



Eco-friendly production of novel antimicrobial sophorolipids from *Meyerozyma guilliermondii* cultivated on jojoba oil cake for combating drug-resistant microbes

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ABSTRACT

Microbial infections in the medical sector often lead to life-threatening systemic disorders due to their resistance to various classical antibiotic families. This work utilized a combination of jojoba oilcake and crude soybean oil for the first time as a low-cost substrate. In addition, a locally isolated and molecularly characterized yeast strain (*Meyerozyma guilliermondii*) was used for the economic production of sophorolipids (SLs). SLs were produced and extracted by methanol, giving a yield of 23.12/100g substrate. They reduced the surface tension (ST) to 39 mN/m (millinewtons/meter) with a critical micelle concentration (CMC) value of 240 mg/l. The produced SLs' characterization investigations using Fourier transform infrared spectroscopy, Proton nuclear magnetic resonance, and Liquid chromatography-tandem mass spectrometry demonstrated the existence of both the acidic and lactonic forms of SLs. Interestingly, the activity of SLs against clinically drug-resistant pathogens was found to be highly suppressive for their growth. The produced SLs demonstrated a strong inhibitory effect against certain drug-resistant pathogens, primarily through mechanisms such as biofilm suppression, increased ROS generation, and membrane destabilization, all with minimal cytotoxicity. This approach could enhance their potential in combating drug-resistant infections.

1. INTRODUCTION

Hospital-acquired infectious diseases are becoming more opportunistic, particularly in immunocompromised

patients and patients with medically implanted devices [1]. Due to their resistance to several traditional antibiotic families, the widespread presence of some microbial pathogens in the medical field typically results in a potentially fatal infection [2]. Common infectious diseases are generally contracted during the surgical and implementation processes, such as candidiasis, pneumonia, and arthritis. They generally occur via skin or bone, or ventilator-associated pneumonia or urinary tract infections, etc. [3].

Antibiotics struggle to inhibit multidrug-resistant (MDR) or extensively drug-resistant (XDR) pathogens due to their biofilm production, which forms an exopolymer substance

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matrix, leading to adherence and colonization, making it difficult for them to penetrate [4]. Thus, features contributed to the resistance level of the infected pathogen, which sometimes had more resistance when compared to their planktonic counterparts. Newer antimicrobial agents that may be effective against both planktonic and biofilm-producing cells are therefore desperately needed. Biosurfactants are naturally synthesized molecules that represent antimicrobial and antiadhesive activities [5]. Sophorolipids (SLs) are a prevalent class of biosurfactants, eco-friendly amphipathic compounds, used in miscellaneous industries, including drugs, cosmetics, and foods. They are produced from non-pathogenic yeast species such as *Wickerhamiella* sp., *Rhodotorulla* sp., *Candida* sp., and *Yarrowia* sp [6]. They primarily consist of a chain of fatty acids linked to a carbohydrate moiety, an oligosaccharide molecule produced from glucose with an atypical β -1,2 glycosidic bond. These special structures are viable natural substitutes for produced antibiotics because of their exceptional antibacterial properties [7]. The biosafety of SL makes it a promising candidate to incorporate to overcome the challenge of biofilm-producing pathogens. The worldwide market for microbial biosurfactants was estimated to be worth USD 1.2 billion in 2022 and is anticipated to rise at an annual rate of 11.2% to reach USD 1.9 billion by 2027. Notably, in 2021, SLs became the most valuable subtype in the biosurfactants market [8].

Synthetic substrates utilized in SLs manufacturing frequently increase costs related to their high pricing and unsustainable nature. Agro-industrial wastes offer one potential way to address this problem. Agricultural and food industry byproducts, such as fruit pomace, spent grains, vegetable wastes, and oil cakes, are abundant, inexpensive resources with fermentable nutrients that promote microbe growth, reducing production costs, mitigating the influence on the environment, and increasing waste valorization. According to reports, microbial fermentation systems can effectively use these renewable resources in place of synthetic inputs. This strategy promotes cost-effective and environmentally friendly manufacturing of SLs, aligning with both economic and environmental objectives [9,10].

Joboba (*Simmondsia chinensis*), a member of the *Simmondsiaceae* family, typically originates in semi-arid regions. Other names for it include oat nut, coffee berry, deer nut, and wild hazel. Southern California, the hills of Arizona, and northwest Mexico are home to the joboba plant. Joboba farming has spread to several nations over the last three decades, including Saudi Arabia, Egypt, Tunisia, and Mexico [11]. The oil cake of joboba is suitable for agro-industrial waste for the microbial production of bioactive compounds.

It is an oil-producing plant that is utilized worldwide for a variety of purposes, mostly in the cosmetics industry [12]. Polaris Market Research stated that the global joboba oil market reached USD 131.33 million in 2022, growing at a 7.5% CAGR to reach USD 270.81 million by 2032. Polaris Market Research & Consulting LLP, 2023. Joboba meal does, however, include anti-nutritional substances such as simmondsin, which can depress appetite, limit nutrient absorption, and suppress its utilization in animal feeding [13]. Therefore, microbial fermentation is considered the most effective method for using this waste to produce SLs economically. The use of the appropriate

fermentation technique and the selection of the robust locally isolated strain are among the most important factors that reduce production costs. To the best of our knowledge, no reports have studied the utilization of joboba oil cake as a substrate for SLs production from *Meyerozyma guilliermondii*.

This study's objective is to assess the economic production of SLs from joboba oil cake as an agro-industrial waste and a newly isolated yeast strain. The produced SLs physicochemical structures will be identified. Furthermore, the effect of produced SLs on inhibiting microbial pathogens and their biofilm production will be investigated. The oxidative stress mechanism produced by SLs will also be included by detecting the oxidation activity level of fatty acid constituents in the cell membrane.

2. MATERIALS AND METHODS

2.1. Substrates

The wheat germ was gathered from El Watania Milling, El Sadat City, Menoufia, Egypt, as a byproduct of the flour grinding process. The Pressing and Extracting Natural Oils unit, NRC, Cairo, Egypt, provided the joboba oil cake. The wastes were frozen and kept at -4°C . The Food Technology Research Institute, Soy Processing Unit, Agriculture Research Center, Giza, Egypt, supplied the crude soybean oil. All chemicals and reagents used in this study were of analytical quality. Operon Technologies, Inc., Netherlands, supplied primers used for yeast identification.

2.2. Preparation of wheat germ oil cake

According to [13], the wheat germ was compressed using a hydraulic press (Carver - USA). The wheat germ was squeezed under pressure at 10,000 lb/in² for 1 hour at 25°C. After a 24-hours air drying period at room temperature, the oil cakes were ground using a blender and then stored in polyethylene bags at -20°C .

2.3. Yeast strains isolation and separation

The wheat germ oil cake was selected for isolating biosurfactant-producing yeast strains. Nine milliliters of sterile distilled water were used to suspend one gram of the oil cake, which was then thoroughly vortexed, and serially diluted up to 10³ using sterile distilled water. From each dilution, 0.2 ml was plated on a medium of Malt Extract (ME) agar amended with chloramphenicol (200 $\mu\text{g}/\text{ml}$). The ME agar medium consisted of 1% glucose, 0.3% yeast extract, 0.5% peptone, 0.3% ME, and 2% agar. For the screening, cultures were incubated for 24–48 hours and then plated on a cetyltrimethylammonium bromide (CTAB)-agar medium adjusted to a pH of 5.5. Colonies demonstrating rapid growth were selected for further analysis.

2.3.1. Molecular identification of yeast isolate

Extraction of Genomic DNA: DNA from the yeast strain (W1), isolated from wheat germ cake and cultured on ME agar, was prepared using the i-genomic BYF DNA Extraction Mini Kit (iNtRON Biotechnology, South Korea) as per the manufacturer's protocol. Purity and concentration of the DNA were assessed utilizing the UV absorbance at 260/280 nm following the method by [14].

2.3.2. PCR amplification and ITS sequencing

ITS rDNA sequences of the yeast isolate (W1) were obtained using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') & ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers, provided by Operon Technologies, Netherlands. PCR was conducted with a reaction mixture containing 1.8 μ M of primers, 40 ng of purified DNA, and polymerase beads to a volume of 25 μ l. The protocol included denaturation at 95°C for 5 minutes, followed by 35 cycles of primer annealing at 55°C for 2 minutes and polymerization at 72°C for 2 minutes, with final cooling at 4°C. A gel documentation system was used to visualize the DNA amplified product after it had been resolved on a 1% agarose gel in TBE buffer for two hours at 100 V, then stained with ethidium bromide (0.5 μ g/ml).

2.3.3. The purification of PCR product

The GeneJET PCR Purification Kit from Thermo K0701 was used to purify the PCR products.

2.3.4. Yeast identification

The sequencing of the purified DNA PCR product was carried out using an ABI 3730xl DNA sequencer (GATC Company, Germany) with the forward primer.

