Antidiabetic impact of novel sophorolipids produced via microbial conversion of *Moringa oleifera* and *Lepidium sativum* oil cakes utilizing locally isolated yeast strains in streptozotocin-induced diabetic wistar rats: role of glucagon-like peptide-1

Mohamed U. Nooman[a], Amr S. Al-Kashef[a], Abd El-Nasser Khattab[b], Kawkab A. Ahmed[c], Mona M. Rashad[a], Maha H. Mahmoud[d]

Departments of [a]Biochemistry, [b]Genetics and Cytology, Biotechnology Research Institute, National Research Centre, [c]Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt, [d]Department of Nutrition and Food Science, Food Industries and Nutrition Research Institute, National Research Centre, Cairo, Egypt

Correspondence to Amr S. Al-Kashef, PhD, Department of Biochemistry, Biotechnology Research Institute, National Research Centre, Cairo 12622, Egypt. Tel: +0106 235 7680; e-mail: amralkashef@gmail.com

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Background
One of the top ten causes of mortality all over the world is diabetes mellitus. Egypt in particular, has one of the highest prevalence of diabetes mellitus in the world
Objective
The purpose of this work is to produce sophorolipids (SLs) economically through microbial conversion of agro-industrial wastes namely *Moringa oleifera* (MO) and *Lepidium sativum* (LS) oil cakes using locally isolated *Saccharomyces cerevisiae* and *Yarrowia lipolytica*, respectively. Finally, the produced SLs will be investigated for their potential use as antihyperglycemic drugs in diabetic rats.
Patients and methods
The SLs were structurally characterized by fourier transform infrared spectroscopy, hydrogen-1 proton nuclear magnetic resonance, and LC-MS/MS. Then the hypoglycemic impact of the produced SLs was evaluated through an experiment on 30 male Wistar albino rats grouped into four diabetic groups induced by a single intraperitoneal dose of streptozotocin; a control positive group, a diabetic group + reference drug (metformin), a diabetic group + MO-SLs and a diabetic group + LS-SLs and the experiment lasted for 4 weeks.
Results and conclusion
The yield of MO-SLs was 18.6 g/100 g substrate. While LS-SLs had a higher yield (43.6 g/100 g substrate). The MO-SLs declined surface tension of water to 38 mN/m at 60 mg/L critical micelle concentration however, LS-SLs levels were 46 mN/m at 50 mg/L. The chemical characterization of MO-SLs indicated the presence of lactonic and acidic forms of SLs and the LS-SLs confirmed the presence of lactonic form only. Data obtained for the hypoglycemic effect revealed that both extracts have a positive impact on the studied biochemical parameters with the best results recorded for body weight change, fasting blood glucose, alanine transaminase, insulin, and glucagon-like peptide-1 for the diabetic group receiving LS-SLs followed by the reference drug, then the MO-SLs group. The results of biochemical markers were confirmed by the histopathological examinations showing the efficiency of the produced LS-SLs in decreasing the fasting blood glucose level.

Keywords:
diabetes mellite, glucagon-like peptide-1, Lepidium sativum, moringa oleifera, saccharomyces cerevisiae, sophorolipids, Yarrowia lipolytica

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Introduction

Diabetes mellitus (DM) is one of the world's top 10 leading causes of death, and the rise in prevalence has reached epidemic proportions posing a significant health challenge and economic burden. It is a chronic disorder in carbohydrate metabolism caused by either insulin insufficiency or reduced insulin sensitivity [1]. DM was diagnosed in over 10.5% of the adult population worldwide in 2021, and this figure is anticipated to climb to 12.2% (783.2 million) by 2045 [2].

Reports confirmed that Egypt ranks ninth in the world's highest prevalence rates of DM, according to the International Diabetes Federation (IDF), with 8 850 400 adult patients or 15.2% prevalence. Even though, these figures might seem high, between 40 and 50% of patients with DM or prediabetes remain undiagnosed. DM commonly affects most of the body organs leading to several complications such as retinopathy which may cause acute blindness, coronary syndrome or cardiomyopathy, and

nephropathy which may lead to chronic renal failure, neuropathy, lower limb amputation, and stroke [1,3]. Furthermore, a gradual increase in glycated hemoglobin (HbA1c) from around 6.3 to 8.0% for a 10-year follow-up was reported according to Turner *et al.* [4]. DM also constitutes a great economic burden as in terms of finances, Egypt's diabetes-related costs are $116/patient annually, which is less than the average yearly cost/patient in other Middle Eastern and North African countries ranging from $160 to $3000/patient annually [1].

Glucagon-like peptide-1 (GLP-1) is a regulator hormone for insulin secretion. It is a 30-amino acids peptide secreted by enteroendocrine L-cells in the intestine. It stimulates the secretion of glucose-dependent insulin from the pancreas postmeal. GLP-1 analogs were introduced recently as therapeutic regimens for type 2 DM such as exenatide, metformin, liraglutide, etc [5].

In general, type 2 DM is treated by one of the following mechanisms or through a combination of two mechanisms of antidiabetic agents, including sulfonylureas, DPP-4 inhibitors, glucose Cotransporter 2 (SGLT2) inhibitors, and metformin which is an anti-diabetic medication used orally that belongs to the biguanide class, it inhibits hepatic gluconeogenesis and opposes the action of glucagon to produce its predominant, glucose-lowering effect. It is specifically used to treat type 2 DM in individuals who are overweight or obese and have normal renal function [6]. Although metformin is the preferred anti-hyperglycemic drug for type 2 DM, yet, it may have a negative impact on renal function and increase the risk of chronic renal disease. Though uncommon, metformin accumulation can lead to the development of lactic acidosis which is a significant adverse effect [7,8]. As a result, researchers are focusing on substituting chemical drugs with natural products that lower blood glucose levels and have fewer adverse effects.

Microbial conversion can produce several valuable bioactive compounds, such as biosurfactants which include different compounds with varied chemical structures. Biosurfactants consist of diverse forms of fatty acids, phospholipids, neutral lipids, lipopeptides, polysaccharide-protein complexes, and glycolipids [9]. They can be synthesized by bacteria, yeast as well as fungi [10]. Sophorolipids (SLs) are a class of glycolipids type of biosurfactant that is comprised of fatty acid linked to sophorose sugar. They possess versatile functions such as emulsifiers, anti-microbial, anti-colitis, anti-cancer, and anti-hypercholesterolemic agents [11–15]. SLs also can substitute chemical surfactants due to their low toxicity, high biodegradability, and stability against drastic conditions that enable them to have many possible applications [16].

Numerous studies have shown that the lipopeptide biosurfactant produced by *Bacillus subtilis* may be a promising therapeutic option for DM and a means of delaying its subsequent complications. The efficacious modulation of the immune system by the biosurfactant reduces β-cell damage, hence lowering blood glucose levels [17]. However, SLs among bioactive compounds are generally expensive therefore, efforts were directed to reduce the production cost of these compounds by using agro-industrial wastes. Large amounts of food waste accumulate in the environment which results in environmental pollution and climate change. They are rich in many nutritive compounds like carbohydrates, protein, and lipids, as well as other bioactive constituents. Hence, they can be used in animal feed or utilized by microbial conversion for food and pharmaceutical industries [18–20].

Among all agricultural products, vegetable oil has the highest trading share (42%) with a global production of 672.0 million tons according to the world's oilseed production from the U.S. Department of Agriculture, [21]. The oil industry simply extracts oil from oily seeds and as a result, it generates large quantities of oilseed cakes. Thus, it is considered one of the most generated agro-industrial wastes worldwide [20]. *Moringa oleifera* (MO) and *Lepidium sativum* (LS) oil cakes are examples of oil seed industry wastes, whereas ~30% wt. of the residual oil remains in the seed cake [22]. They are generally used as animal feed due to their constituents of carbohydrate, protein, lipid, and antioxidant compounds in addition to minerals. These components enabled them to be used as suitable nutrients for the growth of microorganisms to obtain economic value-added compounds that have different applications [19].

Accordingly, this study aims to economically produce SLs via MO and LS oil cakes microbial conversion utilizing locally isolated *S. cerevisiae* and *Y. lipolytica* under solid-state fermentation, respectively. Then, define the chemical structure of MO and LS SLs and assess their surface characteristics. Furthermore, it aims to evaluate the potential antihyperglycemic properties of the produced SLs in diabetic rats induced by streptozotocin (STZ).

Patients and methods

Substrates and materials

For the production of SLs, oil cake of moringa (MO) was received from pressing and extracting natural oils unit, National Research Centre, Cairo, Egypt, while LS seeds were purchased from the local market, in Cairo, Egypt. On the other side, crude soybean oil used in the production process was obtained from the Institute of Food Technology Research (Soy Processing Centre), Agriculture Research Centre, Giza, Egypt. Regarding the yeast strains isolation, rice bran was obtained from rice milling factories, Egypt and jojoba oil cake was collected from the National Research Centre (Pressing and Extracting Natural Oils Unit), Egypt. Rice bran and LS oil cakes were prepared by pressing the rice bran and LS seeds with the hydraulic press (laboratory-type Carver) for 1 h, under 10.000 Ib in^{-2} pressure at 25°C [23], then the oil cakes were air-dried at 25°C for 24 h and milled by mechanical miller (DING CANG DC-500A) at 25 000 rpm and preserved in polyethylene bags at −20°C.

