.35±1.27	15%	698.65±0.42	14%	0.057
3125%	3	655.48±0.21	15%	745.75±0.71	15%	769.3±0.85	16%	
Nystatine	3	635.85±0.42	14%	631.93±0.49	13%	710.43±0.49	15%	
C.albicans	3	573.05±0.14	13%	592.68±0.07	12%	573.05±0.28	12%	
*p-Value		0.049		0.591		0.067		

Table 1. Hydrocarbon index of M. oleifera on the cell surface of C. albicans.
* Kruskal-Wallis, ** One Way Anova.

M. oleifera	N	Hydrophobicity inhibition (mm^3)						** p-value
		24 h (n=21)		48 h (n=21)		72 h (n=21)		
		Mean±SD	Freq	Mean±SD	Freq	Mean±SD	Freq	
50%	3	47.1±0.01	12%	90.28±0.07	18%	78.5±0.42	14%	
25%	3	62.8.6±0.14	15%	86.35±0.14	17%	109.9±0.28	20%	
12.50%	3	70.65±0.01	17%	66.73±0.35	13%	113.8±0.35	20%	
6.25%	3	78.5±0.14	19%	70.65±1.13	14%	66.73±0.35	12%	0.042
3125%	3	39.25±0.01	10%	47.10±0.01	10%	78.5±0.14	14%	
Nystatin	3	86.35±0.14	21%	102.01±0.01	21%	54.95±0.28	10%	
C.albicans	3	23.55±0.14	6%	31.40±0.14	6%	58.88±0.07	10%	
*p-Value		0.031		0.491		0.021		

Table 2. Hydroposity index of *M.oleifera* on the cell surface of *C. albicans*.
* Kruskal-Wallis, ** One Way Anova.

Results

Table 1 shows the value of Moringa oleifera index hydrocarbons on the effect of the hydrophobicity activity of C. albicans cell surfaces. Based on the research findings, C. albicans was only able to limit the influence of hydrocarbon activity at 24 hours (13%), 48 hours (12%), and 72 hours (12%). Increased activity of the test material's hydrocarbons within 24 hours at concentrations of 25% (17%), 6.25% (16%), and 3.125% (15%), Whereas at 48 hours, all concentrations of the test material experienced an increase in hydrocarbon activity, except for the 50% concentration and the positive control. At 72 hours, the movement of the hydrocarbons tends to stabilize towards the hydrophobicity of the C. albicans cell surface. The concentration of 3.125% and positive control have increased.

M. oleifera	N	24 h (n=21)			48 (n=21)			72 (n=21)			**p-value
		Mean± OD	colony (CFU/mL)	Freq	Mean± OD	colony (CFU/mL)	Freq	Mean± OD	colony (CFU/mL)	Freq	
50%	3	0.05±0.002	<150	13%	0.05±0.002	<150	12%	0.06±0.009	<200	16%	
25%	3	0.05±0.003	<150	12%	0.05±0.003	<150	11%	0.05±0.002	<150	12%	
12.50%	3	0.04±0.003	<100	11%	0.05±0.003	<150	11%	0.05±0.005	<150	11%	
6.25%	3	0.04±0.004	<100	11%	0.06±0.004	<200	14%	0.05±0.004	<150	11%	0.352
3.13%	3	0.05±0.005	<150	13%	0.06±0.016	<200	15%	0.06±0.008	<200	13%	
C+ (Nystatin)	3	0.14±0.016	300-500	39%	0.16±0.003	300-500	38%	0.15±0.007	300-500	37%	
C. albicans	3	0.16±0.001	300-500	43%	0.17±0.001	300-500	40%	0.17±0.001	300-500	40%	
*p-value		0.01			0.011			0.012			

Table 3. Distribution and growth frequency of *C. albicans* affected by *M. oleifera*.
* Kruskal-Wallis, ** One Way Anova.