2.3.5. Phylogenetic analysis

The sequence obtained was compared using the Basic Local Alignment Search Tool from the NCBI GenBank database. Sequences were aligned with reference taxa from public databases for comparative analysis.

2.4. SLs production medium

To isolate and identify yeast *M. guilliermondii*, a loopful of a 7-day-old stock culture was inoculated on 50 ml of Yeast-Malt medium [15], sterilized by autoclave (TOMY, SX-700) at 121°C and 0.1 MPa (Megapascal) for 20 minutes. Incubation of the mixture was then carried out for 24 hours at 28°C and 180 rpm in Thermo Scientific, Incubated/Refrigerated Orbital Shaker (USA). The SSF (Solid State Fermentation) medium was prepared in 250 ml flask, as indicated below: Five grams of jojoba oil cake with a particle size of 60–80 mesh, five grams of soybean crude oil, and 5 ml of nutrient solution (g/l): CaCl₂·2H₂O, 0.1; NaH₂PO₄, 0.15; MnSO₄·H₂O, 0.02; peptone, 1.0; MgSO₄·7H₂O, 0.5; NH₄NO₃, 1.0; K₂HPO₄, 2.55. The medium pH was eventually brought to eight with 50% moisture content, then the medium was sterilized as described above. Two milliliters from the overnight culture (4% V/V), which is equivalent to 10⁷ Colony Forming Unit (CFU), were added to each flask with a ratio of 5:1 w/v (substrate-to-inoculum ratio). The cultures were finally incubated for 8 days at 30°C in a static incubator [16].

2.5. Extraction of SLs

A modified technique was used to isolate SLs by the addition of 100 ml methanol to the obtained cultures (solid: solvent ratio of 1:10 w/v); then shaken for 1 hour at 180 rpm and 40°C using an Incubated/Refrigerated Shaker. After passing the extract through a Whatman No. 40 filter, a rotary

evaporator (Heidolph Cooling Analog Vacuum Controller G1, Germany) was used to extract any leftover methanol. The crude SLs extract was then obtained by further drying in an oven at 40°C [17].

2.6. Physicochemical properties of the produced SLs

2.6.1. Assessment of surface tension (ST) & critical micelle concentration (CMC)

The tensiometer-K100 KrÜss Processor (Germany) was used to measure ST & CMC utilizing the ring method. To evaluate CMC, serial SLs concentrations were prepared, and the breakpoint of ST versus SLs concentrations was calculated. The average of replicate readings was then recorded [18]

2.6.2. Fourier transform infrared spectroscopy (FTIR)

Utilizing a diamond disc as an internal reflector, an attenuated total reflectance (ATR)-FTIR analysis was conducted on a Bruker VERTEX 80 (Germany) combined Platinum Diamond ATR at a range of 4,000–400 cm⁻¹ with a refractive index of 2.4 and a resolution of 4 cm⁻¹.

2.6.3. Proton nuclear magnetic resonance (¹H NMR) spectrometry

The NMR spectra were reported using a Varian Mercury VX-300 NMR spectrometer. ¹H spectra were performed at 300 MHz in deuterated chloroform (CDCl₃). Solvent shifts are linked to chemical shifts, which are quoted.

2.6.4. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

An ExionLC AC system was used for separation in the LC-ESI-MS/MS for the produced SLs, and a SCIEX Triple Quad 5500+ MS/MS system was used for detection of electrospray ionization. An Ascentis® C18 Column (4.6 × 150 mm, 3 μ m) and a mobile phase made up of eluents A (0.1% formic acid) and B (LC-grade acetonitrile with 0.1% formic acid) were used for the separation. 10% B for 0–2 minutes, 10%–90% B for 2–30 minutes, 90% B for 30–36 minutes, 10% B again for 36.1 minutes, and 10% B for 36.1–40 minutes was the programming for the mobile phase gradient. The analysis used EMS-IDA-EPI scans for MS1 (100–1,000 Da) in positive ionization mode, with a flow rate of 0.7 ml/min and an injection volume of 10 μ l.

Using EMS-IDA-EPI scans for MS1 (100–1,000 Da) in positive ionization mode, with particular parameters such as 25 psi curtain gas, 5,500 ion spray voltage, and a temperature of 500°C, and MS2 settings of 50–1,000 Da, 80 declustering potential, and 35 collision energy, the analysis included a 10 μ l injection volume and a flow rate of 0.7 ml/min.

2.7. Investigation of the antimicrobial susceptibility of the targeted compounds

One of the primary goals of the current investigation was to estimate whether the generated SLs could function as a microbicidal agent, specifically against microbial infections that are XDR or MDR (MDR or XDR). In this respect, some clinically important microbial pathogens (characterized as

hospital-derived or nosocomial infections) were kindly donated by the Immunology and Microbiology Dep., Faculty of Medicine (Boys), Al-Azhar University and used to determine the efficiency of the produced SLs. All microbiological work involving MDR/XDR isolates was conducted in a Biosafety Level 2 using safety cabinet (Labconco class 2) laboratory following standard biosafety procedures. The investigation against XDR bacteria, Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Acinetobacter baumannii*), and MDR types like *Salmonella typhimurium* (Gram-negative) and *Candida albicans* (Unicellular fungi) was conducted according to the standardized conditions of The Clinical and Laboratory Standards Institute (CLSI) protocol [19]. The pathogenicity and antibiotic sensitivity of the microbial pathogens were carried out and provided by the Microbiology and Immunology Dept, Faculty of Medicine (Boys), Al-Azhar University. Pre-activation of each bacterial pathogen was carried out using Brain Heart infusion broth media (CondaLab, Spain) at 37°C for 24 hours, while *Candida albicans* was activated in Potato dextrose broth medium (CondaLab, Spain) for 24 hours at 28°C under shaking conditions. The size justification of the inoculum for each microbial pathogen was also implemented using the serial dilution method and accurately quantified by CFU to ensure the fixed concentration of each bacterial pathogen throughout all performed tests. Screening of SLs towards the XDR and MDR microbial pathogens took place using the agar-well diffusion technique [2]. A fixed concentration of SLs (80 µg/ml) was selected based on the pre-screening against the tested pathogens using a turbidimetric procedure. Added SL to the previously prepared well was implemented after inoculating each bacterial pathogen over the agar plate and subjecting them to the incubation period individually. Moreover, standard antibiotic agents against microbial pathogens were also applied as a positive control (20 µg/ml) according to the CLSI protocol. In comparison, tween 80 was also added to the agar plate as a synthetic surfactant in order to measure its activity and compare it to the targeted SL. The diameter of the inhibitory zone (mm) surrounding each targeted molecule was used to calculate the observed outcomes of SLs and standard antibacterial drugs [20].

2.7.1 Determination of the minimum inhibition concentration (MIC) for SLs

Accordingly, the produced SLs exhibited a significant antimicrobial activity, thus subjected to determine the MIC value against all microbial pathogens. In this respect, SLs were prepared in a stock solution and then diluted to obtain a desired concentration of 20–200 µg/ml (represented as 5%–50% of the main compound after dilution) according to the standard broth microdilution method [3]. Therefore, the evaluation of the MIC value for SLs was first obtained by the method of turbidometry and then confirmed by the CFU method. All implemented experiments to study microbial-cell growth and death were carried out immediately after the incubation period to avoid cell storage, which is important to prevent cell lysis for a long time. A change in the enumeration of viable cells on the Mueller-Hinton agar plate (i.e., CFU) was assessed by counting the number of colonies formed on the solid medium. Determination of the MIC level for the prepared SLs was defined as the lowest

concentration of each sample that produced a minimum number of CFU compared to the untreated samples [21].

2.7.2 Effect of SLs on the bacterial lipid peroxidation (LPO)

The LPO activity was examined in each bacterial pathogen that had been treated with the produced SLs. A specific reagent, thiobarbituric acid (TBA), was used to determine the fatty acid peroxidation byproduct, malondialdehyde (MDA), by using the LPO colorimetric assay kit. Quantification of pink color complex (MDA-TBA) was considered a positive result and compared to the reference drug [22]. In this respect, SLs around MIC values (20–80 µg/ml, representing 5%–20% of the SL compound) were used to measure the fatty acid oxidation level of the bacterial cell membrane. MDA lysis buffer (300 µl) was mixed with 1 ml of each treated bacterial pathogen at 4°C to assay the LPO. Butylated hydroxytoluene BHT (3 µl) was then added to avoid pigment interference from the breakdown of the lipophilic peroxides. Harvesting of each treated bacterium was then implemented (8,000 rpm for 10 minutes), and the precipitated substances were removed. In brief, combining 200 µl of the clear filtrate with 600 µl of the TBA solution, and incubating for 60 minutes at 95°C was carried out. Immediately, each sample was left at room temperature, and the developed pink color was measured at 532 nm using a Spectrophotometer-Agilent Cary 100 (Germany). As a positive control, 5% hydrogen peroxide was used to treat both bacterial pathogens for 20 minutes. The elevated LPO activity of SL towards the bacterial cell membrane was demonstrated by the rise in malondialdehyde concentration in the tested sample. Using the following formula, the concentration of malondialdehyde was determined:

$$\text{Malondialdehyde (nmol/ml)} = A_{\text{sample}} / A_{\text{standard}} \times 10$$

In the case of the treated bacterial cells, A_{sample} represents the absorbance of LPO, while A_{standard} represents the absorbance of the standard LPO sample. In addition, the LPO efficiency can also be determined according to the following equation:

$$\text{LPO efficiency (\%)} = [(NS - NC) / NC] \times 100$$

where NC (Control) represents the bacterial cells' untreated LPO absorbance and NS (Tested Sample) represents the treated cells' LPO absorbance.