STZ that was injected to induce DM in rats was purchased from Sigma Co., Kits used for different evaluations were obtained as follows: ELISA kits for insulin and GLP-1 were purchased from Elabscience Co., China with cat. No. of E-EL-R3034 and E-EL-R3007 respectively. All kits used for spectrophotometric determinations were obtained from Salusa Co., Netherlands. Metformin was the reference drug (RD) that was obtained from the local market.

Isolation of yeast strains

Yarrowia lipolytica was isolated from rice bran which was previously identified at the molecular level at the accession number ON644535.1 in the GenBank [12]. The yeast strain isolation from jojoba oil cake was achieved as follows: 1 gm of the cake was added to 9 ml of sterile distilled water and then agitated to prepare serial dilutions with water (10^{-3}). From each dilution, 0.1 ml was spread by swab on Malt extract agar medium, containing 0.3% yeast extract, 1% glucose, 0.3% malt extract, and 0.5% peptone in 2% agar and incubated for 24 and 48 h. For the screening, on the cetyltrimethylammonium bromide (CTAB)-agar medium, the culture broth was then spread and adjusted to a pH of 5.5, and finally, the isolation of the robust colonies was carried out.

The molecular characterization of the isolated strain

Extraction of strain DNA

We chose the yeast strain W4-2 for further analysis. This strain was previously isolated from jojoba oil cakes following inoculation on Malt extract agar. Genomic DNA was then extracted from the purified W4-2 culture using the i-genomic BYF DNA Extraction Mini Kit, iNtRON Biotechnology Inc., South Korea, [24].

Partial amplification of PCR and internal transcribed spacer (ITS) sequencing

The molecular identification of yeast strain W4-2 was performed using internal transcribed spacer (ITS) region analysis of the 18 S ribosomal DNA (rDNA) gene. Specific primers flanking the ITS region were employed for PCR amplification [25]. The primers were obtained by Operon Technologies, Inc., Netherlands. To each polymerase chain reaction (PCR) bead, the used primer (12 nanogram) was added to a 40 nanogram purified DNA sample. Then, with sterile distilled water to 25 µl, the total volume of the amplification reaction was completed. the amplification protocol was as follows: 5 min of denaturation at 95°C (35 cycles with the following segments: primer was annealed for 2 min (55°C); the denaturation was carried out for 1 min at 95°C; and the polymerization of DNA was achieved by incubation for 2 min at 72°C. The amplified PCR product was then analyzed using gel electrophoresis. The gel was visualized and documented using a UV transilluminator and gel documentation system.

Identification and purification of isolated yeast strain DNA

Products of PCR were purified using a commercial DNA purification kit (Termo K0701). The purified DNA was then sequenced using a Sanger sequencing method with a forward primer on an ABI sequencer (3730xl DNA, GATC Company, Germany).

Phylogenetic analysis

The DNA sequences of the yeast isolates were analyzed using basic local alignment search tool (BLAST) against the National Center for Biotechnology Information's (NCBI) GenBank database to identify closely related sequences. The sequences were then aligned and a phylogenetic tree was constructed using the Jukes-Cantor model [26] and the neighbor-joining method [27].

Inoculum preparation

A stock culture of *Saccharomyces cerevisiae* and *Yarrowia lipolytica* strains, the two isolated and identified yeasts were inoculated in a sterile inoculum medium (50 ml) according to Wickerham, [28], which was then incubated in a thermo scientific incubated/refrigerated orbital shaker (USA) at 28°C and 160 rpm for 24 h.

Culture medium and cultivation conditions

MO and LS oil cakes culture media were prepared using solid-state fermentation as follows: two ml inoculum of *S. cerevisiae* and *Y. lipolytica* cultures, respectively were transferred to a serialized medium consisting of 5 g MO oil cake, 5 g soybean crude oil with 4 ml solution of the following nutrients: 1.0 gl^{-1} peptone; 0.5 gl^{-1} $MgSO_4.7H_2O$; 1.0 gl^{-1} NH_4NO_3; 2.55 gl^{-1} K_2HPO_4; 0.1 gl^{-1} $CaCl_2.2H_2O$; 0.15 gl^{-1} NaH_2PO_4; 0.02 gl^{-1} $MnSO_4.H_2O$; at final pH 8 then incubated at 30°C for 8 days in a static condition shaker [15].

Extraction of sophorolipids

SLs from both fermented media were extracted according to the procedures modified by Nooman *et al.*, [12]. Methyl alcohol (100 ml) was initially mixed with the fermented culture then shaken at 180 rpm and 40°C for 60 min in a shaking incubator. Later, the mixture was filleted with Whatman filter paper (No. 40). The residual methyl alcohol was then removed via a vacuum cooling controller (heidolph G1), Germany rotary evaporator. For partial purification, both extracts were then exposed to 50 ml of chloroform to discard the residual oil. Further, drying proceeded at 40°C to obtain the MO and LS SLs extracts.

Physicochemical properties of the produced *Moringa oleifera* and *Lepidium sativum* sophorolipids

The surface tension (ST) and critical micelle concentration (CMC) assessment

The surface tension (ST) and critical micelle concentration (CMC) were determined by the tensiometer (KrÜss Processor-K100), Germany, whereas serial concentrations of both produced MO and LS SLs were prepared and measured by the tensiometer for each concentration. The breaking point in the surface tension against the SL compound concentrations was calculated to obtain the CMC of both extracts and the means of three replicates were recorded [29].

The Fourier-transform infrared spectroscopy measurements

Attenuated total reflection fourier transform infrared spectroscopy (Germany) evaluation was implemented for the produced MO and LS SLs using the Bruker VERTEX 80 with ATR platinum diamond, utilizing a range of 4000–400 cm^{-1} diamond disc reflector and a refractive index of 2.4 at a 4 cm^{-1} resolution.

Hydrogen-1 Proton nuclear magnetic resonance spectra analysis

The nuclear magnetic resonance spectra of the produced MO and LS SLs were estimated by a spectrometer (Varian Mercury VX-300 NMR). In $CDCl_3$, ^1H spectra were connected at 300 MHz. The chemical shifts are quoted in and related to solvent bands for both MO and LS SLs.

Liquid chromatography–mass spectrometry

The produced MO and LS SLs were analyzed utilizing liquid chromatography-electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) with an Exion LC AC system for separation and SCIEX Triple Quad 5500+ MS/MS system equipped with electrospray ionization (ESI) for detection. While the separation was performed by an Ascentis C18 column. Two eluents were constituted in the mobile phases as follows: A eluent was 0.1% formic acid while B eluent was: 0.1% formic acid in acetonitrile. The mobile phase program, however, adjusted. In the MS/MS analysis, (0.7 ml/min) flow rate with 10 µl of volume injection. The positive ionization mode was carried out by an EMS-IDA-EPI scan ranging from 100 to 1000 Da for MS1 for both SL extracts. Identification of compounds was conducted by the software MS-DIAL (version 4.70) and the library of the Fiehn HILIC.

Biological evaluation for the produced *Moringa oleifera* and *Lepidium sativum* sophorolipids

Ethics statement

Mice and rats that were used for the present study were purchased from the Central Animal House of the National Research Centre. The experiments on animals were carried out under the guidelines and the approval of the Medical Research Committee of Ethics of the NRC (no. 20-111).

Acute toxicity test

The safety of the produced extracts was evaluated whereas, the extracts were examined for LD50 according to the following procedures: a set of two series each comprising 36 Swiss male albino mice was included for each extract. Six groups were included in each series with six mice in each group. Then, from MO and LS extracts, progressively increasing oral doses of 1, 2, 4, 6, 8, and 10 g/kg body weight were given to the groups. The animals were then observed for 24 h, and the mortality counts were recorded [30].

Induction of diabetes

The hypoglycemic impact of each extract was evaluated by inducing a model of a diabetic rat using STZ as follows: the STZ was freshly prepared immediately before injection by dissolving in citrate buffer (0.1 mol/l) with a final pH of 4.5. Hyperglycemia was induced in a group of 30 male

Wistar albino rats by a single intraperitoneal (IP) administration of the prepared STZ as 50 mg STZ/kg body weight according to Mahmoud *et al.*, [31]. Then, a solution of 10% glucose was given to the injected animals just after the STZ injection and two days after injection to avoid the death of injected animals due to hypoglycemia which is the consequent result of the destruction of beta cells of Langerhans of pancreas by STZ and the release of their content of insulin into the circulation. DM was confirmed by the determination of fasting blood glucose concentrations. The animals with a blood glucose of 200 mg/dl or higher were considered diabetic animals, while others were excluded.

The animal experiment was then started including four groups of diabetic rats and one group of normal rats to serve as a control negative group. The groups were as follows:

- Group 1: Control –ve group that was given the basal diet and received a daily oral injection of saline.
- Group 2: Control +ve group including diabetic rats which received an oral daily injection of saline.
- Group 3: RD group which was a group of diabetic rats receiving 100 mg/kg body weight of metformin (as a RD) daily.
- Group 4: The MO group that was a diabetic group receiving the MO-SLs as an oral dose daily as 200 mg/kg body weight [13].
- Group 5: The LS group which was a diabetic group receiving LS-SLs as an oral dose daily as 200 mg/kg body weight [13].

