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Fungistatic effect of *Moringa oleifera* Lam. on the metabolism changes of *Candida albicans*

[Efecto fungistático de *Moringa oleifera* Lam. sobre los cambios en el metabolismo de *Candida albicans*]

Basri A. Gani^{1*}, Cut Soraya², Vinna Kurniawati Sugieman³, Fitri Yunita Batubara⁴, Dharli Syafriza⁵, Silvia Naliani⁶, Sri Rezeki⁷, Subhani Jakfar⁸, Muhammad Nazar⁹, Kemala Hayati¹⁰

¹Department of Oral Biology, Dentistry Faculty, Universitas Syiah Kuala. Jl. Kopelma Darussalam, Kecamatan Syiah Kuala, Kota Banda Aceh, Indonesia.

²Department of Conservative Dentistry, Dentistry Faculty, Universitas Syiah Kuala. Jl. Kopelma Darussalam, Kecamatan Syiah Kuala, Kota Banda Aceh, Indonesia.

³Department of Oral Biology, Dentistry Faculty, Universitas Kristen Maranatha. Jl. Prof. Drg. M.P.H. No. 65, Bandung, Jawa Barat, Indonesia.

⁴Department of Conservative Dentistry, Dentistry Faculty, Universitas Sumatera Utara. Jl. Alumni No.2, Kampus USU, Medan, Sumatera Utara, Indonesia.

⁵Department of Pedodontics, Dentistry Faculty, Universitas Syiah Kuala. Jl. Kopelma Darussalam, Kecamatan Syiah Kuala, Kota Banda Aceh, Indonesia.

⁶Department of Orthodontics, Dentistry Faculty, Universitas Kristen Maranatha. Jl. Prof. Drg. Surya Sumantri, M.P.H. No. 65, Bandung, Jawa Barat, Indonesia.

⁷Department of Oral Medicine, Dentistry Faculty, Universitas Syiah Kuala. Jl. Kopelma Darussalam, Kecamatan Syiah Kuala, Kota Banda Aceh, Indonesia.

⁸Department of Dental Material, Dentistry Faculty, Universitas Syiah Kuala. Jl. Kopelma Darussalam, Kecamatan Syiah Kuala, Kota Banda Aceh, Indonesia.

⁹Department of Chemistry Education, Faculty of Teacher and Training, Universitas Syiah Kuala. Jl. Tgk. Hasan Krueng Kalee, Kopelma Darussalam, Kecamatan Syiah Kuala, Kota Banda Aceh, Aceh, Indonesia.

¹⁰Department of Dental Radiology, Dentistry Faculty, Universitas Syiah Kuala. Jl. Kopelma Darussalam, Kecamatan Syiah Kuala, Kota Banda Aceh, Aceh, Indonesia.

*E-mail: basriunoe@unsyiah.ac.id

Abstract

Context: *Candida albicans* is a pathological agent that triggers oral candidiasis because *C. albicans* can adapt to changes to increase growth and adhesion through biofilm formation mechanisms. *Moringa oleifera* Lam. has been reported to have fungistatic properties and increases the metabolism change of *C. albicans*.

Aims: To evaluate the fungistatic effect of *M. oleifera* leaves ethanolic extract (MOLE) on the metabolism changes of *C. albicans* cells associated with growth and biofilm formation.

Methods: The assessment of metabolism changes (stress response and metabolic alterations) of *C. albicans* by the action of MOLE was performed by mean of FTIR, growth assessment by spectrophotometry, biofilm formation with 1% crystal violet, also read by spectrophotometry, and observation of biofilm mass with a microscope.

Results: MOLE showed substantial absorption values based on topological polar surface area (<140 Å²). Concentrations of 25% and 6.25% of MOLE increased the stress response (metabolism changes) of *C. albicans* (66-75%), meanwhile 50% and nystatin (100.000 IU/mL) were similar in inducing metabolism changes of *C. albicans*. All concentrations of *M. oleifera* could inhibit the growth of *C. albicans* at all incubation times (24, 48, and 72 h) with an Optical Density (OD) of 0.02-0.05 (<300 CFU/mL) and were able to degrade the biofilm formation of *C. albicans* on a scale substantial at 24 and 48 h (OD>0.4), and moderate scale at 72 h (OD 0.2-0.39).

Conclusions: The extract of *M. oleifera* has increased metabolism changes (stress response) of *C. albicans* cells, which correlate with the ability to inhibit growth and biofilm formation for 24, 48, and 72 h.

Keywords: biofilm; *Candida albicans*; fungistatic; growth; stress response.

Resumen

Contexto: *Candida albicans* es un agente patológico que desencadena la candidiasis oral porque *C. albicans* puede adaptarse a los cambios para aumentar el crecimiento y la adhesión a través de mecanismos de formación de biopelículas. Se ha informado que *Moringa oleifera* Lam. tiene propiedades fungistáticas y aumenta el cambio de metabolismo de *C. albicans*.

Objetivos: Evaluar el efecto fungistático del extracto etanólico de hojas de *M. oleifera* (MOLE) sobre los cambios en el metabolismo de las células de *C. albicans* asociados con el crecimiento y la formación de biopelículas.

Métodos: La evaluación de los cambios en el metabolismo (respuesta al estrés y alteraciones metabólicas) de *C. albicans* por acción de MOLE fue realizada mediante FTIR, la evaluación del crecimiento mediante espectrofotometría, la formación de biofilm con cristal violeta al 1%, también leído mediante espectrofotometría, y la observación de la masa del biofilm con un microscopio.

Resultados: MOLE presentó valores de absorción sustanciales basados en el área de superficie polar topológica (<140 Å²). Las concentraciones de 25% y 6,25% de MOLE aumentaron la respuesta de estrés (cambios en el metabolismo) de *C. albicans* (66-75%), mientras que el 50% y la nistatina (100.000 UI/mL) fueron similares en la inducción de cambios en el metabolismo de *C. albicans*. Todas las concentraciones de *M. oleifera* pudieron inhibir el crecimiento de *C. albicans* en todos los tiempos de incubación (24, 48 y 72 h) con una densidad óptica (DO) de 0,02-0,05 (<300 ufc/mL) y fueron capaces de degradar la formación de biopelículas de *C. albicans* en una escala sustancial a las 24 y 48 h (DO>0,4), y moderada a las 72 h (DO 0,2-0,39).