2.7.3 Biofilm inhibition activity of SLs

Biofilm formation by SLs-treated bacteria was also quantitatively evaluated by the crystal violet assay [23]. Briefly, a 96-well culture plate was used to add 170 µl of Mueller-Hinton broth, which was subsequently inoculated with 10 µl of each microbial pathogen. In this way, SLs around MIC values (20–80 µg/ml, representing 5%–20% of the SL compound) were investigated towards each microbial pathogen in comparison to the negative control [treated with Phosphate-buffered saline (PBS)] and positive control [treated with Ethylenediaminetetraacetic acid (EDTA)]. When the incubation period was completed (48 hours at 37°C), the content of the wells was removed and each of them was then washed using phosphate buffer saline pH 7.2 (200 µl), to eliminate any

microbial debris and dried for 1 hour. Immediately, crystal violet at 0.1% (w/v) was merged (200 μ l) into each well for 1 hour, removing any remaining excess stain, and the plates were dried. Then, 200 μ l of ethanol was combined with elute crystal violet, and the absorbance at 590 nm was recorded using the spectrophotometer.

The biofilm mass inhibition % was calculated according to the following formula:

$$\text{Biofilm mass inhibition (\%)} = [(C - S) / C] \times 100$$

where C is the optical density at 590_{nm} (OD₅₉₀) of the untreated microbial cells, and S is the OD₅₉₀ of the treated bacterial cells.

2.7.4. Measurement of intracellular reactive oxygen species (ROS) inside the treated microbial cells

Generation of ROS by SLs inside the microbial cells was evaluated by dichlorofluorescein diacetate (DCFH2-DA) indicator. Therefore, the susceptibility of SLs to release high levels of ROS inside each microbial pathogen was determined quantitatively. After the cultivation period, each microbial pathogen was physically treated under ultrasonication, and the cell-free extract was collected and combined with DCFH-DA (10 μ m) for 30 minutes at 37°C. In comparison, each microbial pathogen was treated with hydrogen peroxide H₂O₂, which was represented as a positive control [24]. Following that, a Spectrofluorometric device (JASCO FP-6500, light source Xenon arc lamp, Japan) was used to count the intensity of DCF fluorescence. Each sample's fluorescence intensity was measured using wavelengths for excitation and emission at 485 nm and 530 nm, respectively.

2.8. Evaluation of cytotoxic effects on HFB-4 cells

The VACSERA Tissue Culture Unit's human normal melanocyte cells (HFB-4) were cultured at 37°C with 5% CO₂ in DMEM supplemented with 10% FBS, L-glutamine, HEPES buffer, and gentamycin. Cells were seeded in 96-well plates (1 \times 10⁴ cells/well) and, after 24 hours, exposed to serial dilutions of SLs (5%–80%). Vehicle controls received 0.1% Dimethylsulfoxide (DMSO). The 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) test was used to assess viability after a 48-hour incubation period; formazan crystals were solubilized in DMSO, and at 590 nm, the absorbance was read. Percentage viability was calculated relative to untreated controls, and IC₅₀ values were obtained from nonlinear regression of dose–response curves [25].

2.9. Statistical analysis

The findings are shown as mean \pm SD, and each experiment was run in triplicate. The Student's *t*-test was used to assess statistical differences between the groups. *p*-values less than 0.05 were regarded as statistically significant. For all statistical analyses, Origin 2018 was used.

3. RESULTS AND DISCUSSION

3.1. Isolation and screening of SLs-producing yeast

Biosurfactants produced by yeast strains offer antioxidant activity, thermal resistance, and no risk of

pathogenicity, proving their potential use in foods and drug formulations [26]. To achieve the best productivity, it is well recognized that one strategy to reduce production costs is to isolate the microorganism from the local environment. This study employed wheat germ oil cake as an example of agro-industrial wastes found in the Egyptian environment to isolate certain yeast strains for SLs production. SLs-producing yeast strains were screened under CTAB stress conditions, leading to the selection of six yeast-like colonies capable of producing biosurfactants and tolerating CTAB. The W1 strain was further evaluated for molecular identification.

3.2 Molecular characterization of the W1 strain

DNA extracted from the W1 strain was quantified using a spectrophotometer. Amplification of the rDNA ITS region using ITS1 and ITS4 primers produced an approximately 500 bp fragment, as shown in Figure 1.

The PCR purified products were sequenced utilizing an ABI 3730xl DNA sequencer of GATC Company (Germany) with a forward primer. The resulting DNA sequence was identified as belonging to the yeast strain *M. guilliermondii*. This sequence, encompassing the ITS1 region, the 5.8S ribosomal RNA gene, the complete ITS2 region, and a partial 28S ribosomal RNA gene, was submitted to the GenBank database under the accession number ON644534.1.

3.2.1. Evolutionary analysis of the identified strain

The isolated (W1) DNA sequence was analyzed for evolutionary relationships by comparing it with sequences

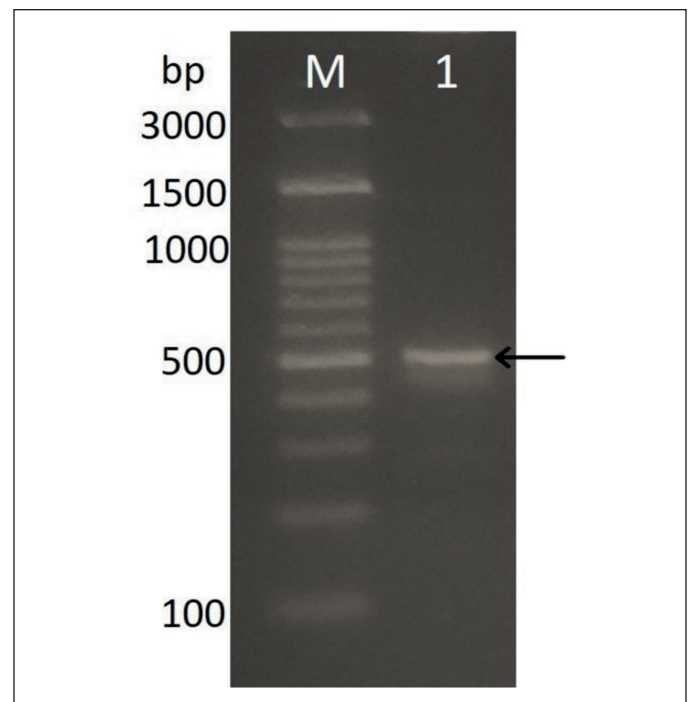


Figure 1. Photograph of the ITS-DNA amplified band for yeast strain (W1) isolated from Wheat germ cake (lane 1), band indicated with arrow. Using ITS1 and ITS4 primers against a 100 bp ladder DNA marker (lane M). The arrow pointing to the band was eluted and gone for sequencing.

available in the NCBI GenBank database (<http://www.ncbi.nlm.nih.gov>). The sequence was aligned with reference taxa retrieved from public databases, and a phylogenetic tree depicts the evolutionary relationships among various strains of *M. guilliermondii*. The tree, based on sequence data from the ITS region, suggests the unknown query sequence likely belongs to the species *M. guilliermondii* as shown in Figure 2. It shares a close evolutionary relationship with the strain MH211588.1. This suggests that the unknown strain may have similar characteristics or properties to MH211588.1. The phylogenetic tree provides a valuable tool for understanding the genetic diversity and evolutionary relationships within the *M. guilliermondii* species. By analyzing the branching pattern and genetic distances, researchers can gain insights into the origins, distribution, and potential applications of these strains.

3.3. Production of *M. guilliermondii* SLs

Isolating microorganisms from their environment and selecting robust ones can reduce manufacturing costs and improve product yield while maintaining product quality. Furthermore, using agricultural waste as a nutritional medium

in the fermentation process assists in reducing production costs [27,28]. Also, there are numerous advantages to using SSF for microbial production, including the ability to imitate their natural habitat, which improves medium adaptation and increases yield; the lack of free water also reduces bacterial contamination [29]. Developments in solid state for the SLs commercial sector, SLs production may provide fermentation solutions with reduced water use, operating costs, and capital requirements [28].