Incubation Times	*Moringa oleifera*	growth	Hydrocarbon Toxicity response	Hydrophobicity Biofilm response	Description
24 h	50%	13%	0.5%	-1.2%	The test material within 24 hours provided a 12% and 88% growth opportunity for *C. albicans*. *C.albicans* cells died as a result of 97.5% toxicity which was influenced by 2.5% hydrocarbon activity, thereby preventing the formation of biofilm-quorum sensing as much as 97.5% which was influenced by 2.5% by hydrophobicity
	25%	12%	4.3%	3.0%	
	12.5%	11%	1.9%	5.9%	
	6.25%	11%	4.2%	7.9%	
	3.125%	13%	1.5%	-3.4%	
	C+	39%	-25.1%	-18.0%	
48 h	50%	12%	2.4%	6.4%	The test material within 48 hours provided a 12% and 88% growth opportunity for *C. albicans*. *C.albicans* cells died as a result of 97.5% toxicity which was influenced by 2.5% hydrocarbon activity, to prevent the formation of 97.5% biofilm-quorum sensing, which controlled by 2.5% hydrophobicity activity
	25%	11%	4.7%	6.6%	
	12.5%	11%	3.8%	2.2%	
	6.25%	14%	1.1%	0.7%	
	3.125%	15%	0.4%	-5.4%	
	C+	38%	-24.7%	-17.0%	
72 h	50%	16%	-0.2%	-1.5%	The test material within 72 hours allowed *C. albicans* 13% and 87% growth opportunity. C.albicans cells died as a result of 98% toxicity which was influenced by 2% hydrocarbon activity, to prevent the formation of biofilm-quorum sensing 96.7%, which controlled by 3.3% hydrophobicity activity
	25%	12%	1.8%	7.6%	
	12.5%	11%	2.9%	9.2%	
	6.25%	11%	3.0%	0.4%	
	3.125%	13%	2.6%	0.6%	
	C+	37%	-21.9%	-26.8%	

Table 4. The growth of C. albicans based on the effect of hydrocarbon activity and hydrophobicity of the ethanol extract of M. oleifera.

M. oleifera	N	Fibroblast cell (%) n=35		
		Mean±SD	Frequency (%)	Recovery Status
50%	5	8,60±1.192	88%	Recovery
25%	5	10,04±1.22	90%	Recovery
12.5%	5	8,20±0.73	88%	Recovery
6.25%	5	7,32±0.50	78%	Moderate Recovery
3.125%	5	7,04±0.71	67%	Moderate Recovery
C+(Nystatine)	5	9.25±0.21	89%	Recovery
C- (Negative)	5	4,97±0.81	51%	No Recovery
*p-value		0.0012		

Table 5. Distribution and frequency of oral mucosal fibroblast cells after infection with *C. albicans* under the influence of *M. oleifera.*
* One Way ANOVA.

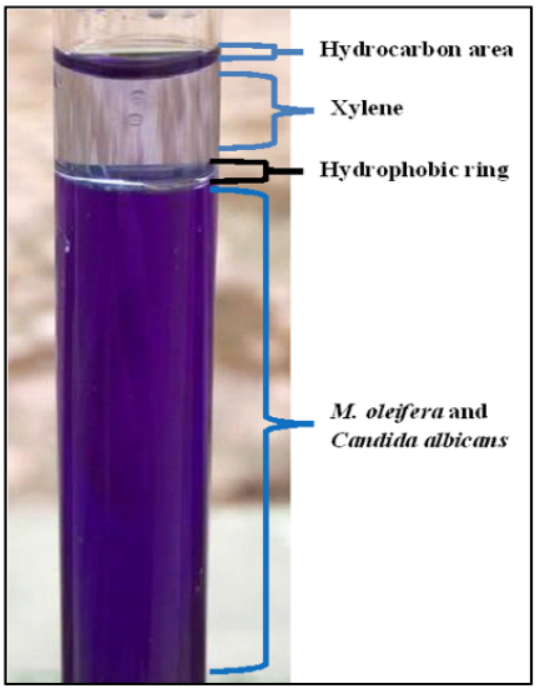

Figure 1. Assessment of the hydrophobicity and formation of C. albicans cell hydrocarbons.