Conclusiones: El extracto de *M. oleifera* ha aumentado los cambios en el metabolismo (respuesta al estrés) de las células de *C. albicans*, que se correlacionan con la capacidad de inhibir el crecimiento y la formación de biopelículas durante 24, 48 y 72 h.

Palabras Clave: biopelícula; *Candida albicans*; crecimiento; fungistático; respuesta al estrés.

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AUTHOR INFO

ORCID: [0000-0002-4592-398X](https://orcid.org/0000-0002-4592-398X) (BAG) [0000-0001-5556-1671](https://orcid.org/0000-0001-5556-1671) (DS) [0000-0001-8361-606X](https://orcid.org/0000-0001-8361-606X) (MN)
[0000-0002-8025-5534](https://orcid.org/0000-0002-8025-5534) (CS) [0000-0002-5846-1200](https://orcid.org/0000-0002-5846-1200) (SN) [0000-0001-5219-6373](https://orcid.org/0000-0001-5219-6373) (KH)
[0000-0002-3688-6718](https://orcid.org/0000-0002-3688-6718) (VKS) [0000-0002-0109-0701](https://orcid.org/0000-0002-0109-0701) (SR)
[0000-0002-4159-9748](https://orcid.org/0000-0002-4159-9748) (FYB) [0000-0002-2233-2239](https://orcid.org/0000-0002-2233-2239) (SJ)

INTRODUCTION

Candida albicans is a causative pathological agent triggering candidiasis infection. This infection can have implications for a decrease in the mucosal defense system. In addition, *C. albicans* has a faster spread if the biological conditions of the oral cavity are not balanced. Changes in temperature, salivary pH, and hormonal disturbances can trigger the development of the fungus, thereby exacerbating the infection (Jabra-Rizk et al., 2016).

Several factors can increase the risk of the growth of the fungus *C. albicans* in the oral cavity. One of the most common causes is overuse of antibiotics, comorbidities such as cancer, AIDS, and diabetes, and wearing dentures that 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 (Singh et al., 2014).

Candida albicans have some virulence factors involved in the pathogenesis of infection. Hydrophilic and hydrophobic properties are two virulent factors that often work when applied to disease pathogenesis. In addition, adhesion factors and biofilm formation are reported to contribute together with hydrophilic properties when penetrating and infecting the host mucosa (Gani et al., 2017a). Increased biofilm formation helps *C. albicans* spread and form new communities to expand the infection. In addition, the intensity of the stress response and changes in metabolism are indicators of the virulence of *C. albicans* working in increasing disorder (Gulati and Nobile, 2016).

Previous studies of antifungal drugs prevent adhesion to the mucosal host by reducing biofilm formation. 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* and host cells, affecting the virulence properties of *C. albicans* cells (Parente-Rocha et al., 2017). In addition, the target antifungal drugs also aim to increase the stress response and changes in cell metabolism so that they can interfere with the cellular activity of *C. albicans* (Chen et al., 2020). Antioxidants sourced from medicinal plants are reported to have a high ability effect on causing damage to cell membranes and disrupting the cellular respiration system, thus disrupting the inter and intercellular communication system. This failure disrupts the mechanism of cell genetic information, which is the forerunner to a decrease in cell function, which ultimately causes stress and cell death. *Moringa oleifera* Lam. (family *Moringaceae*) has been reported to

have fungistatic properties through the membrane synthesis pathway (Unuofin and Lebelo, 2020).

Moringa species contain alkaloids, saponins, tannins, steroids, phenolic acids, glucosinolates, flavonoids, and terpenes. The genus' phytochemical variety makes it useful in pharmacology (Abd Rani et al., 2018). *Moringa oleifera* produces the antifungal protein known as Mo-CBP3, which has been explored as a possible candidate for eradicating fungal illnesses (Pinto et al., 2015). The chemical compounds hexadecanoic acid and oleic acid from *M. oleifera* were reported to have antifungal properties (Lin et al., 2016).

M. oleifera has been reported to be bacteriostatic and fungistatic. As a medicinal plant, this plant is efficacious in increasing immunity and preventing the development of pathogens (Qwele et al., 2013). Our previous study found that the ethanolic extract of *M. oleifera* had the highest cytotoxicity against *Streptococcus mutans* cells and could increase the hydrophilicity on the surface of *C. albicans* cells. This research is a series of studies focusing on the ability of *M. oleifera* leaves ethanolic extract on the stress response and alterations in *C. albicans* cell metabolism associated with growth and biofilm formation. The result of these studies is a reference for formulating fungistatic antifungal drugs from *M. oleifera* through cellular inhibitory pathways against *C. albicans* in the pathogenesis of oral candidiasis infections.

MATERIAL AND METHODS

Chemical and reagents

In this study were used nystatin from Novell Pharmaceutical Laboratories, Bogor, Indonesia, chemical and reagent products from Sigma-Aldrich, an affiliate of Merck KGaA, Darmstadt, Germany, among others, ethanol, alcohol, peptone, and Sabouraud dextrose agar, crystal violet 1%, sodium dodecyl sulfate, and phosphate-buffered saline.

This study used *M. oleifera* as the test material and *C. albicans* as the research subject. The interaction between *M. oleifera* and *C. albicans* was evaluated for stress response and metabolic changes confirmed by growth and biofilm formation on the 96-well plate. Assessment of the functional static properties of *M. oleifera* using concentrations of 6.25%, 12.5%, 25%, and 50%, and nystatin was used as a positive control, and incubation time of 24, 48, and 72 h was used as a determining variable to measure the period of action of *M. oleifera* causing decreased growth and biofilm formation of *C. albicans*.

Plant material

The *M. oleifera* was collected from the District of Aceh Besar, Province of Aceh, Indonesia (5.603444, 95.405863). A sample with voucher number Co2021 was deposited in the Laboratory of Oral Biology, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Indonesia. The plant material was extracted in the Chemical Laboratory, Faculty of Mathematics and Natural Science, Universitas Syiah Kuala, Darussalam Banda Aceh, Indonesia.