In this context, one of the agro-industrial wastes, namely jojoba oil cake, was employed as a nutritional medium under SSF for the growth of the locally isolated *M. guilliermondii* yeast from another waste (wheat germ oil cake). The presence of oils or lipids in a substrate is known to enhance SL production, whereas oils serve as hydrophobic carbon sources that induce biosurfactant production in yeasts like *Candida bombicola* or *Saccharomyces cerevisiae* [5,30,31]. Therefore, soybean crude oil was used as a carbon source and biosurfactant production inducer, resulting in a yield of 23.12 g/100g substrate by methanol extraction. In addition, Parekh and Pandit [32,33] used a combination of glucose and oleic acid with wheat bran to

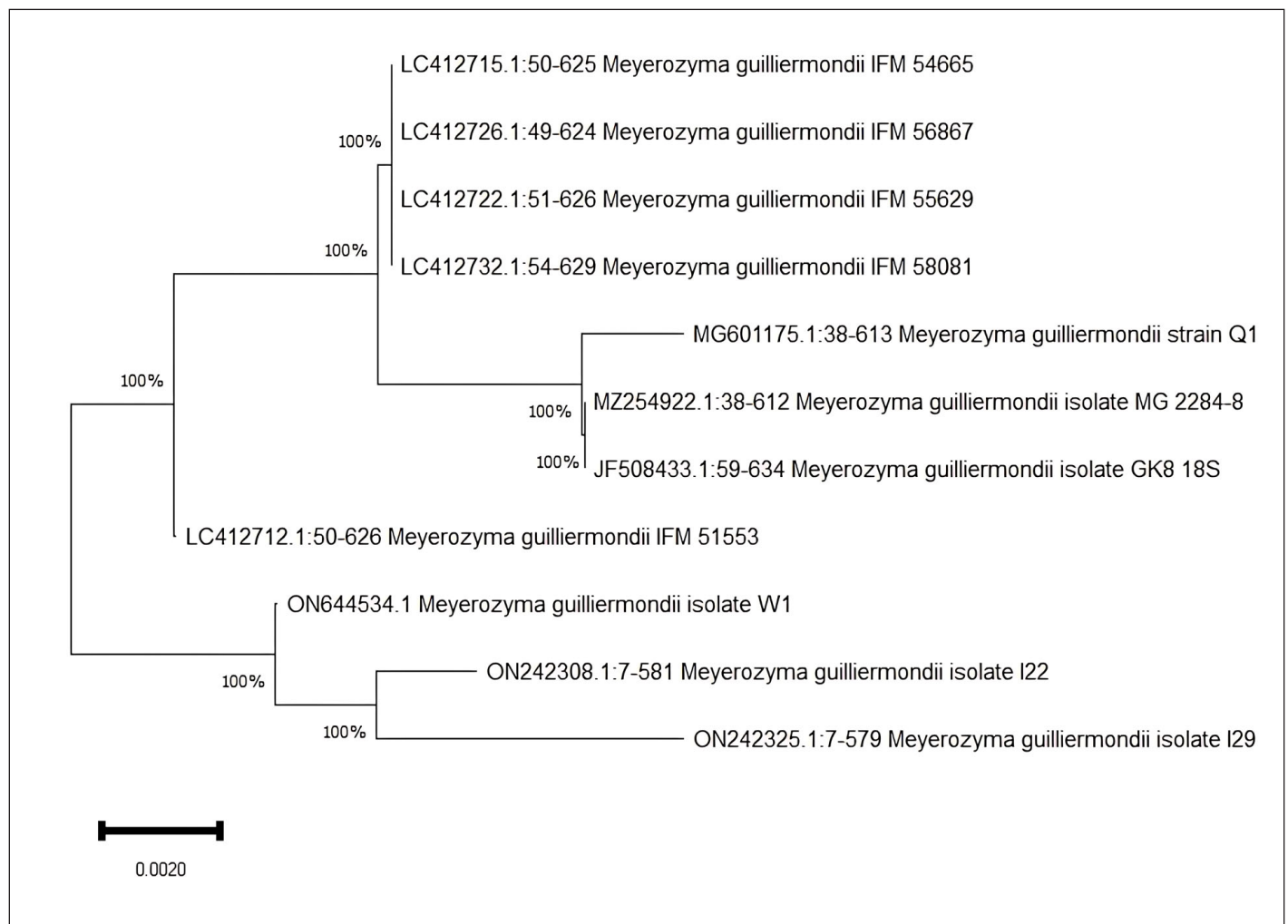


Figure 2. Phylogenetic dendrogram displaying the taxonomic position of the (W1) yeast strain isolated from wheat germ, based on the ITS sequences and other closely related species available from NCBI.

produce *Starmerella bombicola* NRRL Y-17069 SLs reporting a lower yield of SLs (17.48 and 18g/100g substrate). On the other hand, they also utilized mango kernel and wheat bran with oleic acid as a substrate by growing the same strain to produce SLs under solid-state fermentation, respectively. Later, *Saccharomyces cerevisiae* microbially converted *Moringa oleifera* oil cake to produce a similar yield of SLs (18.6/100 g substrate) [34]. Also, [35] obtained a yield close to 20 g SLs /100g substrate from *S. bombicola* grown on winterization oil cake from sunflower oil utilizing SSF. Furthermore, Jiménez-Peñalver *et al.* [36] used winterization oil cake, a leftover oil cake from the oil refining industries, and sugar beet molasses as a co-substrate to produce SLs. growing *S. bombicola* ATCC 22,214 to obtain a similar yield of 23.5g/100 g substrate. Nevertheless, increased yields from other publications have been reported, such as 32.1/100 g of substrate SLs generated by microbial conversion of oil cake from *Moringa oleifera*, which grew the isolated yeast *Yarrowia lipolytica* [28]. However, a higher SLs yield of 39/100 g substrate was produced from *Candida parapsilosis* cultivated on potato peel and frying oil wastes [5]. The potato peel and frying oil wastes were also employed to produce SLs growing the same strain *Candida parapsilosis*, giving a higher yield of 55.3/100 g substrate [31]. Abdel-Latif *et al* [30] also used *S. cerevisiae* grown on banana peels to produce SLs with a yield of 49.04%.

Initial cost studies indicate that using jojoba oil cake can significantly reduce production costs by up to 40%–60% compared to standard SL substrates. This first report on SLs production using locally isolated *M. guilliermondii* highlights the potential for reducing the production cost process and, consequently, the price of the final product, as Pala *et al* [28] reported that the SL business still faces relatively expensive prices (20–30 €/kg). Therefore, the yield (23.12/100 g of substrate) of the produced SLs may be economical.

3.4. Assessment of ST and CMC

The main feature of SLs surface activity is their capacity to reduce ST and CMC values. The CMC estimated values are crucial to identifying the possible uses of

biosurfactants because it is frequently used to assess surface activity. To ascertain whether a compound is economically related to its productivity, CMC is also a significant indicator [5]. The SLs from *M. guilliermondii* lowered the ST from 72 to 39 mN/m with a CMC level of 240 mg/l (Fig. 3). The obtained ST value was similar to that observed for the produced SLs by *Yarrowia lipolytica* and *Saccharomyces cerevisiae* cultivated on *Moringa oleifera* oil cake (39 and 38 mN/m) at CMC values of 62.5 and 60 mg/l, respectively [25,31]. Additionally, [32,33] showed lower STs (32.6 and 35 mN/m), although Rashad *et al.* [38] reported greater ST for SLs (45 mN/m) utilizing *Candida bombicola* grown in sunflower oil cake. Nonetheless, SLs were shown to have a range of CMC values from 62.5 mg/l to 5 g/l [5,27,28,39].

Therefore, the obtained results from the production, ST, and CMC levels it can be concluded that, when considering its economic value, the new compound's yield and low ST make it fairly acceptable in various industries.