The experiment was terminated after 4 weeks during which the change in body weight and blood glucose were followed. The fasting blood samples were obtained under slight anesthesia by sodium pentobarbital IP. At the end of the experiment, after the animals' sacrifice, the organs namely the pancreas, liver, kidney, and heart were separated from the animals, washed with saline carefully, and finally, they were immersed in 10% formalin solution for further histopathological analysis. The following biochemical analyses were carried out in the obtained serum: fasting blood glucose (FBG) by glucometer, urea, creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) that were obtained from Salusa Co., Netherlands, while blood hemoglobin was determined in whole blood by a kit obtained from Biodiagnostic Co., Egypt. Also, serum insulin and GLP-1 were determined by ELISA technique using ELISA kits with cats. No. of E-EL-R3034 and E-EL-R3007, respectively, were purchased from Elabscience Co., China. All biochemical analysis was done according to the instructions of the manufacturers as well as histopathological examination for the pancreas, heart, kidney and liver.

Histopathological examination

The specimens of the liver, pancreas, kidneys, and heart were obtained from all animals, fixed in 10% formalin, washed, dried, cleared, and then merged in paraffin. The blocks of paraffin were cut into 4–5 μm thickness for the microscopic examination (Olympus BX50, Tokyo, Japan). Then, the sections were stained with hematoxylin and eosin (H and E) according to Bancroft and Gamble [32]. Histopathological changes were graded as (0) indicating no changes, (1), (2), and (3) indicating mild, moderate, and severe changes, respectively [33].

Statistical analysis

The computer application SPSS version '20' was used to statistically evaluate the results. After doing the one-way ANOVA test, the Duncan *post hoc* test was conducted. Also, the GraphPad Prism statistical program, version '6.0' was used (GraphPad software, Inc., San Diego, CA, USA). The results were displayed as mean ± SE. The significance level was considered at '*P*' value less than 0.05, otherwise were considered not significant.

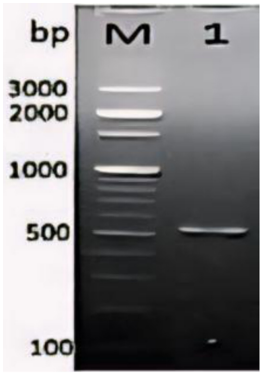
Results and discussion

The isolation and evaluation of the selected yeasts

In this study, yeast strains used in the production of SLs were isolated from jojoba oil cake to ensure their ability to grow in harmony with other oil processing residues, confirm their robust growth to achieve economical production, and recycle these environmentally harmful wastes.

The yeast colonies that have been grown on jojoba oil cake were examined for the production of SLs, where the robust one was selected and coded as W4-2. For the molecular level identification of the isolated strains, the DNA of the selected one was isolated. The primers were utilized for amplifying the rDNA area repeat unit that included the ITS from the yeast strain genomic DNA and estimating its concentration. The data in Fig. 1 revealed the amplification of around 550 bp. Followed by DNA primer sequencing related to the purified PCR. Finally, a comparison was made between the obtained DNA sequence and the known yeast strains (Fig. 2) that were implemented and kept in the database of the GenBank under the accession number: Seq. [organism =*Saccharomyces cerevisiae*]

Figure 1



ITS-DNA amplified band photograph of yeast strain (W4-2) isolated from jojoba oil cake (lane 1) using internal transcribed spacer (ITS) primers in comparison with 100 bp ladder DNA marker (lane M).

W4-2, ITS1, partial sequence; 5.8 S ribosomal RNA gene, and ITS2, complete sequence; and 28 S ribosomal RNA gene, partial sequence (GenBank accession number ON644537.1). However, the selected robust yeast colony that was grown on *Lepidium sativum* cake (coded as W2) has been identified previously as *Yarrowia lipolytica* under the GenBank accession number ON644535.1 [12].

Production of sophorolipids from *Moringa oleifera* and *Lepidium sativum* oil cakes

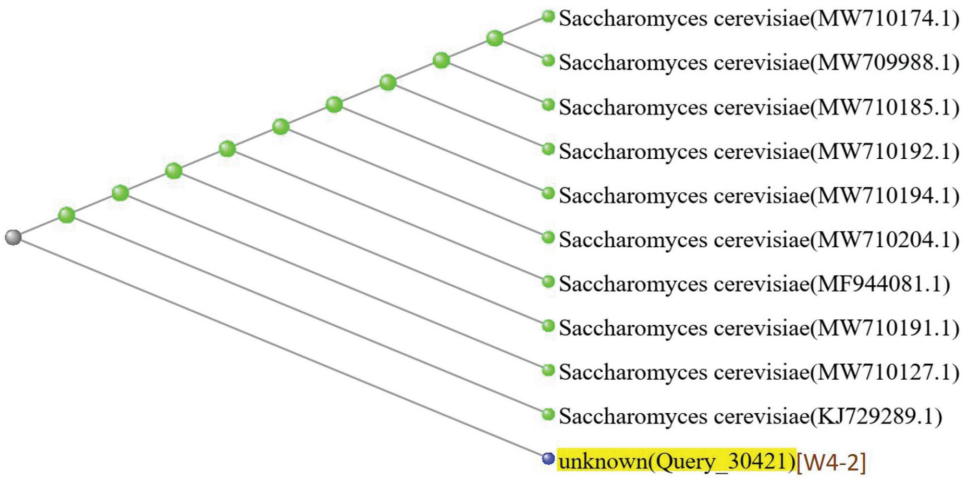
The isolated strains (*S. cerevisiae* and *Y. lipolytica*) were cultivated on oil residues polluting the environment, namely MO and LS oil cakes, respectively. The cultivation of *S. cerevisiae* on MO residue actually, produced 18.6 g/100 g substrate, while *Y. lipolytica* produced 43.6 g/100 g substrate when cultivated on LS residue. Previously, many reports discussed the production of SLs by different yeast strains cultivated on different substrates, Al-kashef *et al.*, [11] stated that *Candida parapsilosis* grown on a mixed substrate containing potato peels and frying oil wastes produced SLs with a yield of 39 g/100 g substrate, while Nooman *et al.*, [12] produced 32.1 g/100 g substrate of SLs by the yeast strain of *Y. lipolytica* cultivated on MO oil cake. A higher yield of SLs was obtained (49.04 g/100 g substrate) from *S. cerevisiae* cultivated on banana peels extracted by both methanol and ethyl acetate in two successive steps [34]. On the other hand, a lower level (20 g/100 g substrate) of SLs was produced by growing *Starmerella bombicola on* winterization oil filtration cake and sugar beet molasses [35]. Earlier, Rashad *et al.*, [15] reported a yield of 49.5 g/100 g substrate by utilizing sunflower oil cake fermented by *Candida bombicola* after applying a new approach for extraction as methanol was used at the first, followed by ethyl acetate extraction from the same media.

Functional characterization of produced sophorolipids

The surface tension and critical micelle concentration measurements

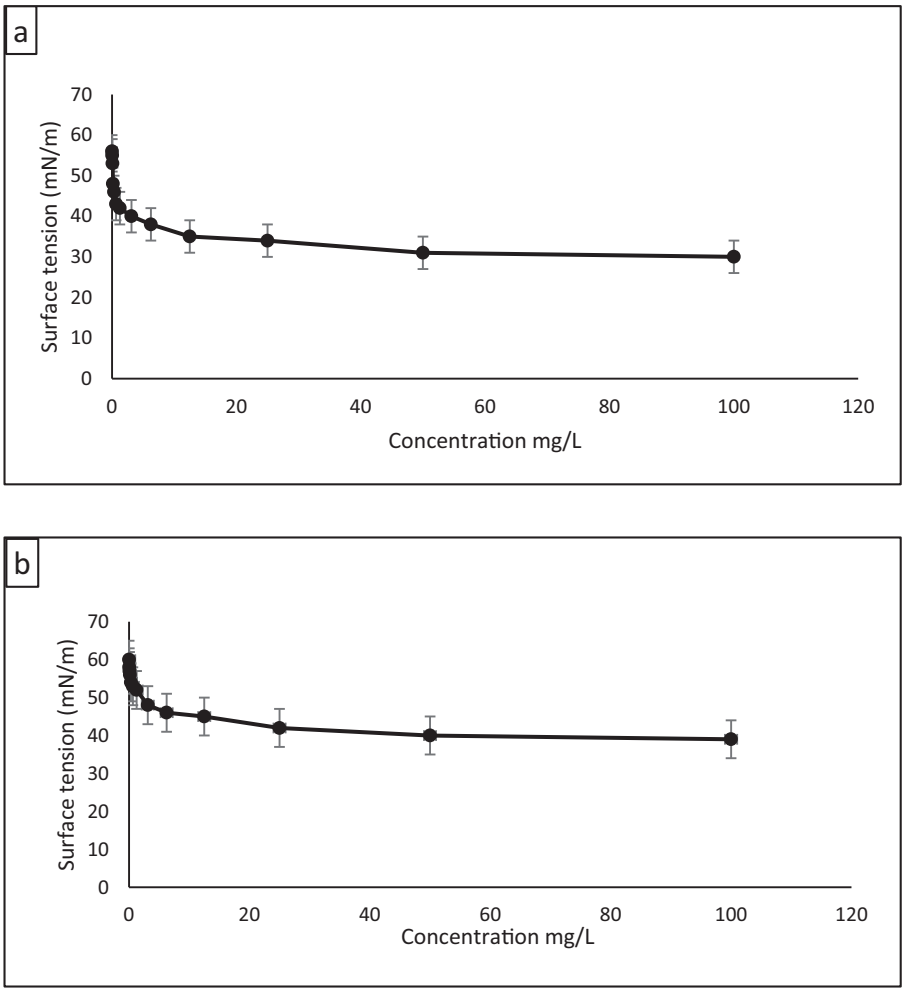
The CMC is the point of sophorolipid concentration at which micelles start to aggregate [11]. The SLs from *S. cerevisiae* cultivated on MO showed the ability to decrease the ST of water to 38 millinewton per meter (mN/m) at a CMC point of 60 mg/l, while the *Y. lipolytica* grown on LS decreased the ST to 46 mN/m with CMC of 50 mg/l (Fig. 3a and b). Generally, varied levels were reported concerning the reduction of ST caused by SLs ranging from 32.5 to 48 mN/m, with different CMC values (62.5 mg/l – 5 g/l),

Figure 2



The phylogenetic position of the isolated yeast strain W4-2, obtained from jojoba oil cake, was determined using the neighbor-joining method based on its 16 S ribosomal DNA (rDNA) sequence and sequences of closely related species retrieved from a database.