Table 2 shows the value of the hydrophobicity index of Moringa oleifera on the influence of the surface hydrophobicity activity of C. albicans cells. Based on the results of the study, it was shown that C. albicans was only able to maintain changes in cell surface hydrophobicity activity at 24 hours (6%), 48 hours (6%), and 72 hours (10%). At all incubation times, it can generally inhibit the activity of C. albicans cell surface hydrophobicity. At 24 and 48 hours, the ethanol extract of Moringa leaves increased by 15% respectively; at 72 hours, it grew to 16%. In particular, at 24 hours, the concentration of 6.25% (19%) is better than other concentrations. At 48 hours, the concentration of 50% (18%) was the best to prevent the activity of C. albicans cell surface hydrophobicity. Meanwhile, at 72 hours of incubation, concentrations of 25% and 12.5% had better performance, each having an inhibitory effect on the surface hydrophobicity activity of C. albicans cells of 20%.

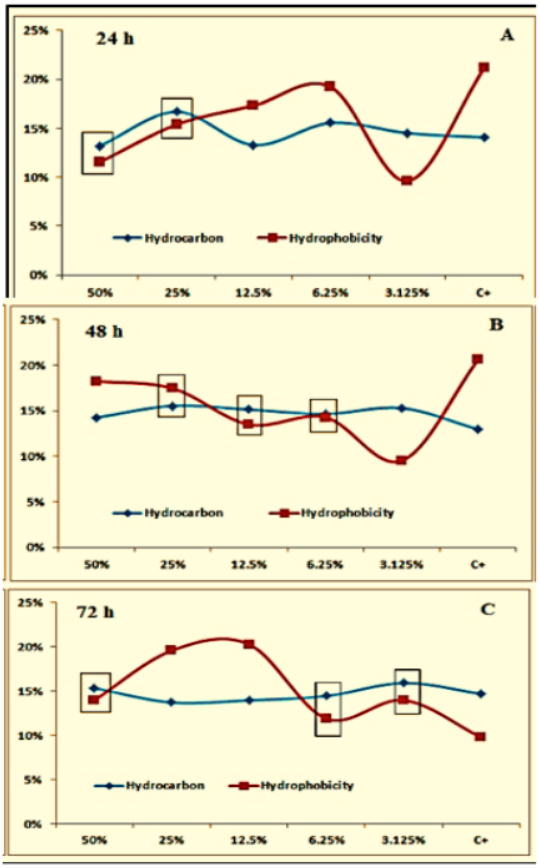

Figure 2. Distribution of the hydrophobicity and hydrocarbon activity of *M. oleifera* in influencing changes in the cell surface of *C. albicans*. In general, at all concentrations *M. oleifera* had a good effect on suppressing the surface hydrophobicity of *C. albicans* cells while increasing the activity of *C. albicans* cell surface hydrocarbons. The hydrophobicity graph line correlates with the activity of the hydrocarbons. (A) 25% and 50% concentration and (24 hours). (B) Concentrations of 25%, 12.5%, and 6.25% (48 hours). and (C). Concentration 50%, 6.25%, and 3.125% (72 hours). Data were taken in three repetitions for each concentration. Horizontal (M. oleifera mg/mL) and Vertical (correlation value).