3.5. Molecular characterization of produced SLs

3.5.1. FTIR

FTIR spectroscopy was utilized to determine the chemical structure of the produced SLs. The FTIR spectra (Fig. 4) FTIR analysis of the obtained SLs showed absorption at 2924.48 and 2853.59 cm^{-1} , which validated the methylene group's asymmetrical stretching (vas CH_2). These findings are consistent with Kumari *et al.*, [39], who produced SLs from a novel yeast (*Metschnikowia churdharensis*), obtaining a mixture of both acidic and lactonic forms. In addition to the FTIR analysis of the SLs obtained from microbial conversion of banana peels using *Saccharomyces cerevisiae* [30]. The absorption at 1644.16 and 1157.87 cm^{-1} which correlated with the C (=O)–O–C = of the lactone group, while the absorption of the carboxylic group of fatty acid C–O–H was detected at 1455.45 cm^{-1} , which was in agreement with what have been found by Kumari *et al.*, [39] and Abdel-Latif *et al.*, [30]. The absorption noted at 1712.38 cm^{-1} proved the C=O stretch of esters, ketones, aldehydes, or acids. Furthermore, the sugar

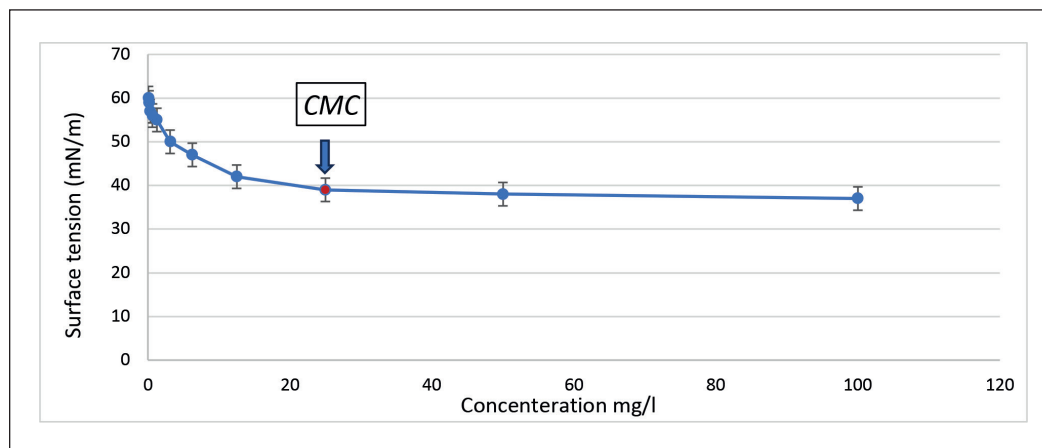


Figure 3. CMC and minimum ST of the produced SLs from *Meyerozyma guilliermondii*. *Data were expressed by means of three triplicates.

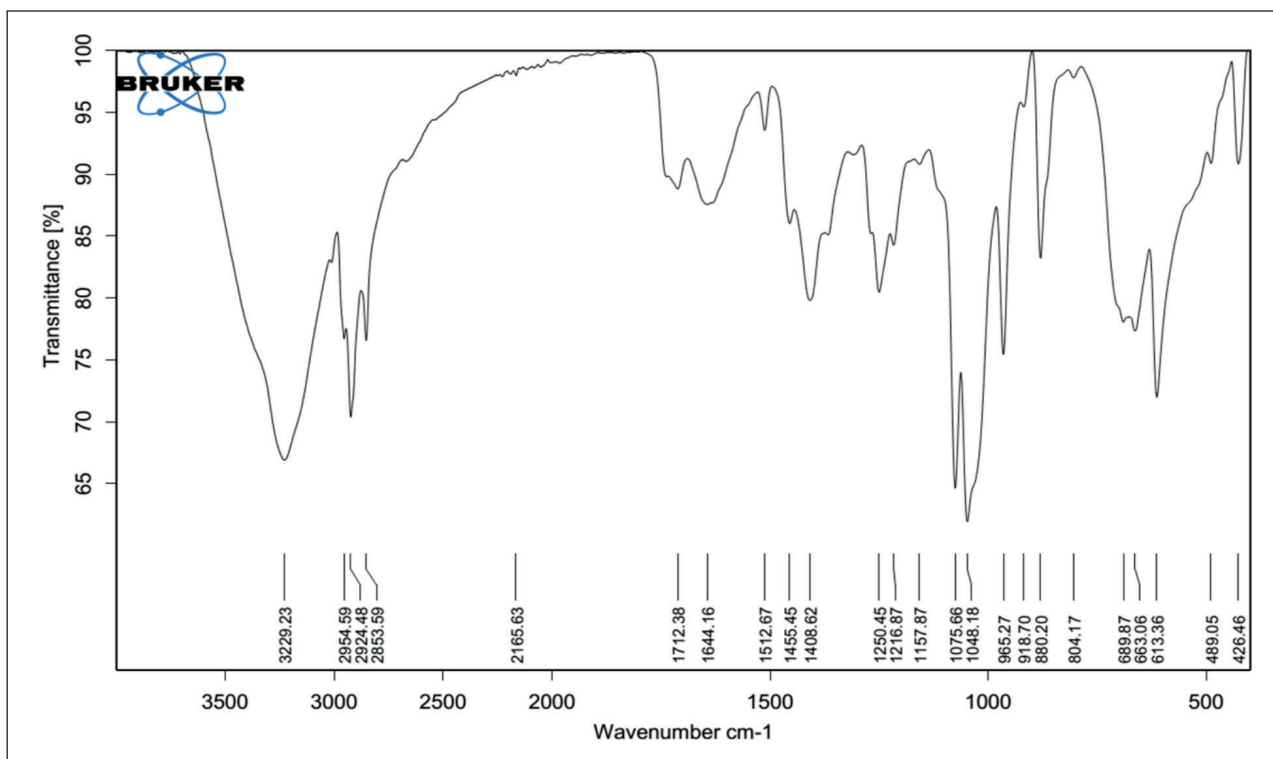


Figure 4. FTIR spectroscopy for the produced SLs from *Meyerozyma guilliermondii* grown on jojoba waste as a substrate.

molecule (sophorose) is also one of the determining factors for the presence of the SLs molecule in the obtained extract. In the present study, the FTIR analysis proved the existence of sophorose moiety C–O stretch from C–O–H groups at 1080–1098 cm^{-1} [17,30,34].

The presence of surface-active glycolipid structures, which are compatible with SLs or related biosurfactants, is confirmed by this FTIR. This identification is supported by the essential characteristics (O–H, C=O, C–O–C, and long aliphatic chains).

3.5.2. ^1H NMR analysis of the produced SLs

To confirm the presence of essential substances that determine the presence of sphorolipids in the extract, an analysis was performed using ^1H NMR spectrometer.

The occurrence of the vinyl group (–CH=CH) was demonstrated by the ^1H NMR spectra, which showed signals from 5.33 to 5.34 ppm. The presence of the fatty acid moiety was shown by the several signals that emerged between 1.23 and 1.27 ppm, while the signals at 2.28 ppm validate the presence of the –CH₂–COO–group. Nevertheless, the signals at 2.03 ppm indicate the presence of the –CO–CH₃ group, whilst the glucose protons were found at 4.13 and 4.26 ppm. The obtained results were similar to the chemical characterization using ^1H NMR analysis for SLs produced from *Candida bombicola* grown on different substrates, including sunflower oil cake, soybean crude oil, motor oil waste, and corn oil [17,38–40].

These signs point to the existence of sophorose linked to fatty acid units, which are typical structural characteristics of biosurfactants based on glycolipids.

3.5.3. LC-MS/MS spectroscopy of the produced SLs

The spectra of SLs were implemented using LC-ESI-MS/MS (Fig. 5A and B), where the retention times of 3.72 and 26.77 minutes, acidic and lactic SLs peaks were observed at m/z 700.04 and 688.24, respectively. The peaks at m/z 344.07 and 362.03 match hydroxy fatty acid fragments (C18:2), while the peak at m/z 407.83 indicates the existence of a sophorose fragment. Furthermore, peaks at m/z 500.91, 519.97, and 537.95 confirmed the existence of sophorose linked to C13, hydroxy fatty acid, and polyunsaturated fatty acid C15:3, respectively. Lactic SL was also found during the retention time of 26.77 minutes (688.24 m/z), whereas the peaks at m/z 620.50 and m/z 671.64 verified the presence of lactic SLs with varying fatty acid lengths. Additionally, the peak at m/z 401.76 highlighted the existence of the sophorose sugar moiety at the same retention duration. The peaks at m/z 575.26 and 589.38 confirmed the presence of polyunsaturated fatty acids linked to sophorose (C15:3 and C18:2), while sophorose with C13 appeared at m/z 492.74. However, different lengths of hydroxy fatty acids from C16 to C22 were confirmed by the peaks at m/z 255.24; 281.14; 312.98, and 356.87 [39,41–43].

However, chemical structure investigations using LC-MS/MS, ^1H NMR, and FTIR spectroscopy verified that the SLs produced from the locally isolated *M. guilliermondii* cultivated on a sustainable source (jojoba oil cake) had a mixture of both lactic and acidic SL structures.