Figure 3



Critical micelle concentration and surface tension of the produced *Moringa oleifera* sophorolipids (a) and *Lepidium sativum* sophorolipids (b).

this in fact, due to the diversity of utilized yeasts and substrates [10–12,15,36,37].

Characterization of the produced *Moringa oleifera* and *Lepidium sativum* sophorolipids structures

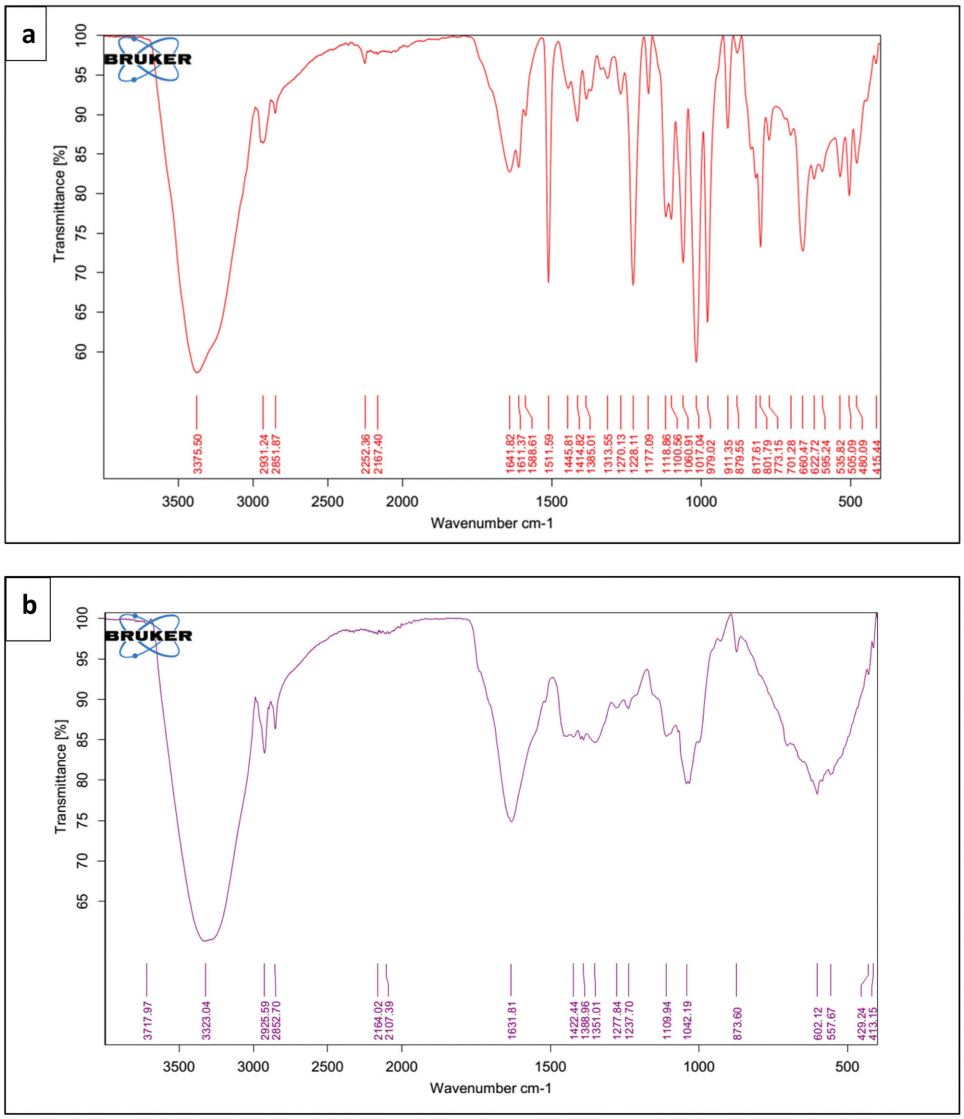
FTIR analysis of the SLs produced from *S. cerevisiae* cultivated on MO (Fig. 4a) showed absorption at 2931.24 cm^{-1} and 2851.87 cm^{-1} confirming the presence of methylene group asymmetrical stretching (νas CH_2), in addition to absorption occurred at 1641.82 cm^{-1} which related to lactone C=O group [36]. However, the CO group of sugar stretch of COH groups appeared at 1049 cm^{-1} according to Sen *et al.*, [10], the results also, observed the existence of bands at 3375.5 cm^{-1} and 1445.81 cm^{-1} that matched the stretching of O-H and C-O-H related to the carboxylic acid. The data analysis of the produced MO-SLs confirmed that the mixture contains both acidic and lactonic structures with the majority of the acidic SLs [36,38].

Furthermore, produced LS-SLs were also analyzed by FTIR (Fig. 4b), and the results indicated the presence of H-O-H vibrational bonding at the peak 3323.04 cm^{-1} and a band at 3717.97 cm^{-1} related to the OH stretching group [39]. However, the absorption at 2925.6 cm^{-1} complies with the asymmetrical stretching (ν_{as} CH_2) of the methylene [36]. The band at 1631 cm^{-1} represents the C&9552;C group while the acetyl esters lactones stretch of the C-O band of C(=O)-O-C from sophorose moiety, appeared from 1277.8 to 1237.7 cm^{-1} and 1042.19 cm^{-1} [10]. Moreover, the band at 1422.44 cm^{-1} related to O-H and C-O-H carboxylic acid stretching. In conclusion, the data obtained from the FTIR analysis confirmed that the produced LS-SLs are a lactonic type of biosurfactants [36,38].

Hydrogen-1 proton nuclear magnetic resonance analysis

SLs produced from *S. cerevisiae* cultivated on MO were analyzed by Hydrogen-1 proton nuclear magnetic

Figure 4



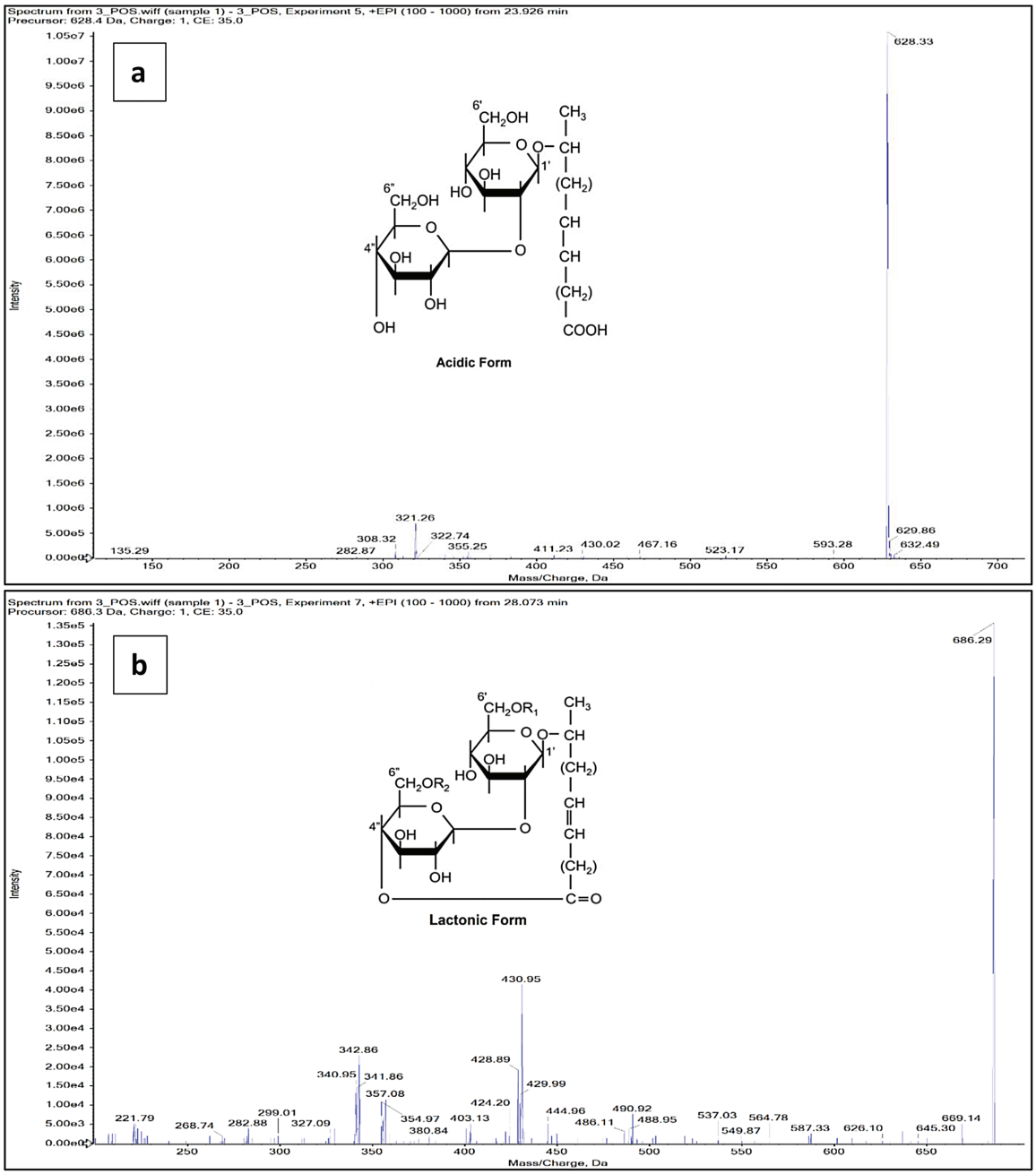
FTIR spectra analysis of the produced sophorolipids by microbial conversion of *Moringa oleifera* oil cake (a) and *Lepidium sativum* oil cake (b).