Extraction and GC-MS of *Moringa oleifera*

The *M. oleifera* leaves of 1 kg were washed and dried for two days till wilted. The *M. oleifera* leaves were blended into powder and stored airtight, then soaked in 100 mL of 96% ethanol in a clean flat-bottomed glass container. They changed the solvent for three days and remade residue and filtrate. The filtrate is concentrated with a rotary vacuum evaporator at 50°C and 75 mmHg to acquire the extract (Soraya et al., 2021). The GC-MS analysis of *M. oleifera* leaf ethanol extract was performed using a Shimadzu Japan QP2010PLUS with polymethyl silicon-coated fused GC column (2010). Conditions: 80–200°C, 5 min, 200°C for 20 min. The flame ionization detector (FID) temperature was 300°C, the injection temperature was 220°C, and the nitrogen carrier gas was a flow rate of 1 mL/min. 116.9 kPa. 30 m column, 0.25 mm diameter, 50 mL/min flow rate (Yusuf et al., 2021). The chromatograms obtained from GC-MS should be displayed in Fig. 1.

Bioactivity assay compounds of *Moringa oleifera*

Utilizing the Molinspiration property engine software, the efficiency and molecular properties were calculated. Chemdraw professional v.16 Cambridge software was used to create the Compound structures, and Chem3D v.16 Cambridge software was used to convert the 2D designs into 3D. In addition, the software mol-inspiration properties engine v. 2018.10 was used to calculate the bioactivity and molecular scores of *M. oleifera* compounds. The structure of the two compounds is transferred to the mol-inspiration canvas in the first step. Clicking the computed properties button then calculates the number of atoms, molecular weight, particles, and essential groups such as N, O, OH, and NH. In the second phase, the effectiveness of the two test materials in response to the host or infectious agent was determined (Alberga et al., 2018).

Culture and growth assay

ATCC 10231 *Candida albicans* was recultured on Sabouraud Dextrose Agar medium for 48 h in an aerobic atmosphere at 37°C. Then, one colony was re-

cultured in Peptone medium for 48 h at 37°C. The McFarland 0.5 (1.5×10^8 CFU/mL) was then standardized. The amount of *M. oleifera* gel administered to *C. albicans* was then determined to observe its effect on the stress response and changes in cell metabolism, growth, and biofilm formation (Gani et al., 2017b).

In a 96-well plate, 50 μ L of a *C. albicans* suspension was put in each well (triple series). After that, it was allowed to sit at room temperature for 15 min before one hundred microliters of *M. oleifera* extract were added to each of the different concentrations. In addition, the *C. albicans* suspension was modified with the test material for 10 min at a speed of 200 rpm. After that, the cultures were grown in an aerobic environment at 37°C for 24, 48, and 72 h. Furthermore, the growth inhibition was assessed by spectrophotometry (xMark™ Microplate Absorbance Spectrophotometer, Biorad, California, USA) based on turbidity. Scale for rating growth based on optical density (OD). OD 0.05 nm (<150 CFU/mL), 0.08-0.1 nm (McFarlan 0.5; <300 CFU), OD 0.11-0.29 nm (McFarland 1; 300-600 CFU), OD 0.3-0.49 nm (McFarland 2; 600-1200 CFU). These scales were adopted by McFarland Standard for *in vitro* use only, catalog No. TM50-TM60, Scott Sutton, Measurement of Microbial Cells by Optical Density, 2011 (Sutton, 2011).

Biofilm assay

A total of 25 μ L of saliva was collected on a 96-well plate for 15 min. Then discarded and continued with the addition of 30 μ L *C. albicans* on the bottom, which had been covered with saliva on each well-plate and allowed to stand for 15 min. Then, *M. oleifera* extract gel was added to each well of 100 μ L and then adapted to room temperature for 10 min at 300 rpm. The interaction of *C. albicans* biofilm formation ability was assessed after incubation for 24 and 48 and 72 h at 37°C. Assessment of the inhibition of biofilm formation begins by removing the mixed solution of *C. albicans* and the test material. Then added, 1% sodium dodecyl sulfate (SDS) solution 100 μ L for 15 min and discarded and washed once with phosphate-buffered saline (PBS). Then, 150 μ L of glycerol was added to each well-plate and washed once with 200 μ L of PBS. Visualization of the biofilm mass by adding 150 μ L of 1% crystal violet for 10 min, then washing with 76% ethanol (150 μ L) and PBS once. Quantitative data reading with spectrophotometry at 520 nm (Asmah et al., 2022).

Preparation of whole-cell *Candida albicans*

The fungus *C. albicans*, equivalent to McFarlan 0.5 (<300 CFU/mL) taken 50 μ L, was included in the test material with various concentrations. Furthermore, adaptation was carried out at room temperature for

15 min at a speed of 200 ×g. The whole cell extraction process of *C. albicans* was adopted from He et al. (2009) research with several modifications. Homogenization of the fungus with the test material was checked based on changes in turbidity. Furthermore, the mixture of the two was incubated for 48 h in an aerobic atmosphere. The whole cell *C. albicans* extraction process was initiated by centrifugation for 5 min at 2000 rpm (separating *C. albicans* cells in the test material). Then the supernatant (*C. albicans* cells) was collected and incubated for 30 min at 37°C, vortexed again for 30 s, and 0.1 M HCl (200 mL in 1 mL) was added. Then it was vortexed for 30 s and incubated at 37°C for 5 min. Then it was centrifuged at 300 ×g for 10 min. The precipitate was collected, 500 mL PBS was added, vortexed for 30 s, and centrifuged 3000 ×g for 10 min. It was followed by washing the residue by adding 70% ethanol (1:3) and centrifuging 2000 ×g for 15 min. Furthermore, the deposit was collected as an extract whole (surface protein and endo-protein). Next, a 2 mL sample buffer solution was added. Then, the FTIR (IRPrestige 21, Merk Shimadzu, Kyoto, Japan) device examined the stress response and metabolic changes of *C. albicans* cells under *M. oleifera*.

Stress response and metabolism changes assay

Fourier transform infrared spectroscopy (FTIR) assessment of the stress response of *C. albicans* under the influence of saliva was analyzed based on the functional groups contained in the sample. This analysis uses FTIR-ATR with a wave number of 4000 cm⁻¹-400 cm⁻¹, producing transmittance spectra. In the first stage, an example of *C. albicans* is placed on the surface of a transparent Infrared (IR) prism with a refractive index that is always higher than the sample (the refractive index of bacterial cells is estimated at 1.39). The radiation beam is directed at one of the prism walls for the prism-sample interface at an angle higher than the barrier. Under these conditions, complete reflection occurs on the side of the internal prism. The reflected light exits through the walls of the second prism, where the light intensity and absorption spectrum are recorded (Alvarez-Ordonez et al., 2011). Stress response assessment based on window peaks. The absorbance value of the intensity (wave) with the area value of each peak becomes the basis for measuring the stress response and changes in the metabolism of *C. albicans* cells. Stress response and metabolic changes are assessed based on changes in constituent cell elements such as lipids, proteins, carbohydrates, and nucleic acids.