3.6. Investigation of the antibacterial susceptibility of the produced SLs

The main target of the prepared SLs is to investigate their ability to prevent the proliferation of the

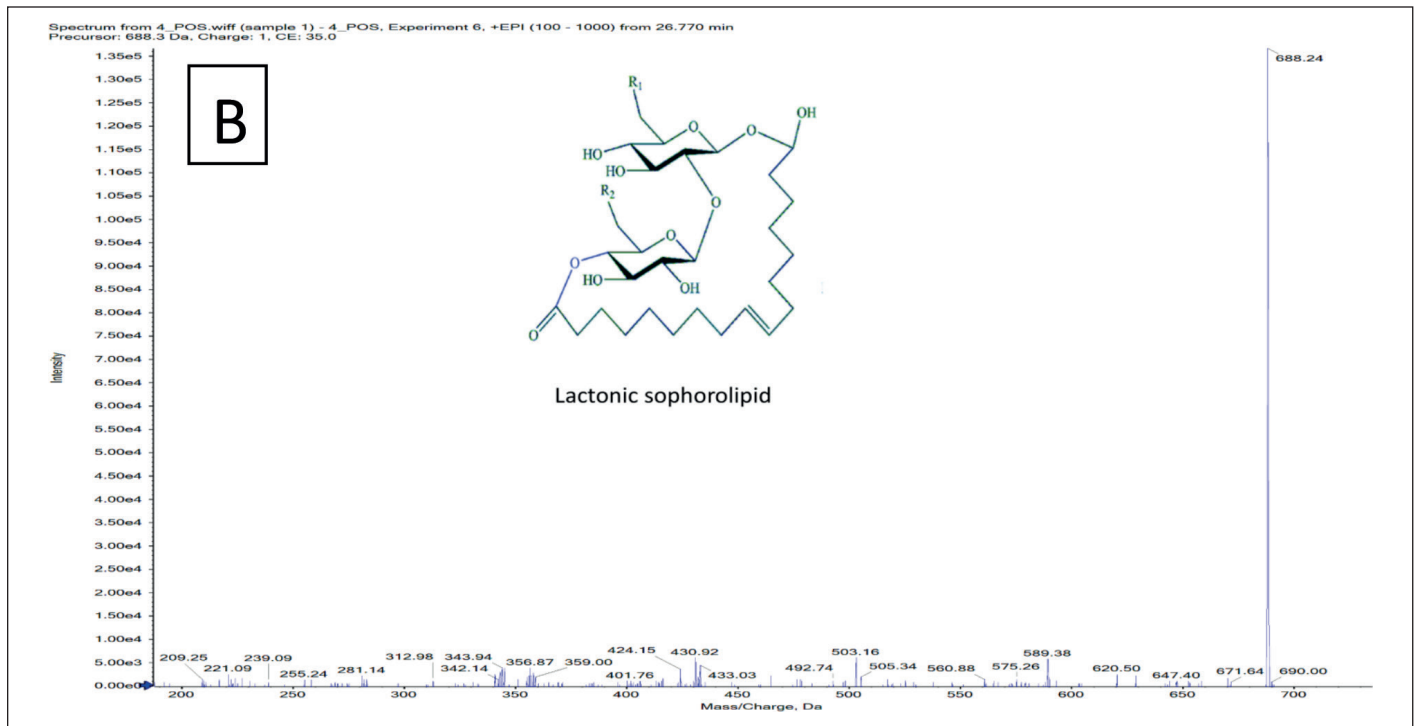


Figure 5. (A, B) Mass spectra of the SLs produced by *Meyerozyma guilliermondii* by LC-MS/MS in positive electrospray ionization mode (+ESI). A: At the retention times of 3.72 minutes, acidic SLs peaks were observed at m/z 700.04. B: At 26.77 minutes, the lactonic form at m/z 688.24, and its fragments are noticed at the same retention times.

MDR and XDR microbial pathogens. This may contribute to next-generation antimicrobials as alternative or adjunct antimicrobial agents.

Therefore, the antimicrobial test was explored against a range of MDR and XDR pathogens isolated from clinical facilities (characterized as hospital-derived infection). A detectable virulence of the targeted pathogens was sharply noted by the antibiotic sensitivity profile (Table S1 and S2), which reached more than 50% of the resistance ratio for all tested antibiotic agents.

Screening of the SLs activity was initially carried out using the turbidimetric method (data not shown), and a significant concentration (i.e., 20%) was applied using agar-well diffusion and compared to several classical antibiotic drugs. As shown in Table 1 and Figure 6, the inhibition activities of SLs were relatively different between the tested pathogens. Whereas, the potent inhibitory activity was obtained against *S. aureus* (9 ± 1.08 mm), followed by *S. typhimurium* (6 ± 0.71 mm) and *A. baumannii* (4 ± 0.22 mm). Otherwise, a moderate inhibition activity was noted against the unicellular fungal pathogen, *C. albicans* (3 ± 0.02 mm). Notably, the SLs activity was sharply observed against Gram-positive bacteria, greater than Gram-negative bacteria, and *C. albicans*, which may be related to the cell membrane synthesis inhibition. The antibiotic activity profile proved the superiority of the lower groups among them, like Ciprofloxacin and Amphotericin B against bacterial and fungal strains, respectively. However, a distinguishable activity of SLs appeared in the case of *S. aureus* when compared to the potent antibiotic drug (Ciprofloxacin, 5 ± 0.55 mm), and close

to the inhibition zone value in the case of the Gram-negative ones (Approx 4 and 6 mm for *A. baumannii* and *S. typhimurium*, respectively). These results provide a strong indication of the promising effectiveness of SLs when utilized against clinically derived pathogens. Therefore, determining the MIC value of SLs could be the next investigative step to evaluate the efficacy at different concentrations.

To determine the MIC value of the produced SLs, for each microbial pathogen, varying doses were used. For this purpose, the microdilution method was performed, and the microbial activity was evaluated according to turbidity and confirmed by the CFU method Table 2. The lower concentration of SLs notably yielded a minimum CFU in the case of *S. aureus* (51.5 ± 1.05 $\mu\text{g/ml}$) and *S. typhimurium* (72.5 ± 3.92 $\mu\text{g/ml}$). Meanwhile, relative increases in MIC values were detected in the case of *A. baumannii* (84.4 ± 2.58 $\mu\text{g/ml}$) and *C. albicans* (28.9 ± 2.18 $\mu\text{l/ml}$). In addition, Ciprofloxacin (13.9 ± 2.15 $\mu\text{g/ml}$) also had a higher MIC value when compared to SLs (51.5 ± 1.05 $\mu\text{g/ml}$) against *S. aureus*, confirming the anticipated function of SLs against this specific bacterial pathogen. All bacterial pathogens were affected by SLs at lower concentrations not exceeding 100 $\mu\text{g/ml}$, while displaying a distinctive activity higher than 100 $\mu\text{g/ml}$ against *C. albicans*. Therefore, it is assumed that the prepared SLs based on their lactonic and acidic mixture content could induce a significant antimicrobial activity.

This study was performed on a limited number of MDR and XDR clinical isolates, which may not fully represent the wide diversity of resistant pathogens encountered in clinical

Table 1. Antibacterial activity of the *Meyerozyma guilliermondii* SLs using agar-well diffusion.

Compound code	Inhibition zone (mm)			
	<i>Staphylococcus aureus</i> (XDR)	<i>Acinetobacter baumannii</i> (XDR)	<i>Salmonella typhimurium</i> (MDR)	<i>Candida albicans</i> (MDR)
SL (1)	9±1.08 ^a	4±0.22 ^a	6±0.71 ^a	3 ± 0.02 ^b
Tween 80 (2)	ND	ND	ND	ND
Fluconazole	ND	ND	ND	ND
Amphotericin B	ND	ND	ND	ND
Nizoral	ND	ND	ND	ND
Cephadrine b	ND	ND	ND	ND
Ciprofloxacin	5 ± 0.55 ^b	4 ± 0.61 ^a	6 ± 1.02 ^a	ND
Ampicillin	ND	ND	ND	ND
Polymyxin B	ND	3 ± 0.33 ^b	3 ± 0.06 ^b	ND
Erythromycin	ND	ND	ND	ND
Kanamycin	3 ± 0.91 ^c	2 ± 0.22 ^c	ND	ND
Sulfadiazine	ND	ND	4 ± 0.88 ^b	ND

Fluconazole, Amphotericin B, and Nizoral were used as standard antifungal agents, b Kanamycin, Ciprofloxacin, Ampicillin, Polymyxin B, Erythromycin, and Cephadrine were used as standard antibacterial agents at 20 µg/ml, b ND: not determined.

Note, the SLs were used at a concentration of 20% in the screening test.

The mean ± SD ($n = 3$) is used to express values. ND: not determined. Within each column, different superscript letters indicate statistically significant differences among compounds ($p < 0.05$, one-way ANOVA).

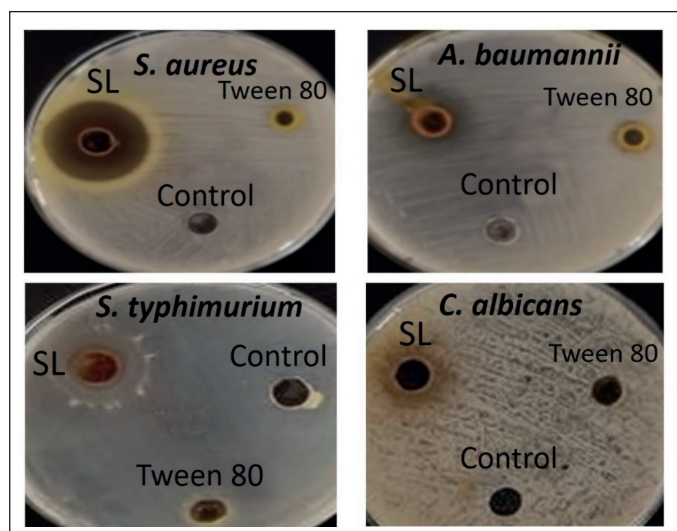


Figure 6. Antibacterial activity of *Meyerozyma guilliermondii* SLs using agar-well diffusion method.

practice. Furthermore, the experiments were implemented under *in vitro* conditions, and therefore, the efficacy and safety of SL need to be further validated through *in vivo* studies and clinical trials. Moreover, the underlying molecular mechanisms of SL activity were not deeply investigated in this work, which warrants future mechanistic and genomic studies to better understand its mode of action. Despite these limitations, the outcomes of this study highlight the potential of SL as a promising antimicrobial agent against drug-resistant pathogens. Its ability to induce oxidative stress and LPO in resistant bacteria

suggests a novel mechanism that could be exploited either as a standalone therapeutic or in combination with conventional antibiotics to enhance their efficacy. Such applications may open new avenues for developing alternative or adjunctive strategies to overcome the global challenge of antimicrobial resistance.