resonance (^{1}H NMR) spectrum where the signals from 5.31 and 5.34 ppm, demonstrated the presence of a vinyl group. However, the -CH_2-COO- group was confirmed by the signal at 2.28 ppm. Moreover, the fatty acid was represented by signals that were observed at 1.23 to 1.27 ppm. The signals at 2.02 ppm prove the existence of the -CO-CH_3 group in the structure. However, methylene of lactone was revealed at 2.19 ppm, while the protons of glucose appeared at 4.11–4.14 ppm [15,37,38]. Furthermore, ^{1}H NMR analysis of the SLs produced from *Y. lipolytica* cultivated on LS was carried out, showing a signal at 2.16 ppm of the lactone methylene group and protons of glucose resonated at 4.15 ppm. A signal (2.30 ppm) was also detected confirming the existence of the -CH_2-COO- group, in addition to the signals resonated between 1.24 and 1.29 ppm corresponding to fatty acids. Finally, signals from 5.33 to 5.38 ppm, indicated the existence of a vinyl group [13,38].

The produced sophorolipids analysis by liquid chromatography-mass spectroscopy

Moringa oleifera SLs spectrum produced from *S. cerevisiae* was accomplished using LC-ESI-MS/MS (Fig. 5a and b). The peaks observed at m/z 628.4 and m/z 686.29 indicated the existence of acidic and lactonic SLs at the retention time of 23.92 and 28.07 min, respectively [12,36,40]. The peaks at m/z 299.01 in the lactonic form and 282.8 m/z in both forms correspond to fatty acid fragments C18:3 and C18:0, respectively [36,40]. Furthermore, sophorose moiety appeared in the lactonic SLs at the same retention time (28.07 min) at m/z 403.13, while sophorose fragment from the acidic SLs appeared at

Figure 5



Liquid chromatography–mass spectroscopy characterization of the sophorolipids produced by *Saccharomyces cerevisiae* grown on *Moringa oleifera* oil cake acidic form (a) and lactonic form (b) and their fragments.

m/z 4011.23 [12]. The peak at m/z 537.03 emphasized the existence of sophorose plus C15:3 in the lactonic form and finally peaks at m/z 355.25 and 321.74 were evidence for the hydroxy fatty acids fragments from the acidic SLs [12,36,40].

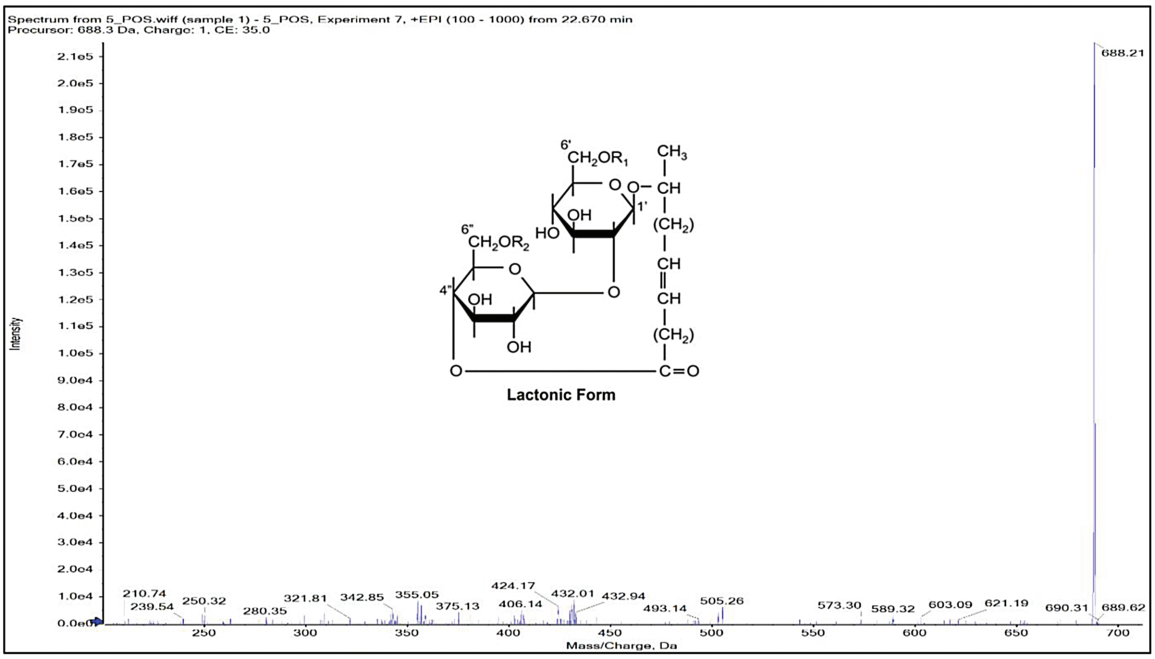
As for the LC-MS/MS spectroscopy analysis conducted on the generated SLs from *Y. lipolytica* cultivated on LS (Fig. 6), the data revealed the presence of the lactonic type of SLs at m/z 688.26 at 24.8 min retention time. Peaks at m/z 587.25 and 531.24 were evidence for the presence of C18:2 and C15:3 fatty acids, respectively. However, peaks at m/z 281.96 and 269.06 corresponded to the hydroxy fatty acids. Moreover, disaccharide moiety (sophorose) appeared at m/z 410.2 [36,40].

Biological evaluation of the produced sophorolipids

Acute toxicity test

The obtained MO and LS sophorolipid compounds were found to be safe since they had no toxicity as evidenced by the LD50 test. No mortality rate was recorded for all the evaluated doses up to 10 g/kg BW of the mice. Hence, the above-mentioned two extracts

Figure 6



Liquid chromatography–mass spectroscopy characterization of the lactonic form of sophorolipids produced by *Yarrowia lipolytica* cultivated on *Lepidium sativum* oil cake and its fragments.

are safe and can be tested for their health promotional benefits.

Effect of produced sophorolipids from *Moringa oleifera* and *Lepidium sativum* on streptozotocin-induced diabetic rats

A very marked and significant reduction in body weight as seen in Table 1 was recorded for the control positive diabetic group to the extent that the body weight change for this group was −17.00±5.84 g meaning that the final body weight is less than the initial one by 17 g comparing to the group of the control negative that recorded an increase in body weight as 53.50±4.49 g. This result seems to be in accordance with previous studies [3,31]. This can be attributed to the hypoinsulinemia that will be discussed later in this study which leads to a lack of utilization of the ingested carbohydrates. The obtained results for fasting blood glucose (Table 1) reinforce this explanation since a state of hyperglycemia was noticed for the control positive group, recording 377.20±30.57 mg/dl compared to the control negative with a value of 87.83±5.96 mg/dl meaning that although the circulating blood glucose is very high yet, it can not be transported into the cells as a result of insufficiency of the secreted insulin. Similar results were stated earlier by Vasiljević *et al.*, [41]. Anyway, an improvement was noticed for the three diabetic groups with intervention for both body weight change and fasting blood glucose levels with the best result being for the diabetic group that received a daily oral dose of LS-SLs. The potency of the LS-SLs may be attributed to the content of SLs with their antioxidant effect [12].