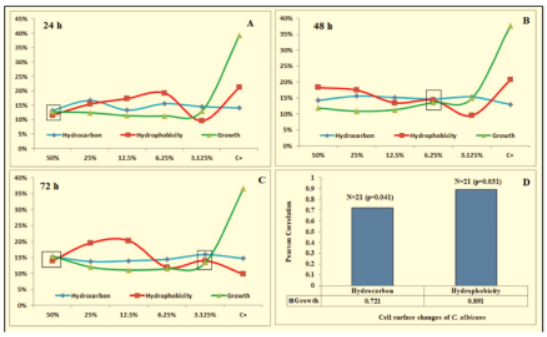

Figure 3. Correlation between the growth of *C. albicans* and the hydrocarbon activity and hydrophobicity of Moringa oleifera. In general, at all concentrations of Moringa oleifera there was a relationship between the growth quantity of *C. albicans* and the activity of hydrocarbons and cell surface hydrophobicity of *C. albicans*. Line chart of C. albicans growth correlated with hydrocarbons and hydrophosphoricity. (A) 50% concentration (24 hours), (B) 6.25% (48 hours), (C) 50% and 3.125% (72 hours), (D) Growth correlation with hydrocarbons and hydrophobicity. Data were taken in three repetitions for each concentration. Horizontal (*M. oleifera* in mg/mL interacted with *C. albicans*) and Vertical (correlation value).

Table 3 shows the incubation times of 24, 48, and 72 hours indicating the growth intensity of C. albicans with an average OD of 0.05-0.05 nm. Based on the Mc Farland standard this value is equivalent to <150 CFU or Mc. Farland 0.5 ($1.5x10^8$). Optical Density 0.05 nm (<150 CFU/mL), 0.08-0.1 nm (Mc Farlan 0.5; <300 CFU), OD 0.11-0.29 nm (Mc Farland 1; 300-600 CFU); OD 0.3-0.49 nm (Mc Farland 2; 600-1200 CFU). These scales were adopted by McFarlad Standard for in vitro use only, Catalog No. TM50-TM60, Scott Sutton, Measurement of Microbial Cells by Optical Density, 2011). [16]

Based on the Kruskal Wallis test analysis, it was shown that the growth of C. albicans affected by the ethanol extract of M. oleifera did not lead to a significant difference based on the incubation time p>0.05; 0.352). Meanwhile, based on the concentration, there were significant differences at 24 hours (p=0.01), 48 hours (p=0.011), and 72 hours (p=0.12). The Spearman's rho correlation shows that the growth of C. albicans with time has a weak relationship (r = 0.272). Meanwhile, there is a relatively strong relationship with concentration (r = 0.722). It means that the growth capacity of C. albicans is affected by the total concentration of M. oleifera.

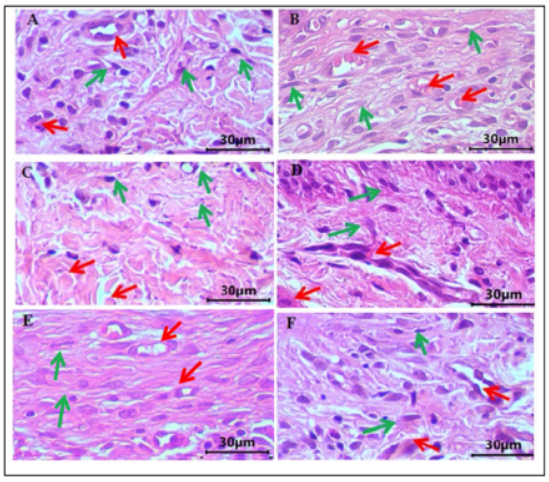

Figure 4. Histopathological profile of fibroblast cells and angiogenesis. The concentration of ethanol extract of Moringa leaves can induce an increase in fibroblast cells and angiogenesis in the oral mucosa. (A) 50%; (B) 25%, (C) 12.5%, (D) 6.25%, (E) 3.125% and (F) Negative Control. Green arrow (fibroblast) and red arrow (angiogenesis). 500x magnification.

Table 4 shows the growth of C. albicans which is affected by the activity of the hydrocarbons and the hydrophobicity of the ethanol extract of Moringa oleifera. The value of the toxicity response affected by the action of the hydrocarbon and the value of the response biofilm affected by the activity of the hydrophobicity were both obtained as a result of the percent growth value minus the percent value of the hydrocarbon index and the hydrophobicity index. At the same time, the value of the description of C albican growth for each treatment time (24 hours, 48 hours, and 72 hours) was obtained from the average increase, hydrocarbon, and hydrophobicity.