There are many trials to incorporate SLs in food and pharmaceutical sectors due to their activity properties against several microbial types, like viral, bacterial, and fungal cells [44]. Moreover, the encouragement of these studies is related to the lower toxicity, safe susceptibility, and biodegradability of SLs, especially when secreted from non-pathogenic yeast. In accordance with the present findings, SLs were extensively used as antibacterial agents against Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. aureus*) pathogens [45]. Furthermore, the efficiency of SLs toward unicellular and multicellular fungal pathogens also showed significant activity by interrupting their growth and preventing the biofilm formation by *Candida* species [4].

3.7. Antibiofilm activity of the targeted molecules

Microbial biofilm is a significant tool to increase its virulence via resistance towards several hindrance conditions, like different antibiotic groups. Quantitative assay procedures were applied to assess the efficiency of SLs in preventing biofilm formation by the targeted bacterial and fungal pathogens. The initial screening for microbial biofilm development was conducted at a fixed dose of 80 µg/ml. The target concentration was found to be stressful against some bacterial pathogens, as carried out in the case of *S. aureus* and *S. typhimurium*. Thus, lower concentrations of SLs could provide

a true result about the ability of microbial pathogens to secrete the biofilm without the high impact on cell proliferation. As detected from the MIC test, concentrations from 20 to 80 µg/ml may provide indications about the biofilm production process through the incubation period. Table 3 shows the biofilm generation for each microbial pathogen at the final stage of the incubation period. The concentrations of SLs showed a gradual inhibition of biofilm formation, increasing with concentration, possibly as a result of the cells' rapid disintegration. At a lower SLs concentration, evaluation of biofilm production could demonstrate a significantly superior capacity to suppress the biofilm formation in both Gram-positive & negative bacteria compared to unicellular fungi. The bacterial biofilm inhibition percentage reached more than 50% at the targeted concentration (80 µg/ml), which displayed a good antibiofilm agent. Otherwise,

it was noteworthy that SLs had a moderate antibiofilm efficacy against *Candida albicans*, which was lower than 40% at 80 µg/ml. Noticeably, differences between the tested pathogens for biofilm production in the presence of SLs may be due to the differences between them in the cell membrane composition. Therefore, the LPO activity was subsequently investigated for each microbial pathogen.

Each bacterial type produces exopolymer molecules in multiple phases, which make up the biofilm matrix. Cell adhesion mechanisms may be facilitated and prevented when treated with a strong molecule that can inhibit more than 50% of the microbial biofilm development [44]. Microbial biofilms increase the resistance to most of the current antimicrobial agents. Therefore, several reports have demonstrated the SLs activity against the biofilm formation by inhibiting the adhesion of microbial pathogens

Table 2. The minimum inhibitory concentration (MIC) of the potent *Meyerozyma guilliermondii* SLs.

Sample No.	Minimum inhibitory concentration (MIC, %)			
	<i>Staphylococcus aureus</i> (XDR)	<i>Acinetobacter baumannii</i> (XDR)	<i>Salmonella typhimurium</i> (MDR)	<i>Candida albicans</i> (MDR)
SL	12.8 ± 1.05 ^b	21.1 ± 2.58 ^c	18.12 ± 3.92 ^c	27.9 ± 2.18 ^c
Tween 80	ND	ND	ND	ND
Fluconazole a	ND	ND	ND	>62.5 ^b
Amphotericin B	ND	ND	ND	18.4 ± 0.14 ^a
Nizoral	ND	ND	ND	46.7 ± 3.21 ^b
Cephadrine b	>62.5 ^c	>62.5 ^c	>62.5 ^d	ND
Ciprofloxacin	13.9 ± 2.15 ^a	10.6 ± 1.88 ^a	6.7 ± 1.02 ^a	ND
Ampicillin	>62.5 ^c	>62.5 ^c	>62.5 ^d	ND
Polymyxin B	37.8 ± 3.75 ^b	21.8 ± 2.42 ^b	27.8 ± 3.11 ^b	ND
Erythromycin	>62.5 ^c	44.8 ± 2.05 ^b	34.5 ± 3.32 ^b	ND
Kanamycin	21.9 ± 2.22 ^a	31.8 ± 1.78 ^b	>62.5 ^d	ND
Sulfadiazine	>62.5 ^c	>62.5 ^c	12 ± 2.17 ^a	ND

Not, SLs concentration was prepared from the main compound 100% (5%–50%)

The mean ± SD ($n = 3$) is used to express values. ND: not determined. Within each column, different superscript letters indicate statistically significant differences among compounds ($p < 0.05$, one-way ANOVA).

Table 3. Antibiofilm activity (quantitative method) using the crystal violet method at the MIC value

SL concentration (%)	Biofilm inhibition (%)			
	<i>Staphylococcus aureus</i> (XDR)	<i>Acinetobacter baumannii</i> (XDR)	<i>Salmonella typhimurium</i> (MDR)	<i>Candida albicans</i> (MDR)
5	24.04 ± 3.05 ^c	16.22 ± 4.15 ^c	27.11 ± 2.52 ^c	11.5 ± 1.25 ^c
10	48.2 ± 1.95 ^b	28.39 ± 1.45 ^b	36.9 ± 3.16 ^b	18.4 ± 5.16 ^b
20	76.9 ± 2.66 ^a	51.5 ± 2.61 ^a	67.4 ± 2.44 ^a	38.6 ± 2.02 ^a
Amphotericin B (20 µg/ml)	-	-	-	69.2 ± 4.03 ^a
Ciprofloxacin (20 µg/ml)	58.24 ± 1.33 ^b	62.83 ± 3.27 ^a	53.94 ± 2.75 ^b	-

The mean ± SD ($n = 3$) is used to express values. Within each column, different superscript letters indicate statistically significant differences among treatments ($p < 0.05$, one-way ANOVA).

to solid surfaces [4]. In consistency, the potent antibiofilm activity of SLs was clearly obtained against Gram-positive bacteria more than Gram-negative bacteria [46]. The antibiofilm ability of SLs was observed against *E. coli* and *S. aureus* via reduced adhesion activity, reflecting the high disruption of biofilm formation [47]. In addition, SLs were also proven to decrease the attachment of *S. aureus* to the coating materials, suggesting their efficacy for application in medical devices [48]. Moreover, the reduction of biofilm production by *C. albicans* was also significantly indicated at 15 µg/ml of SLs, which remains around 55% of its production as compared to the untreated cells [4].

The biofilm inhibition activity was also indicated even against the resistant bacterial strains, which contributed to the dispersed biofilm formation only without any inhibition activity for the bacterial growth [49]. Vasudevan and Prabhune [50] showed the higher antibiofilm activity of curcumin-SL nanoparticles against *Pseudomonas aeruginosa* by quorum quenching, which contributed to the rapid destruction of the bacterial proliferation.

3.7.1 LPO activity of the potent molecules (LPO)

The oxidation level in the fatty acid content of the cell membrane is one of the common tools to indicate the oxidative stress on the bacterial cells, and the possibility of the tested molecule to disrupt the cell membrane. The cell membrane inhibition degree commonly occurs according to the oxidative stress level caused by the targeted molecule, which could provide some interpretations about the eradicated pathway of the targeted bacterial cells. Here, the oxidation level of the bacterial cell membrane by SLs was sharply increased against all bacterial pathogens. The oxidation activity of the lipid content in the cell membrane was strongly indicated in the higher concentrations of SLs, in which *S. aureus* was found to be the most affected bacterial pathogen, followed by *S. typhimurium* and *A. baumannii* Table 4. Obviously, there were differences in the LPO activity between bacterial pathogens, where the potent oxidation was obtained at higher concentrations against all bacteria. However, the fatty acid oxidation level in the case of Gram-positive, *S. aureus* was higher than that detected for the standard antibiotic. In contrast, the impact of higher SLs concentrations was higher than that of the standard antibiotic, suggesting their efficacy against Gram-positive bacteria. In the

scope of Gram-negative bacteria, the LPO activity of SLs was notably higher than *S. typhimurium*, which was carried out on *A. baumannii*, revealing the ability of SLs to induce oxidative stress and remarkably targeted disruption of the cell membrane. It is clearly noted that the ability of the produced SLs to cause severe oxidation of cell membrane fatty acids gradually increases as the concentration increases.