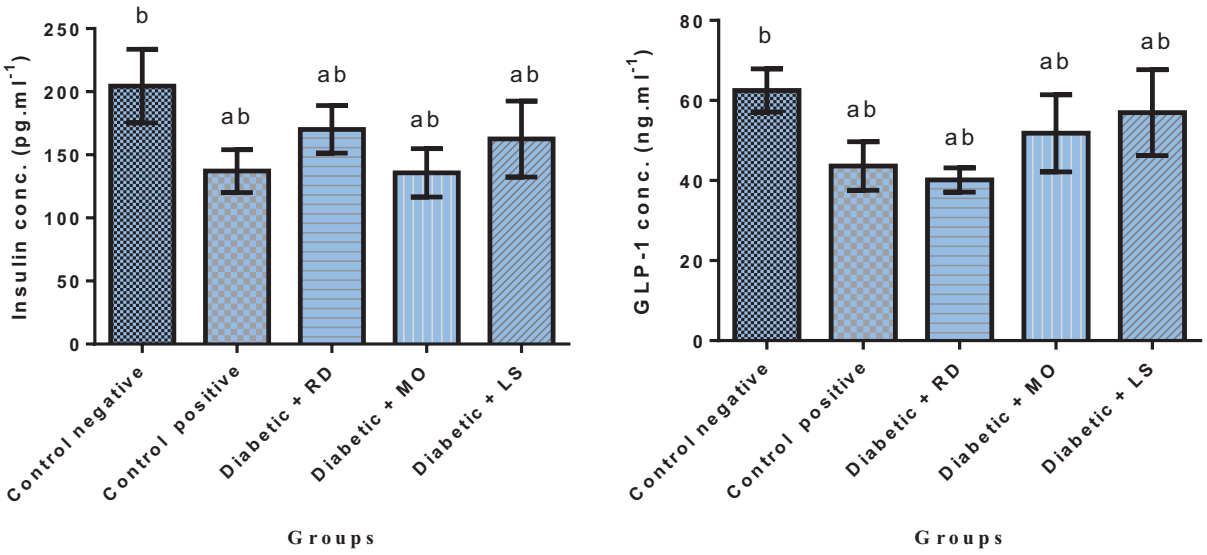
Table 1 Body weight change, and fasting blood glucose for the control and the different treated groups

Groups	FBG (mg/dl)	B. wt. change (g)
Control –ve	87.83±5.96 [a]	53.50±4.49 [c]
Control +ve	377.20±30.57 [c]	−17.00±5.84 [a]
Diabetic + RD	296.20±41.62[b c]	−3.88±8.16 [a b]
Diabetic + Mo-SLs	330.83±17.53[b c]	−9.25±5.33 [a b]
Diabetic + Ls-SLs	284.60±29.09[b]	7.38±10.54 [b]

Diabetic + Ls-SLs, diabetic rats receiving SL from *Lepidium sativum*. Data are illustrated as mean±SE and values that have different letters are considered significant while those sharing the same letters are nonsignificant; Diabetic + MO-SLs, diabetic rats receiving SL from *Moringa oleifera*; Diabetic + RD, diabetic rats receiving reference drug.

A state of hypoinsulinemia was observed in the diabetic control positive group evidenced by a marked reduction of serum insulin for this group comparing to the group of the control negative one (Fig. 7). This decrease was more or less restored to some extent in the diabetic groups that received either the RD or the LS-SLs confirming that the latter is as powerful as the RD, while those diabetic rats that received MO-SLs did not show any improvement in serum insulin level comparing to the group of control positively. In this respect, Koksal, [42] mentioned in his meta-analysis study that plasma insulin levels showed a reduction in

Figure 7



Insulin concentration and Glucagon-like peptide-1 (GLP-1) concentration in all studied groups. RD: reference drug, MO: *Moringa oleifera,* LS: *Lepidium sativum.* Bars that have different letters are significant, while bars sharing similar letters are nonsignificant.

the case of the diabetic groups that were injected with STZ comparing to the group of control negative in all the studies in his meta-analysis survey which is in the same line with this study. Also, El-Shobaki *et al.,* [3] reported a reduction in the insulin level in the diabetic group that was injected with STZ, while the diabetic group that received the formula which was a mixture of edible plants showed a restoring insulin level. The serum GLP-1 also showed a reduction in the group of control positive comparing to the group of control negative (Fig. 7). This reduction didn't show any change in the diabetic group that was given the RD, while it recorded an improvement in the two diabetic groups that received the extracts of either MO or LS SLs to the extent that the latter became more or less near the control negative value. It is worth mentioning that GLP-1 is considered as a regulator hormone for the secretion of insulin since GLP-1 acts to stimulate the secretion of glucose-dependent insulin from beta-cells of the pancreas. GLP-1 is secreted in the intestine after ingestion of a meal. GLP-1 analogues are used recently as a therapy for type 2 DM, since it leads to improvement in the function of β-cells of the pancreas [5]. Thus, it can be said that both extracts; MO and LS have a positive impact on GLP-1 secretion and, hence can be used as hypoglycemic therapies.

A significant decline was noticed in hemoglobin concentration for the group of control positive comparing to the group of control negative. This may be attributed to the toxic effect of STZ on hepatocytes which synthesize the globin required for hemoglobin formation [43]. This decline was restored in both diabetic groups that received either the RD or the MO-SLs, but the diabetic group that received the LS-SLs did not record any improvement in hemoglobin concentration. This result may be explained based on the potent antioxidant properties of both MO and RD that were able to counteract the increased oxidative stress on hepatocytes resulting from the toxic action of STZ, while the relatively lower antioxidant potency of LS was not able to counteract the increased oxidative stress from STZ injection. Regarding liver function, a significant increase in the activity of alanine aminotransferase was found in the control positive group reaching more than two folds of rats of the control negative group. This elevation was fully normalized in the two diabetic groups that received either the MO-SLs or the LS-SLs where their values became non-significantly changed from the group of negative control, however, the diabetic group that received the RD showed no significant change compared to the group of positive control. Whereas the activity of the aspartate aminotransferase did not show any significant change among different groups. Hyperglycemia was known to generate a state of elevated oxidative stress all over the body, MO and LS extracts contain SLs that are well-known for their potent antioxidant activity [12,34]. The increased oxidative stress affects the hepatic cells rendering their cell membrane fragile because of the peroxidation of the cell membrane fatty acids that leads to the leaking of the hepatic enzymes into the circulation [44]. The SLs produced from either MO or LS with their antioxidant activity protect cell hepatic membranes

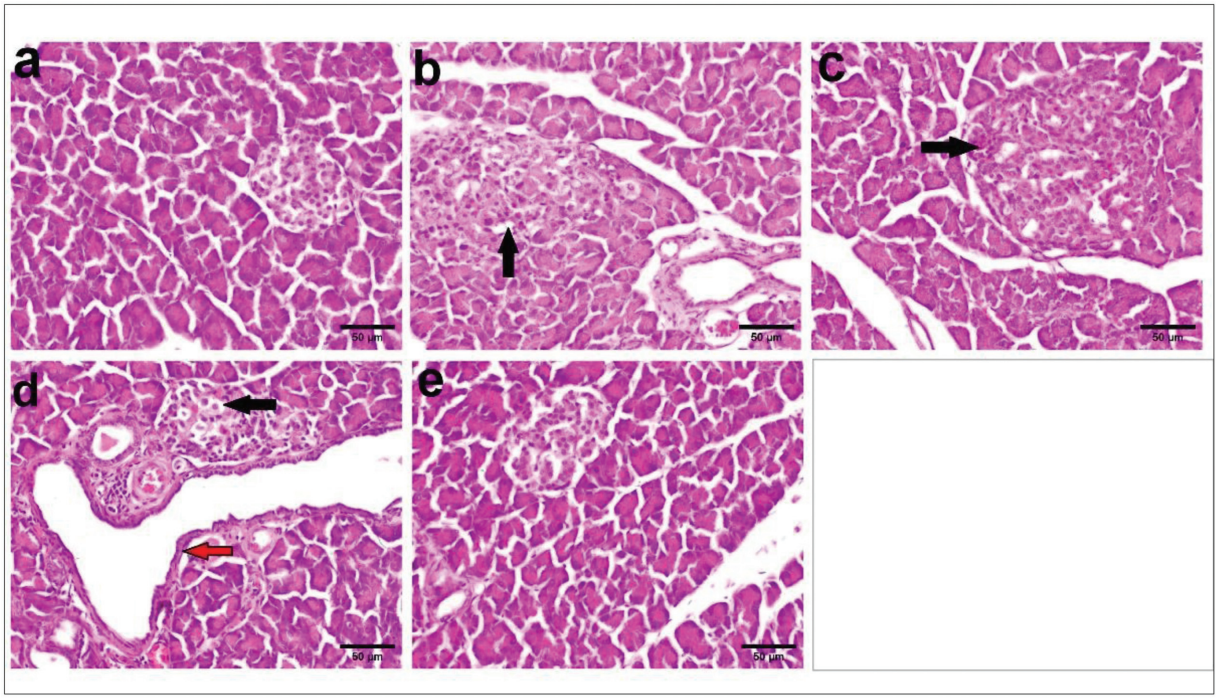
Table 2 Concentration of hemoglobin, activity of alanine aminotransferase and aspartate aminotransferase and concentration of urea and creatinine