Table 5 reports the distribution and frequency of oral mucosal fibroblast cells after infection with C. albicans under the influence of M. oleifera. Concentrations of 50%, 25%, and 12.5% have antifungal properties by reducing the growth of C. albicans (Table 3). The decrease in C. albicans growth was in line with the infection

reduction, assessed based on the increase in mucosal fibroblast cells. Fibroblast cells work to help improve tissue repair to achieve healing. Figure 4 shows the number of fibroblast cells in the mucosa of mice that have been given M. oleifera for seven days.

Figure 2 shows no difference in the activity of M. oleifera hydrocarbons based on the incubation time of 24 hours, 48 hours, and 72 hours. In general, the hydrophobic activity of M. oleifera on the surface hydrophobicity of C. albicans cells increased at concentrations of 12.5% and 6.25% (24 hours). At 48 hours, the increase occurred at a concentration of 50%, and at 72 hours, the hydrophobic inhibition of C. albicans occurred at 25% and 12.5%. In comparison, the activity of M. oleifera's hydrocarbons and hydrophobicity closely disrupted the surface hydrophobicity of C. albicans cells, especially at concentrations of 50% and 25% (close relationship) at 24 hours of incubation. At an incubation time of 48 hours, the concentrations of 12.5% and 6.25% (very close relationship) had a close relationship between hydrocarbons and hydrophobicity. At 72 hours of incubation, the concentrations of 50% and 3.125% had a close relationship between the activity of hydrocarbons and the hydrophobicity of M. oleifera to prevent the hydrophobic activity of C. albicans cell surfaces.

Based on the One Way ANOVA analysis, it was shown that there was no significant difference between the activity of hydrocarbons and hydrophobicity on the surface of C. albicans cells affected by the ethanol extract of M. oleifera based on incubation time (Hydrocarbon: p> 0.05; 0.975 and hydrophobicity: p> 0.05; 0.940). Meanwhile, based on the concentration, there was no significant difference either for hydrocarbons (p>0.05; .0.406) or for hydrophobicity (p>0.05; 0.364). The Pearson correlation shows that the activity of hydrocarbons has a weak relationship with incubation time (r = 0.058), while there is no relationship with concentration (r= -0.062). It means that the quantity of the hydrocarbon is not affected by the amount of concentration.

Figure 3 shows the relationship between the growth of C. albicans and the hydrocarbon and hydrophobicity properties of M. oleifera. At 24 hours, it was 50% and 3.125%. At 48 hours, it was only 6.25%, while at 72 hours, the concentration was 50% and 3.125%. A growth chart line between the hydrocarbon and hydrophobicity graph lines indicates a good relationship. Both can control the growth of C. albicans except for positive control.

Discussion

This study generally reported that Moringa oliefera gave good results for increasing changes on the C. albicans cell surface on hydrophobicity activity and hydrocarbon response, as well as a good effect on C. albicans growth and increasing the healing of mucosal infections triggered by C. albicans. Onsare (2015) reported that the bioactive components of the M. oleifera seed coat had demonstrated anti-biofilm potential against test organisms classified as Gram-positive, Gram-negative, and yeast. [17].

Moringa oleifera increased the hydrocarbon value and decreased the hydrophobicity of the cell surface of C. albicans, as seen in Tables 1 and 2 during 24 hours (13%), 48 hours (12%), and 72 hours (12%) after exposure to hydrocarbons, the ethanol extract of M. oleifera demonstrated tolerance for C. albicans. Within 24 hours, concentrations of 25% (17%), 6.25 % (16 %), and 3.125 % (15 %) of the test material's hydrocarbons exhibited increased activity. At 48 hours, all concentrations of the test material experienced increased hydrocarbon activity, except for the 50% concentration and the positive control. At 72 hours, the movement of the hydrocarbons tends to stabilize towards the hydrophobicity of the C. albicans cell surface. The concentration of 3.125% and the positive control increased, thus indicating that the most negligible concentration still had an excellent effect in increasing changes on the cell surface of C. albicans.