The reading of the stress response under the influence of *M. oleifera* used the ATR-FTIR (cm⁻¹) mode approach. Assessment of bacterial stress response based on FTIR assessment adopted from Zarnowiec et

al. (2015) based on windows zone (W). W1- lipids (3,000-2,800 cm⁻¹), W2- proteins (1,700-1,500 cm⁻¹), W3-nucleic acids (1,500-1200 cm⁻¹), W4-carbohydrates (1200-900 cm⁻¹), W5 -fingerprints regions (900-500).

Statistical analysis

Data on growth and biofilm formation were analyzed by One Way ANOVA. At the same time, data on stress response and changes in metabolism were described descriptively based on the frequency value of each concentration of *M. oleifera*. The limit of significance (p<0.05).

RESULTS

Fig. 1 shows the chromatogram of *M. oleifera*. As much as there 17 chemical compounds were exposed based on the GC spectrum, and we reported 13 chemical compounds that were believed to have potential as antioxidants compounds and worked to prevent the development of fungi, including *C. albicans* (Senthilkumar et al., 2015). The peaks show up at 5.43 min of retention until 33.82 min. The GC analysis was in tandem with a mass spectrometer to obtain the relative mass of each compound. The data from the instrument was then compared with the NIST compound database. A total of 13 combinations from varied compound classes were detected and tabulated in Fig. 2. which reports the GC-MS results of several chemical compounds contained in the extract of *M. oleifera* and also reports the rate times and percentage of these chemical compounds of *M. oleifera*.

Fig. 3 shows that the stress indicator experienced by *C. albicans*, based on changes in absorbance (peak), occurred due to changes in nucleic acid synthesis, thus disrupting lipid synthesis, protein, and carbohydrates. At the same time, areal fingerprints can be ascertained that there is a change in virulence properties due to the stress response. The authors assume that the changes in the fingerprints introduced can indicate the assessment of growth and biofilm formation by *C. albicans*. Although these results are debatable because this study correlates with the inhibition of growth and formation of *C. albicans* biofilms, the fingerprints area can certainly be used as a reference for assessing the virulence properties of the pathogen.

Fig. 4 reports the results of the evaluation and analysis of FTIR data based on the intensity value (wavelength) given to *C. albicans* cells that have been affected by *M. oleifera* extract gel. At the same time, the area value is the change in the metabolic properties of *C. albicans* cells after interacting with *M. oleifera*. The intensity and area values in the FTIR assessment of biological products must be synergistic. The small-

er the wave given, the higher the energy produced to obtain the target value (changes in cell metabolism). This study showed a close relationship between the intensity and area values for *C. albicans* cells, which were affected by *M. oleifera* extract concentrations of 6.25%, and 25%. In contrast, 50% and the nystatin group had similarities in inducing changes in the metabolism of *C. albicans* cells.

In Fig. 5, it was reported that at all concentrations of *M. oleifera*, it caused a loss of *C. albicans* biofilm mass or caused a decrease in the morphology of the

biofilm mass. It can be assumed that *M. oleifera* has disrupted the metabolism of *C. albicans* cells to reduce biofilm formation. It is related to the stress response experienced by *C. albicans* during interaction with the test material (Table 1). Fig. 6 shows that *M. oleifera* caused changes in *C. albicans* to growth and biofilm formation at all incubation times. These findings indicate that the inhibition of biofilm formation affects the growth inhibition of *C. albicans*. It means that disrupting the formation of biofilms has interfered with the growth of *C. albicans*.

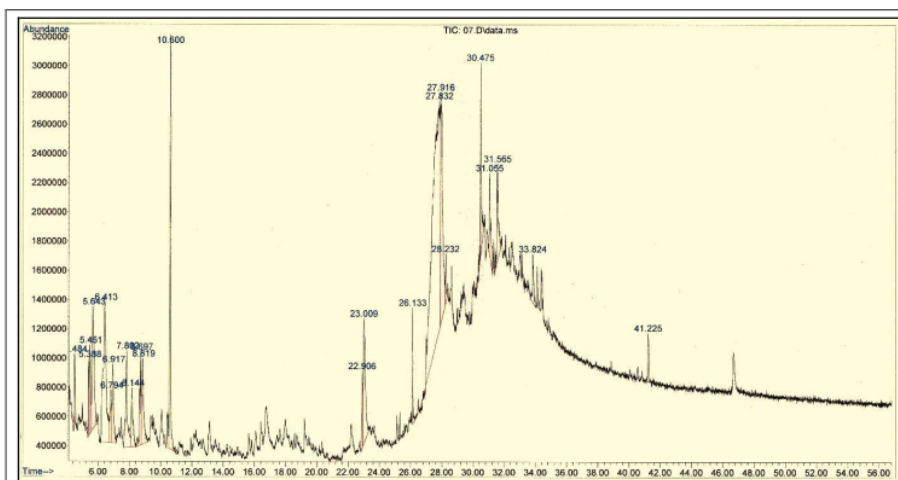


Figure 1. Gas Chromatography spectrum of *Moringa oleifera*.

Three significant components with a quantity greater than 5 percent are compounds (glycerol, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, and 1,3,4,5-tetrahydro-cyclohexane carboxylic acid.