Groups	Hb (g/dl)	ALT (U/l)	AST (U/l)	Urea (mg/dl)	Creatinine (mg/dl)
Control –ve	13.80±0.20^{bc}	25.67±4.33^{a}	63.63±8.51^{a}	40.48±2.43 ^{ab}	0.68±0.04 ^{a}
Control +ve	12.35±0.28^{a}	57.67±6.19^{b}	65.20±10.73^{a}	64.06±3.94 ^{c}	0.84±0.20 ^{a}
Diabetic + RD	13.48±0.28^{b}	47.67±8.16^{b}	69.00±10.40^{a}	58.82±4.62 ^{c}	0.84±0.09 ^{a}
Diabetic+ MO-SLs	14.30±0.30^{c}	21.40±5.50^{a}	48.33±7.14^{a}	34.08±9.18 ^{a}	0.70±0.09 ^{a}
Diabetic+ LS-SLs	12.35±0.25^{a}	27.16±4.78^{a}	64.80±8.74^{a}	53.56±5.70 $^{b\ c}$	0.71±0.08 ^{a}

Diabetic + LS, diabetic rats receiving SL from ***Lepidium sativum***. Data are represented as mean±SE and values that share different letters are significant while those sharing the same letters are nonsignificant; Diabetic + M, diabetic rats receiving SL from moringa; Diabetic + RD, diabetic rats receiving reference drug.

from peroxidation and hence, prevent the leakage of enzymes into the circulation. It is worth mentioning that the results obtained for both produced SLs are even better than the result obtained for the RD indicating that these two extracts exert an ameliorative effect on the hepatic tissue of the diabetic rats more efficiently than the RD. These outcomes in fact are in the same line with the findings of Abdel-Latif *et al.*, [34], who reported that SLs produced by *S. cerevisiae* grown on banana peels exhibited hepatoprotective properties against rats' doxorubicin-induced hepatotoxicity. DM in fact can cause some complications if it is not treated well or ignored, for example, there are several different types of chronic kidney disease associated with diabetes, such as hypertensive nephrosclerosis, diabetic nephropathy, and ischemic nephropathy linked to vascular disease [45]. However, no significant change was noticed for serum creatinine among the different groups, while there was a significant rise in urea concentration for the group of control positive which was improved in the diabetic three groups that received the intervention with the best result corresponding to the MO-SLs. Earlier, Bhat *et al.*, [5] stated that biosurfactants reduce blood glucose levels by modifying the immune system, and minimizing beta cell damage, and this may decrease the effect of diabetes on the kidneys. Also, the increased oxidative stress of the hyperglycemia in this study affects the nephrotic tissue which leads to altering its function and hence increased serum urea concentration significantly, but this increased oxidative stress was more or less overcome by the antioxidant

Figure 8



Sections of pancreatic tissue photomicrographs stained with H and E of rats with a scale bar of 50 µm: a) Control –ve group; showing the normal histological architecture. b) Control +ve group; showing the formation of vacuoles in islets of Langerhans's cells (arrow). c) Diabetic group + RD; showing congestion of islets blood capillaries (arrow). d) Diabetic group + MO-LSs; showing the formation of vacuoles in islets of Langerhans's cells (black arrow) and pancreatic duct dilatation (red arrow). e) Diabetic group + LS-SLs; showing no histopathological lesions.

potency of the SLs in the two extracts. These results may be explained based on the anti-inflammatory to the antioxidant properties of SLs [12], Table 2.

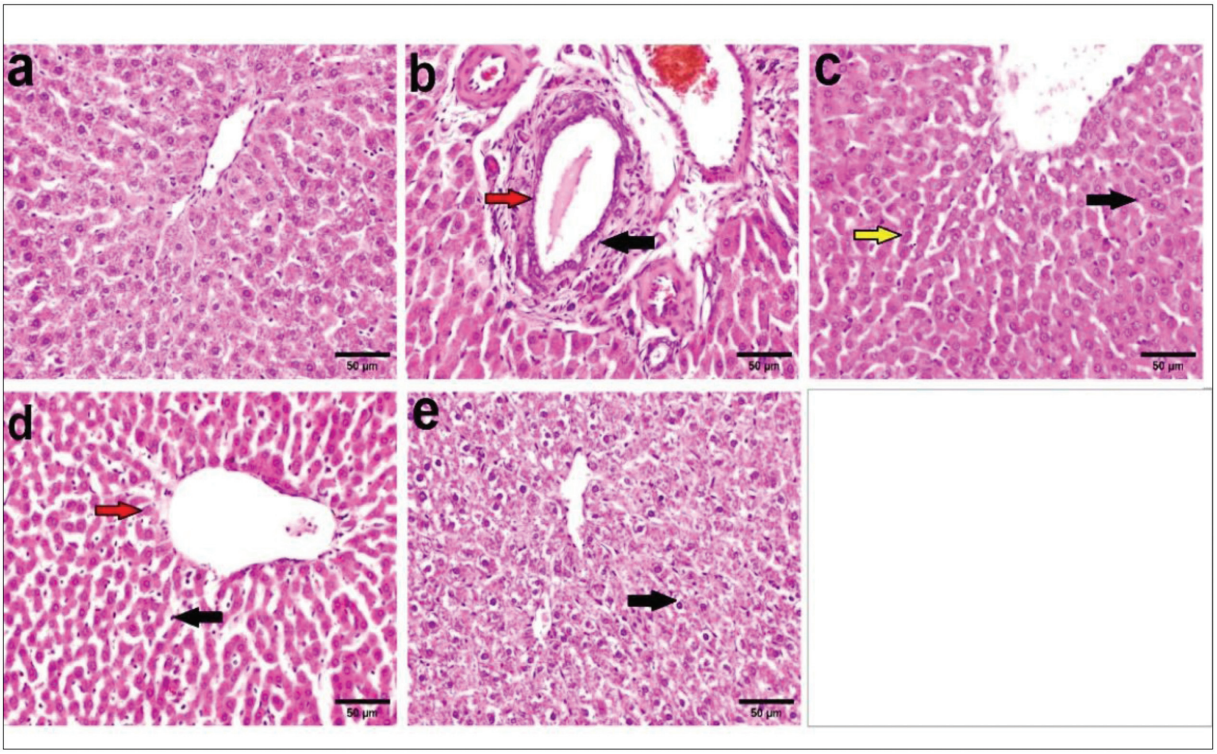
Histopathology

The findings of histopathological examinations of various organs (Figs 8–11), including the pancreas, liver, kidney, and heart agreed with and even reinforced the results of biochemical markers. Microscopic examination of the pancreas (Fig. 8) showed an improvement in groups treated with both extracts, but recovery was more obvious in the RD group followed by the LS-SLs group and then the MO-SLs group (Fig. 8). Giri [46] revealed that the SL has increased the number and area of islets in the pancreas of treated rats, improved β-cell morphology, and decreased apoptosis. It also, reduced inflammatory markers in pancreatic tissue. Over all enhancement of pancreatic structure and function. According to Adewole *et al.*, [47], using yeast quercetin as a biosurfactant has a positive effect on pancreatic tissues exposed to STZ-induced oxidative stress by directly decreasing lipid peroxides and indirectly increasing the production of endogenous antioxidants.

Moreover, microscopic examination of the liver tissue (Fig. 9) also showed a positive effect of the two extracts in the process of regenerating damaged hepatic cells as a result of diabetes, but the improvement in the group that was treated with LS-SLs was better than the diabetic group that received MO-SLs treatment. These results were in the same line as the obtained biochemical results for the liver enzymes of both extracts. This effect may be due to the antioxidants and anti-inflammatory nature of the SLs compounds [12,34].

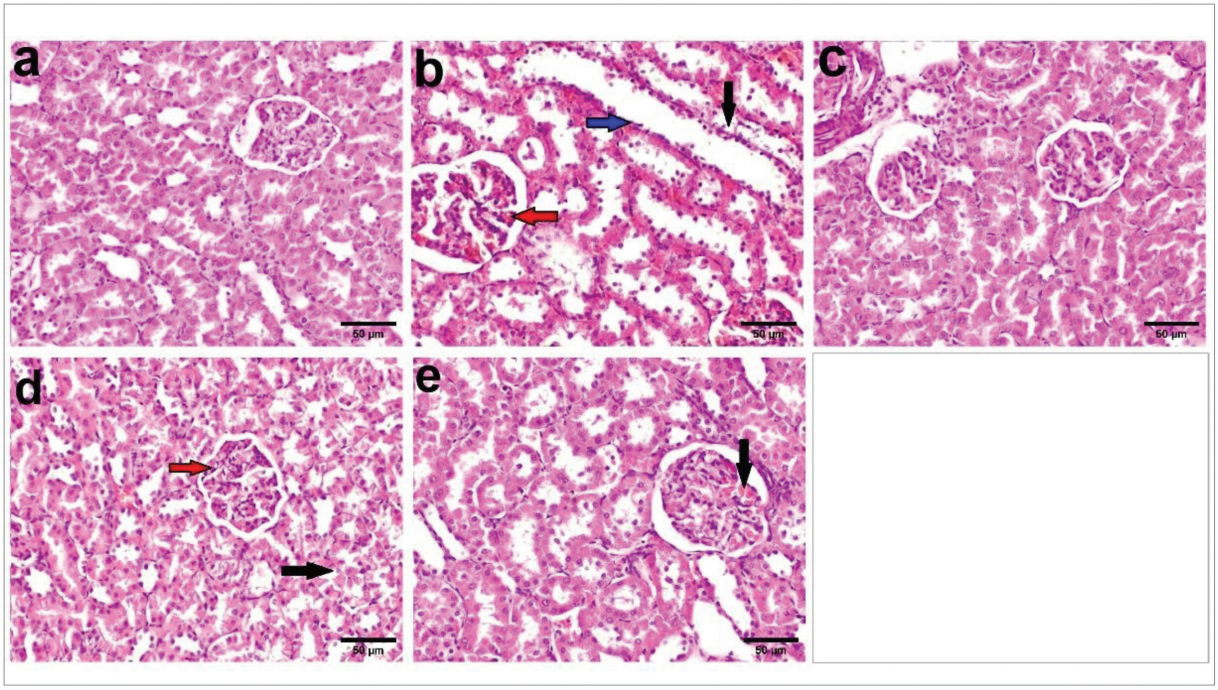
However, the kidney microscopic examination (Fig. 10) also showed a kind of improvement in the renal tissue of both diabetic groups treated with LS and MO-SLs, from the damage resulting from elevated blood glucose, but the improvement was to a lesser extent than the RD. Also, the microscopic examination of heart tissue showed the success of both examined extracts in removing damage resulting from diabetes. Meanwhile, the results showed the superiority of the