In this respect, the evaluation of bacterial cell membranes after treatment with SLs was studied by many authors. The antibacterial activity of SLs was reflected in the rapid induction of the disruption that occurred in the cell membrane towards the Gram-positive and Gram-negative bacteria [51]. The SLs interaction with the bacterial cell membrane could be indicated by the alteration in the morphological and structural features, and the membrane permeability increases, resulting in a disturbance of membrane integrity [46]. Accordingly, penetration of SLs into the cell membrane actually leads to the release of more intracellular substances, causing cell membrane ruptures. The facility of SLs penetration into Gram-positive bacteria was greater than Gram-negative bacteria, as indicated in the present findings. The differences between them in the cell membrane composition were discussed, which may be due to the presence of a lipopolysaccharide layer around the Gram-negative outer membrane. However, the effectiveness of SLs on cell membrane rupture was found to be identical at the same concentration against both bacterial types [44].

3.7.2. Quantification of ROS induced by SLs

The increased potential to kill bacterial pathogens may be related to the excitation process of SLs that results in the release of high concentrations of ROS. Hence, quantification of ROS inside the treated microbial cells was estimated to confirm the predicted inhibition mechanism. As can be seen in Table 5, the generation of different ROS was indicated by the selected SLs concentration, and the elevated level of ROS was sharply indicated at the higher concentration of SLs. In this respect, ROS inside *S. typhimurium* was found to be relatively close to that obtained by the standard drug, which reflected their ability to penetrate the cell membrane via ROS produced by SLs. Decreases in ROS amount were clearly related to the lower SLs concentration (5%), which is inconsistent with the

Table 4. LPO activity of the targeted molecules against bacterial pathogens at the MIC value.

SL concentration (%)	Bacterial pathogens					
	<i>Staphylococcus aureus</i> (XDR)		<i>Acinetobacter baumannii</i> (XDR)		<i>Salmonella typhimurium</i> (MDR)	
	Malondialdehyde (nmol. ml ⁻¹)	LPOP efficiency (%)	Malondialdehyde (nmol. ml ⁻¹)	LPO efficiency (%)	Malondialdehyde (nmol. ml ⁻¹)	LPO efficiency (%)
5	3.61 ± 0.15 ^c	113.24 ± 2.05 ^c	2.52 ± 0.22 ^c	81.4 ± 1.31 ^c	3.4 ± 0.55 ^c	104.6 ± 1.75 ^c
10	5.03 ± 0.41 ^b	196.6 ± 4.15 ^b	3.79 ± 0.33 ^b	166.2 ± 2.35 ^b	4.2 ± 0.25 ^b	153.3 ± 2.55 ^b
20	6.31 ± 0.02 ^a	258.2 ± 2.22 ^a	4.25 ± 0.51 ^b	206.6 ± 3.31 ^b	6.23 ± 0.18 ^a	247.4 ± 2.88 ^a
Ciprofloxacin	5.62 ± 0.65 ^b	231.7 ± 3.11 ^a	5.05 ± 0.27 ^a	262.9 ± 2.22 ^a	6.51 ± 0.24 ^a	293.3 ± 4.36 ^a

The mean ± SD (*n* = 3) is used to express values. Within each column, different superscript letters indicate statistically significant differences among treatments (*p* < 0.05, one-way ANOVA followed by Tukey's test). MDA: Malondialdehyde.

Table 5. ROS determination of SL toward the bacterial pathogens.

SL Concentration (µg/ml)	<i>Staphylococcus aureus</i> (XDR)	<i>Acinetobacter baumannii</i> (XDR)	<i>Salmonella typhimurium</i> (MDR)	<i>Candida albicans</i> (MDR)
5	166 ± 1.82 ^b	112 ± 2.05 ^b	130.5 ± 2.17 ^b	149.5 ± 3.04 ^b
10	208.4 ± 3.11 ^c	148.3 ± 0.83 ^c	201.9 ± 1.66 ^c	169.6 ± 1.84 ^c
20	226 ± 2.01 ^d	217 ± 1.93 ^d	283 ± 1.75 ^d	221.5 ± 0.71 ^d
Ciprofloxacin	279 ± 1.22 ^e	253 ± 3.18 ^e	301 ± 1.22 ^e	295 ± 1.09 ^e
Control (Untreated)	94.2 ± 0.67 ^a	89.57 ± 0.43 ^a	97.3 ± 0.91 ^a	107.4 ± 1.66 ^a

The mean ± SD ($n = 3$) is used to express values. Within each column, different superscript letters indicate statistically significant differences among treatments ($p < 0.05$, one-way ANOVA followed by Tukey's test). MDA: Malondialdehyde.

Table 6. Inhibitory activity of SLs against Human normal melanocytes.

Sample conc. (%)	Viability % (Mean ± SD)	Inhibitory % (Mean ± SD)
100	11.94 ± 0.28 ^e	88.06 ± 1.04 ^e
80	34.75 ± 0.93 ^d	65.25 ± 1.13 ^d
60	49.87 ± 0.09 ^c	50.13 ± 0.95 ^c
40	76.08 ± 0.41 ^b	23.92 ± 1.21 ^b
20	95.63 ± 0.27 ^a	4.37 ± 0.85 ^a
10	99.41 ± 0.17 ^a	0.59 ± 0.22 ^a
5	100 ± 0.00 ^a	0 ± 0.00 ^a
2.5	100 ± 0.00 ^a	0 ± 0.00 ^a
0 (Control)	100 ± 0.00 ^a	0 ± 0.00 ^a

The mean ± SD ($n = 3$) is used to express values. ND: not determined. Within each column, different superscript letters indicate statistically significant differences among compounds ($p < 0.05$, one-way ANOVA).

MIC value for all tested pathogens. Furthermore, a considerable amount of the ROS level was also detected at 10%, particularly against *S. aureus* and *S. typhimurium*, where the detected DCF counted more than 200. In contrast, the ROS induced by SLs at both concentrations (5 and 10%) was found to induce a lower ROS activity inside *A. baumannii*. Overall, results showed that the ROS release from SLs could increase the antibacterial efficiency. This finding may be related to the capability of this biosurfactant agent to improve the oxidative stress on drug-resistant pathogens.

3.8. Cytotoxicity of SLs on Human normal HFB-4 cell line

The safety profile of the diluted SLs was subsequently evaluated against human normal melanocytes cells HFB-4 using complementary approaches. The MTT assay was used to measure the targeted cells' quantitative vitality, and the resulting viability and inhibitory percentage, along with distinct IC_{50} values, are summarized in Table 6. In this regard, SLs at lower concentrations exhibited negligible toxicity even at 10%, where the cell remained in the viability state and the inhibitory effect did not exceed 1%. Likewise, the tested cells could be proliferated at full capacity, with only a very small degree of inhibition observed in the presence of SLs at 20%. The activity of SLs against HFB-4 showed moderate toxicity when the

concentration was used at 40%, with 23.92% of the cells being inhibited. Interestingly, SLs represented an IC_{50} value at 60%, which is still higher than three or fourfold that obtained by the MICs test against microbial pathogens (12%–27%). This finding significantly supported the safety profile of SLs, which emphasizes a robust therapeutic window and good tolerability at clinically relevant doses. In general, our findings confirmed the broadest safety index of SLs and considered it a promising candidate against drug-resistant pathogens.

4. CONCLUSION

This study encourages the sustainable production of SLs, minimizing waste accumulation and improving the manufacturing economics and environmentally friendly aspects. Therefore, this investigation proved the production of novel SLs economically with a yield of 23.12 g/100 g substrate utilizing the locally isolated and molecularly identified *Meyerozyma guilliermondii* cultivated on agro-industrial waste (jojoba oil cake). It was discovered that the produced SLs had an acceptable ST of 39 mN/m (millinewtons/meter) and a CMC level of 240 mg/l. Also, the structure characterization studies by FTIR, ¹H NMR, and LC-MS/MS demonstrated that the produced SLs consist of an acidic and lactonic mixture. The antimicrobial and antiadhesion activity of SLs against drug-resistant pathogens was successfully obtained compared to the antimicrobial agents. Interestingly, the inducible generation of ROS levels inside the microbial pathogens, along with lower toxicity toward normal human cells, makes SLs a more effective candidate against some drug-resistant pathogens. As far as we are aware, this is the first study demonstrating the role of SLs in inhibiting drug-resistant pathogens, providing a basis for the future development of medications based on SLs as natural and environmentally friendly alternatives to conventional antibiotics for combating infectious microbes.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

13. SUPPLEMENTARY MATERIAL

The supplementary material can be accessed at the Link here: [https://japsonline.com/admin/php/uploadss/4768_pdf.pdf].

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