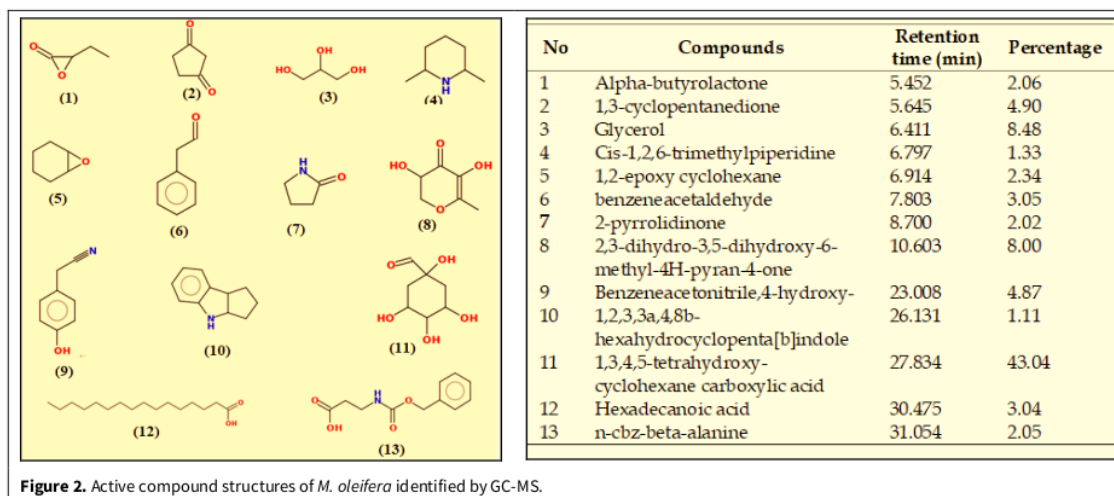


Figure 2. Active compound structures of *M. oleifera* identified by GC-MS.

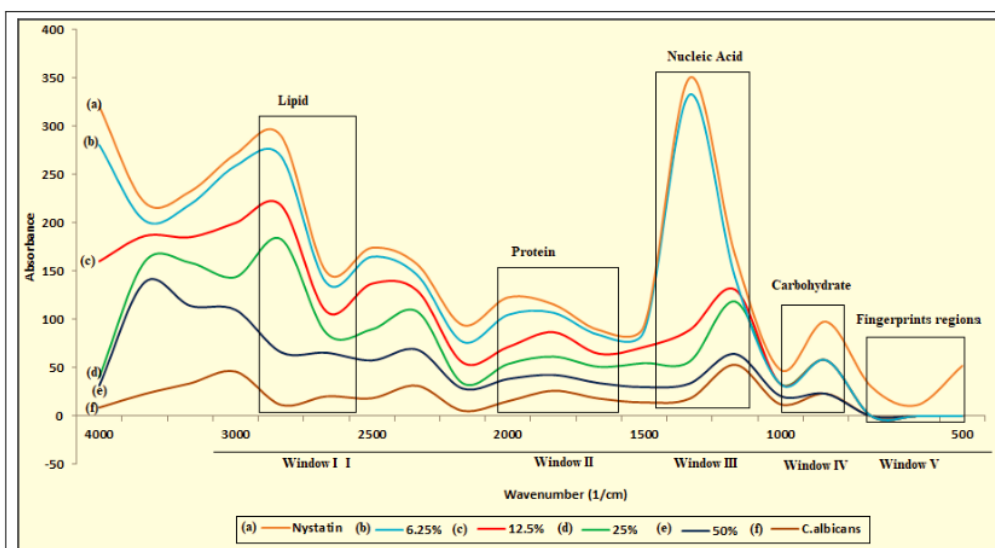


Figure 3. The FTIR was performed on the stress response of *C. albicans* after interacting with *M. oleifera* (4000-400 wavenumbers; cm^{-1}). In biological assessment, the representation of stress response values is obtained from the frequency range (wavenumbers/ cm^{-1}) based on window 1 (lipid), window 2 (protein), window 3 (nucleic acid), window 4 (carbohydrates), window 5 (fingerprints regions). Changes in absorbance of *C. albicans* cells affected by *M. oleifera* and nystatin from each window were used as a measurement value to analyze the response of *C. albicans* to the environment (stress response).

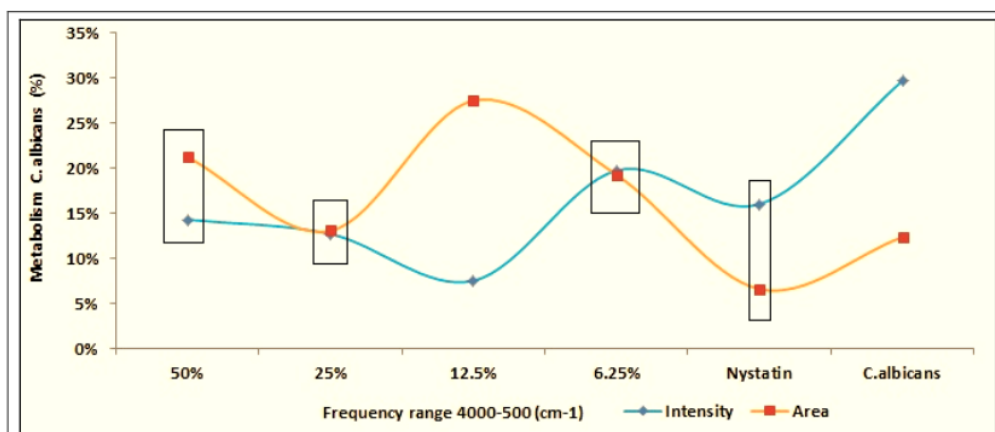


Figure 4. The FTIR graph pattern of metabolic changes of *C. albicans* after being induced with *M. oleifera* at a frequency range of 4000-500 cm^{-1} .

The value of intensity (wavelength) and area (interaction) between *M. oleifera* and *C. albicans* became a reference for changes in *C. albicans* cell metabolism. Concentration groups of 25% and 6.25% had a better ability to interfere with the metabolism of *C. albicans*.

Table 2 shows the percentage quantity of each chemical compound contained in *M. oleifera*. 1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid compounds have the highest amount among other chemical compounds (43.04%). The assessment of drug properties using the Molinspiration property engine v2018.10 software. The topological polar surface area (TPSA), milogP, molecular weight, and working volume assess work as a drug ingredient. The TPSA value

shows that all TPSA values are below 140 \AA^2 , meaning that all these compounds have excellent absorption values. While the milogP value, all chemical compounds were below 4, except for Hexadecanoid compounds, which had a milogP of 7.06. In addition, all these compounds have a volume above $75 \text{ mm}^3/\text{mol}$. The volume quality of the compounds correlates with pharmacokinetic activity.