Figure 9



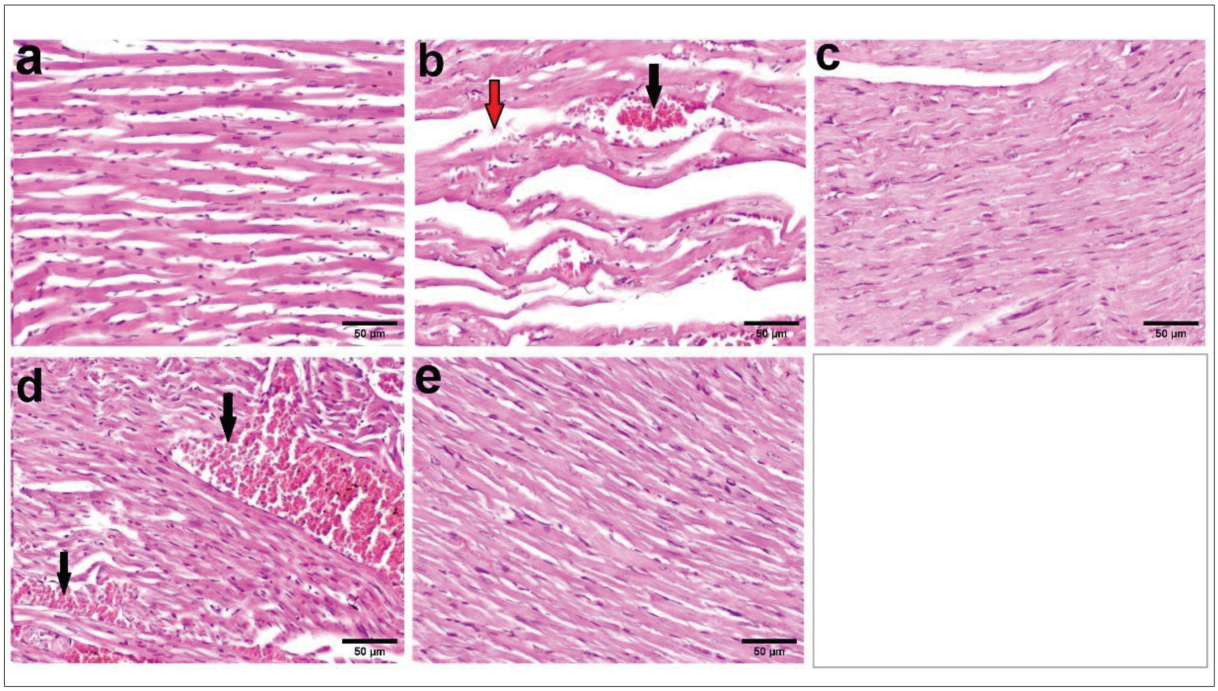
Sections of hepatic tissue photomicrographs stained with H and E of rats with a scale bar of 50 µm: a) Control –ve group; showing the normal histological architecture. b) Control +ve group; showing fibroplasia in the portal triad (black arrow) and cystic dilatation of bile duct (red arrow). c) Diabetic group + RD; showing proliferation of the kupffer cells (black arrow) and some hepatocytes binucleation (yellow arrow). d) Diabetic group + MO-SLs; showing Kupffer cells proliferation (black arrow) and necrosis of sporadic hepatocytes (red arrow). e) Diabetic group + LS-SLs; showing slight hydropic degeneration of some hepatocytes (black arrow).

Figure 10



Sections of renal tissue photomicrographs stained with H and E of rats with a scale bar of 50 µm: a) Control –ve group; showing the normal histological architecture. b) Control +ve group; showing vacuolar degeneration of epithelial lining renal tubules (black arrow), cystic dilatation of renal tubules (blue arrow), and congestion of glomerular tuft (red arrow). c) Diabetic group + RD; showing no histopathological alterations. d) Diabetic group + MO-SLs; showing slight vacuolation in some epithelium of renal tubules (black arrow) and slight congestion of glomerular tuft (red arrow). e) Diabetic group + LS-SLs; showing slight congestion of glomerular tuft (black arrow).

Figure 11



Sections of cardiac tissue photomicrographs stained with H and E of rats with a scale bar of 50µm: a) Control –ve group; showing normal cardiac myocytes. b) Control +ve group; showing congestion of myocardial blood vessel (black arrow) and intramyocardial edema (red arrow). c) Diabetic group + RD; showing no change in histopathological pattern. d) Diabetic group+ MO-SLs; observing congestion of myocardial blood vessels (arrow). e) Diabetic group+ LS-SLs; showing no

Table 3 The histopathological lesion scores in different groups

Organ	Histopathological lesion	Normal	Diabetes	D+RD	D+M	D+LS
Pancreas	Vacuolation of cells of islets of Langerhans's	0	3	1	2	0
	Cystic dilatation of pancreatic Duct	0	2	0	2	0
	Congestion	0	2	1	0	1
Liver	Kupffer cells activation	0	3	1	2	1
	Hepatocellular necrosis	0	2	0	1	0
	Hydropic degeneration of hepatocytes	0	2	0	0	1
	Fibroplasia in portal triad	0	2	0	0	0
Kidneys	Vacuolations of tubular epithelium	0	2	0	1	0
	Congestion of glomerular tuft	0	2	0	1	1
Heart	Congestion of myocardial blood Vessels	0	3	0	2	0
	Intermyocardial edema	0	2	0	1	0

LS-SLs and RD in complete recovery of the heart tissue, compared to the MO-SLs.

The data in Table 3 summarizes the histopathological lesion score for all diabetic groups comparing to the group of control negative. As shown in the table, the control positive group shows the highest lesion score, while the diabetic group that received the RD shows the best improvement as the lowest lesion score followed by the diabetic group that received the LS extract. On the other hand, the diabetic group that received the SLs from MO extract still has a moderate lesion score for both the pancreas and liver, while it records a mild lesion score in kidney and heart. Consequently, a specific improvement in the pancreas, liver, kidney, and heart organs was observed when using any of the produced SLs either from MO or LS. This confirmed the general benefits of SLs on the body's different organs, whether on growth and improving the general condition, as well as stimulating the liver, pancreas, kidneys, and heart through many systems of influence [13,14,34,48,49]. Moreover, Darne *et al.* [50], observed that all the animal organs (heart, liver, kidney, lung, spleen) were completely normal with no unusual legions or necrosis after the curcumin–sophorolipid nanoconjugates application through the rat as an animal model.

Conclusion

Based on the previously mentioned results and discussion, this study directed to the production of SLs economically depending on the microbial conversion of two agro-industrial wastes namely MO and LS oil cakes utilizing locally isolated *Saccharomyces cerevisiae* and *Yarrowia lipolytica*, respectively. The produced MO SLs gave a yield of 18.6 g/100 g substrate, while LS SLs gave 43.6 g/100 g substrate with surface tension activities of 38mN/m and 46 mN/m, respectively. However, the chemical structure analyses conducted by fourier transform infrared spectroscopy, ^{1}H NMR, and LC-MS/MS confirmed the presence of both forms of sophorolipid structures (lactonic and acidic) in MO SLs. While the produced LS SLs confirmed the exitance of lactonic form only. The impact of both extracted compounds as antihyperglycemic agents in STZ-induced diabetic rats compared to metformin as a RD proved the efficiency of the LS SLs in decreasing the body weight change and fasting blood glucose level as the examined RD and so did the extract of MO SLs to a lesser extent. Furthermore, screening for the serum insulin, serum GLP-1, liver, and kidney functions indicated that the RD or the LS SLs diabetic groups showed restoring these parameters to some extent, while the MO SLs improved the same parameters but to a lower extent. The histopathological examinations including the pancreas, liver, kidney, and heart confirmed the results obtained by biochemical markers. Results in fact may indicate that the produced SLs could be a cheap alternative as an adjuvant-safe treatment for diabetes.

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writing original draft and writing review and editing; M. M. Rashad, M. U. Nooman, A. S. Al-Kashef, A.E. Khattab, K. A. Ahmed; M.H. Mahmoud.

Ethical considerations: The animal experiments were carried out by the criteria of the Medical Research Committee of Ethics under the approval number 20-111.

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Conflicts of interest
There are no conflicts of interest.

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