Several studies reported that the antifungal effect of several active ingredients from plants could interfere with the surface of fungal cells [18,19]. This pathogenesis begins by interfering with communication between fungal cells to suppress toxic release [20]. Furthermore, the antifungal action damages the extra and intra-cell active transport systems, disrupting nutrient intake and the intra-cell fluid metabolism system. Another antifungal ability possessed by natural products such as M. oleifera is to interfere with the protein synthesis system involved in the response of the cell wall to its environment, such as HWP1 and ALS3. [21]. These two cell wall

proteins are involved in biofilm formation and adhesion to the host mucosa [22]. So the impact of disrupting the hydrophobic surface mechanism of C. albicans cells causes a decrease in adhesion ability. Hydrophobicity is another characteristic that is usually associated with the formation of biofilms [23].

Table 2 shows the value of the hydrophobicity index of Moringa oleifera on the influence of the surface hydrophobicity activity of C. albicans cells. Based on the study's results, it was shown that Moringa oleifera could reduce the surface hydrophobicity of C. albicans cells. This ability aligns with the decreased growth of C. albicans (Table 4). It means that the hydrocarbon and hydrophobicity properties of M. oleifera affect the growth quantity of C. albicans. These results indicate that M. oleifera affects changes in the cell wall's surface, thus having implications for decreasing its growth.

Previous studies reported that hydrocarbons adsorbed on the surface of microbial cells could be transported across the membrane into the cell mainly by passive or active transport. [24]. The hydrophobicity of the cell surface (CSH) plays an essential role in the adhesion of microorganisms to biotic and abiotic surfaces, which is positive for the growth rate of C. albicans [25]. Several research results have described the potential of Moringa oleifera leaves as an active ingredient to prevent the growth and activity of C. albicans cell surface hydrophobicity. [26]. Based on the growth value of C. albicans, M. oleifera is fungistatic against C. albicans. The hydrocarbon activity of M. oleifera can indicate cytotoxic activity against C. albicans cells. Meanwhile, the hydrophobicity of M. oleifera on the surface of C. albicans cells can suggest that M. oleifera can prevent quorum-sensing and biofilm formation by C. albicans [26]. Some plant antifungal compounds have been reported to be toxic to fungi such as C. albicans. The mechanism of antifungal toxicity occurs by disrupting cell membranes by increasing the pressure on permeability, resulting in higher penetration of intracellular fluids to extra cells. [27].

Table 5 reported that M. oleifera was able to increase the development of fibroblast cells. This means that this test material can reduce infection and improve mucosal healing. Reddy (2013) reported that fibroblast cells help improve tissue repair to achieve recovery [28]. In addition, M. oleifera can increase the development of fibroblast cells as an indicator of increased healing of mucosal infections due to infection by C. albicans [29]. This potential can illustrate that M. oleifera can maintain the metabolism of C. albicans as a commensal involved in the pathogenesis of oral candidiasis infection. Fibroblasts play an essential role in regulating extracellular matrix turnover under normal conditions. In injured tissue, fibroblasts are activated and differentiate into myofibroblasts, which contract and participate in healing by reducing wound size and secreting ECM proteins. [30]

The fungus C. albicans was reported as a causative pathological agent that triggers oral candidiasis infection. Moringa leaves (Moringa oleifera) have antifungal properties and contain antioxidant compounds that can potentially prevent interactions between C. albicans receptors and ligand proteins of epithelial cells and fibroblast cells. This ability is related to antioxidant properties that can increase the surface hydrophobicity of epithelial and fibroblast cells. Increasing the hydrophobicity of M. oleifera can reduce the interaction of the ROS (Reactive Oxygen Species) system of C. albicans cells.

Conclusions

M. oleifera can increase hydrocarbons and reduce the hydrophobicity of the C. albicans cell surface and reduce growth. In addition, M. oleifera can increase the healing of oral mucosal infections after being infected with C. albicans, which is indicated by an increase in fibroblast cells.

Declaration of Interest

The authors report no conflict of interest.

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