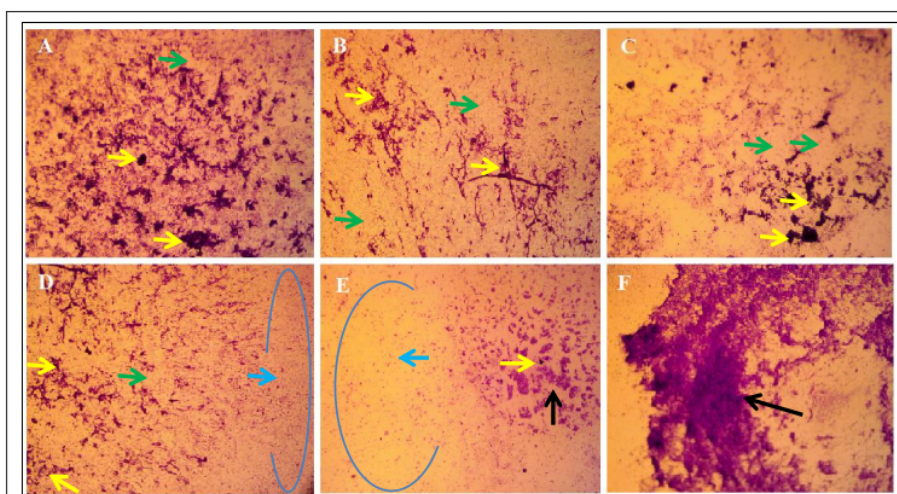


Figure 5. Mass of *C. albicans* biofilm after interaction with *M. oleifera*. The degradation of the biofilm mass of *C. albicans* is representative of 24 h of incubation.

(A) 50% concentration, (B) 25% concentration, (C) 12.5% concentration, (D) 6.25% concentration, (E) Positive control (nystatin), and (F) *C. albicans* biofilm mass. Green arrows (residual biofilm area), yellow arrows (deactivated biofilm mass), blue arrows (lost biofilm), and black arrows (active biofilm mass). 1% crystal violet stain, 200× magnification.

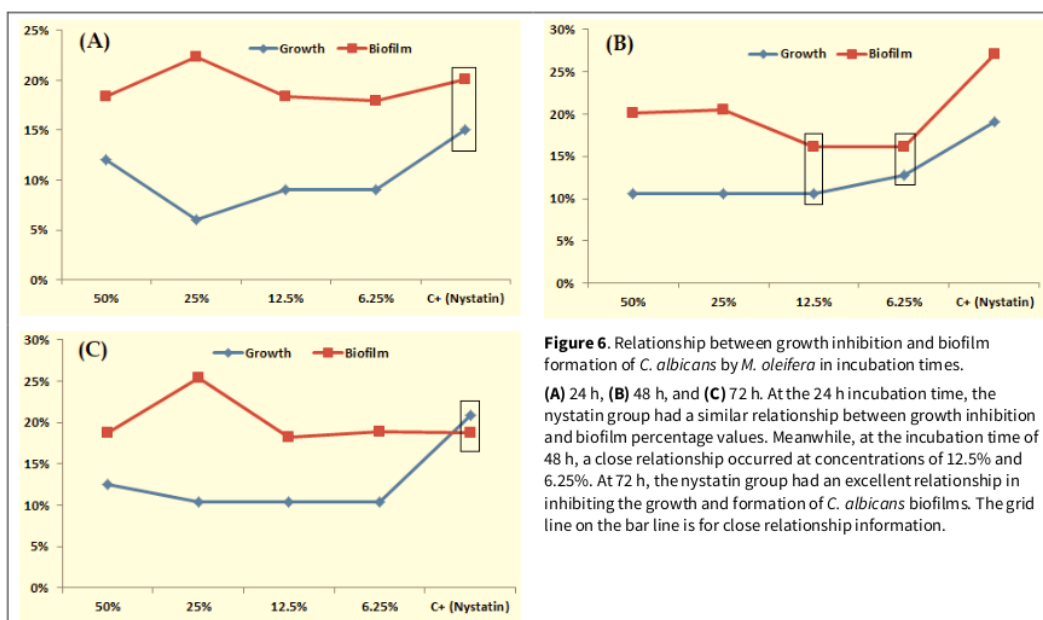


Figure 6. Relationship between growth inhibition and biofilm formation of *C. albicans* by *M. oleifera* in incubation times.

(A) 24 h, (B) 48 h, and (C) 72 h. At the 24 h incubation time, the nystatin group had a similar relationship between growth inhibition and biofilm percentage values. Meanwhile, at the incubation time of 48 h, a close relationship occurred at concentrations of 12.5% and 6.25%. At 72 h, the nystatin group had an excellent relationship in inhibiting the growth and formation of *C. albicans* biofilms. The grid line on the bar line is for close relationship information.

Table 1 shows that the ethanolic extract of *M. oleifera* at all concentrations can increase the stress response of *C. albicans*. At a concentration of 25%, it had a better ability to increase the stress response of *C. albicans* and was similar to the positive control group of nystatin (74%). Table 3 shows the incubation times of 24, 48, and 72 h showing the growth intensity of *C. albicans* with an average OD of 0.06-0.02 nm. This value equals <math><150\text{ CFU/mL}</math> or McFarland 0.5 ($1.5 \times$

10^8). Based on the One Way ANOVA analysis results, the concentration of *M. oleifera* affected the growth of *C. albicans* at incubation times of 24, 48, and 72 h. All concentrations showed similar properties in reducing the growth of *C. albicans* at OD values of 0.02-0.05, meaning that *C. albicans* colonies ranged below 300 CFU/mL. This ability indicates that *M. oleifera* is fungistatic.

Table 4 shows that *M. oleifera* extracts can reduce biofilm formation (biofilm static). In general, all concentrations of *M. oleifera* extract gel can reduce biofilm on a strong scale (OD > 0.4), except at 72 h, there is a moderate decrease. OD (0.2-0.39), except 25% concentration. Assessment of the biofilm inhibition scale using the reference of Syafriza et al. (2021). The anti-

biofilm assessment according to OD spectrophotometry, OD 0.4 (strong); OD=0.2-3.9 (moderate); OD=0.05-0.1 (low); OD < 0.05 (no biofilm formation). Fig. 4 reports that *M. oleifera* can degrade *C. albicans* biofilm at all concentrations with various frequencies. As a comparison, *C. albicans* has a substantial quantity of biofilm mass.

Table 1. The FTIR peak assessment of the stress response of *C. albicans* cells after interaction with *M. oleifera*.

<i>M. oleifera</i>	Frequency range (cm ⁻¹)	Bands	Absorbance (A)			Area (%)			Stress response	Comments
			Value	Corr	Freq	Value	Corr	Freq		
Conc _{50%}	3240.41-451.34	16	10.56	0.166	14%	795	6.46	21%	65%	Increase
Conc _{25%}	3240.41-462.92	17	9.39	0.197	13%	489	9.84	13%	75%	Increase
Conc _{12.5%}	3240.41-470.63	15	5.56	0.297	8%	1,028	10.25	27%	66%	Increase
Conc _{6.25%}	3240.41-470.63	15	14.59	0.156	20%	721	6.26	19%	62%	Increase
Nystatin	3240.41-451.34	20	11.84	0.103	16%	381	6.59	10%	74%	Increase
<i>C. albicans</i>	3240.41-462.92	17	21.95	0.143	30%	463	5.96	12%	58%	Standard

Table 2. The assessment of medicinal properties of chemical compounds of *M. oleifera*.

NO	Compounds of <i>M.oleifera</i>	GC-MS (Percent area)	Drugs properties assessment			
			milogP	TPSA(Å ²)	MW (kDa)	Vol (mm ³ /mol)
1	Alpa-butyrolactone	2.06	0.90	29.6	86.09	79.95
2	1,3-Cyclopentanedione	4.90	-0.68	34.145	98.1	90.17
3	Glycerol	8.48	-1.60	60.68	92.09	87.12
4	Piperidine-1,2,6-trimethyl-cis	1.33	1.67	12.03	113.2	131.38
5	1,2-Epoxy-cyclohexane	2.34	1.55	12.53	98.14	100.8
6	Benzeneacetaldehyde	3.05	1.94	17.07	120.15	119.83
7	2-Pyrrolidinone	2.02	-0.18	29.1	85.11	83.58
8	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	8.00	-0.46	66.76	144.13	123.4
9	Benzeneacetonitrile,4-hydroxy-	4.87	1.42	44.02	133.15	125.72
10	1,2,3,4-Tetrahydro-cyclopentan[b]indole	1.11	2.47	12.03	159.23	158.82
11	1,3,4,5-Tetrahydro-cyclohexane carboxylic acid	43.04	-1.76	97.98	176.17	153.44
12	Hexadecanoic acid	3.04	7.06	37.3	256.43	291.42
13	n-cbz-beta-alanine	2.05	1.06	75.63	223.23	201.82

Table 3. The growth inhibition of *C. albicans* by *M. oleifera*.

<i>M. oleifera</i>	N	24 h				48 h				72 h			
		OD 620 nm	SD	Colony (CFU/mL)	Freq	OD 620 nm	SD	Colony (CFU/mL)	Freq	OD 620 nm	SD	Colony (CFU/mL)	Freq
Conc _{50%}	3	0.04	0.002	<150	12%	0.05	0.002	<150	11%	0.06	0.009	<200	13%
Conc _{25%}	3	0.02	0.003	<100	6%	0.05	0.003	<150	11%	0.04	0.002	<150	10%
Conc _{12.5%}	3	0.03	0.003	<150	9%	0.04	0.003	<150	11%	0.0	0.005	<150	10%
Conc _{6.25%}	3	0.03	0.004	<150	9%	0.06	0.004	<200	13%	0.05	0.004	<150	10%
C+ (nystatin)	3	0.05	0.016	<300	15%	0.09	0.003	<150	19%	0.10	0.007	≤300	21%
<i>C. albicans</i>	3	0.16	0.01	300-600	48%	0.17	0.01	300-600	36%	0.17	0.02	300-600	35%
*p-value		0.025				0.0421				0.031			

*One Way ANOVA; SD: Standard Deviation; Freq: Frequency.

Table 4. Inhibition of biofilm formation of *C. albicans* by *M. oleifera*.

<i>M. oleifera</i>	N	24 h			48 h			72 h		
		OD (520 nm)	SD	Freq	OD (520 nm)	SD	Freq	OD (520 nm)	SD	Freq
Conc _{50%}	3	0.412	0.011	18%	0.512	0.003	20%	0.311	0.019	19%
Conc _{25%}	3	0.501	0.002	22%	0.523	0.003	21%	0.422	0.022	25%
Conc _{12.5%}	3	0.411	0.005	18%	0.411	0.002	16%	0.302	0.015	18%
Conc _{6.25%}	3	0.403	0.024	18%	0.411	0.005	16%	0.315	0.014	19%
Nystatin	3	0.450	0.012	20%	0.691	0.002	27%	0.212	0.027	19%
*p-value		0.062			0.041			0.050		

*One Way ANOVA; SD: Standard Deviation; Freq: Frequency.

DISCUSSION

This study demonstrates and evaluates the potential for the fungistatic capabilities of *M. oleifera* to influence the stress response and metabolic alterations in *C. albicans* cells. Changes in the development and ability of *C. albicans* to build biofilms verified this effect. Figs. 1 and 2 depict the GC spectra of *M. oleifera* with the number of chemical components as a reference for evaluating its fungus function. Glycerol, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, and 1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid are three chemical compounds derived from *M. oleifera* reported having antifungal potential (Chakraborty et al., 2021; Senthilkumar et al., 2015). This glycerol molecule increases the permeability of fungal cells and inhibits the chlamydospore system as a toxin against host cells (Chen et al., 2018). This compound is reported as an antioxidant that influences the development of bacteria and fungi (Ruta and Farcasanu, 2021).

M. oleifera can increase the stress response of *C. albicans* (Table 1). It is indicated by changes in *C. albicans* cell metabolism (Fig. 3), which has implications for decreased growth and increased degradation of biofilm mass (Fig. 5 and Table 4). Changes in *C. albicans* cell metabolism after exposure to *M. oleifera* (antifungal) cause disruption of the protein synthesis system and response to virulence proteins against antifungals (da Silva Neto et al., 2020). This failure causes a decrease in adaptation to extreme environments so that cells undergo biological and physical changes. Biologically, the cell wall changes, and a leak occurs, allowing antifungal compounds to enter the cell membrane (Araújo et al., 2019). Physically, the antifungal effect of *M. oleifera* causes an excessive change in the hydrophobic pressure response of the membrane, causing *C. albicans* cells to have difficulty growing and forming quorum sensing as a prerequisite for infection (Araújo et al., 2019).

The ability of *M. oleifera* to cause an increase in stress response and changes in the metabolism of *C. albicans* is related to the cellular nature of the action of the chemical compound *M. oleifera* in increasing its effect on *C. albicans* cells. The analysis of drug properties of *M. oleifera* using the Molinspiration software (Table 2) shows that *M. oleifera* has a topological polar surface area (TPSA) value below 140 Å², meaning that all these compounds have excellent absorption values (Prasanna and Doerksen, 2009). The absorbance of these compounds is related to the ability of the active compound to penetrate the damaged target or the pharmacokinetic target of the drug. So it can be assumed that all these compounds have a perfect effect on reducing growth and inhibiting the virulence of *C. albicans* biofilm formation. The TPSA assessment relates a molecule of the active compound as the sum of the surfaces on all polar atoms or molecules, primarily oxygen and nitrogen, including the hydrogen atoms attached to the compounds (Lim et al., 2018).

The chemical compound of *M. oleifera* evaluated a milogP value, below 4, except for the hexadecanoid compounds, which had a milogP of 7.06. The LogP value of the chemical compound *M. oleifera* is considered to be active. Suppose the LogP is more significant than one or less than 4 in this value range. In that case, the *M. oleifera* compound is considered to have optimal physicochemical properties for oral drugs (Van De Waterbeemd and Gifford, 2003). Czyski (2019) reported that the compound's Log P value indicates the drug's permeability to reach the target cells and tissues in the body. This ability is related to the volume of each *M. oleifera* compound, which has a volume above 75 mm³/mol, meaning that the total volume of the active compound can be in line with the pharmacodynamic and pharmacokinetic properties of the drug.

The *M. oleifera* has chemical compounds with varying molecular weights 80-256 kDa (Table 2). High molecular weight can be considered to increase chemical resistance - up to a certain point and be an indica-

tor of the viscosity properties of the drug (Broseta et al., 1990). The increase in the viscosity of the *M. oleifera* compound can complicate the process of destroying the cell structure of *C. albicans*. Chae et al. (2005) reported that molecular weight could be considered absorption in the intestine. Using lower molecular weight standards of chemical compounds provides good fungistatic properties because the absorption of chemical compounds co-occurs with a decrease in infectious agents because the function of active compounds guarantees or controls changes in the virulence properties of pathogenic cells (Bickerton et al., 2012).

Cellularly, it is inevitable that the compounds contained in *M. oleifera* have the potential to interfere with changes in cell components such as nucleic acids, lipids, proteins, and carbohydrates. This element is reported as a building block for the integrity of a cell (Ercan et al., 2021). Changes in these four chemical elements can be used as a basis for referring to the stress response of cells (Fig. 3). These findings show that the cellular activity of *C. albicans* tremendous changes after interacting with *M. oleifera* is a nucleic acid, lipid, protein, and carbohydrate. Fulda et al. (2010) reported that changes always influence environmental cell activity. The response to these changes manifests in cell defense by expressing several proteins, lipids, and carbohydrates. This increase in activity can be used as a response to environmental changes (stress response) to adjust to the environment (LaDage, 2015).

Alterations in the stress response are linked to metabolic alterations, which can affect cell proliferation and the growth and biofilm formation of *C. albicans* (Li et al., 2015). The data in Table 3 shows a decrease in the growing intensity of *C. albicans* after interaction with *M. oleifera*, which was related to the inhibition of biofilm formation by *C. albicans* (Table 4). This decrease is related to the role of active compounds that interfere with the protein synthesis of *C. albicans*. As a result, it causes a change in the response of the cell membrane to the surface environment, causing leakage of the membrane and releasing intracellular fluid so that the cell becomes lysed and undergoes premature death before dividing (Rock and Kono, 2008).

The decrease in biofilm formation is related to the active compound *M. oleifera* increasing the pressure on the membrane permeability of pathogens, thereby disrupting the hydrophobicity of the membrane to form biofilms (Soraya et al., 2022). Işinde et al. (2021) reported that the hydrophobicity of the cell surface of *C. albicans* can be a determinant of the formation of quorum sensing on the mucosal surface and that the hydrophobicity is a determinant of the development

of biofilms on the mucous membrane. In addition, *M. oleifera* could degrade and reduce the morphology of the biofilm mass (Fig. 4). Mishra et al. (2020) reported that phytochemical compounds of herbal plants play an essential role in inhibiting bacterial adhesion and suppressing genes associated with biofilm formation. According to Nobile et al. (2008), this phenomenon reported that many antifungal drug compounds work to describe the structure of compounds (proteins) that make up biofilms, such as HWP1 or ALS1-3 proteins. The decomposition of biofilm protein compounds can cause a decrease in the biofilm matrix, causing mass degradation and even lowering the tension of spreading biofilms by disrupting communication between pathogenic cells during quorum sensing (Tits et al., 2020).

M. oleifera was responsible for the decline in biofilm production, which corresponded with the organism's slower rate of growth (Fig. 6). It indicates that the height of the biofilm generated has a significant role in determining the development of *C. albicans*, which *M. oleifera* influences. The fungistatic effect of *M. oleifera* and reducing the virulence of *C. albicans* also interfere with protein synthesis. It can limit adaptability to the antifungal environment, disrupting growth and interaction with the surrounding environment (Santos et al., 2021). Meanwhile, *C. albicans* cells cannot increase or make morphological changes at this stage. The antifungal activity of *M. oleifera* can cause *C. albicans* cells to die prematurely. This potential can serve as a reference for using active material to limit the infection caused by *C. albicans*, which plays a role in the pathogenesis of oral candidiasis.

CONCLUSION

The extract of *M. oleifera* has increased the metabolism changes (stress response) of *C. albicans* cells, which have implications for decreasing growth and inhibiting biofilm formation for 24, 48, and 72 h.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Gani BA	Soraya C	Sugiaman VK	Batubara FY	Syafriza D	Naliani S	Rezeki S	Jakfar S	Nazar M	Hayati K
Concepts or ideas	x									
Design	x	x		x	x		x	x	x	
Definition of intellectual content	x	x	x	x			x	x	x	
Literature search	x	x		x	x		x	x	x	x
Experimental studies	x				x					
Data acquisition	x		x	x	x	x		x	x	x
Data analysis	x									
Statistical analysis	x									
Manuscript preparation	x			x	x	x				
Manuscript editing	x			x	x	x		x	x	x
Manuscript review	x	x	x	x	x	x	x	x		x

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