Evaluating the Anti-yeast, Anti-diabetic, Wound Healing Activities of *Moringa oleifera* Extracted at Different Conditions of Pressure *via* Supercritical Fluid Extraction

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Plants represent a great source of medicines, and for their components to be discovered, extraction processes must be developed, especially methods based on green technology. Supercritical fluid extraction (SFE) was employed as a green method for *Moringa oleifera* extraction in the present investigation. The maximum yield of extraction was obtained at 25 MPa. Moreover, the extraction at 25 MPa induced the release of various phenols and flavonoids, as analyzed *via* high-performance liquid chromatography. The investigation revealed the concentrations of chlorogenic, gallic, rosmarinic, and coumaric acids to be 150.59, 89.90, 44.75, and 29.41 µg/mL, respectively at 25 MPa. However, their concentrations were 0.73, 1.53, 0.24, and 0.04 µg/mL, respectively at 15 MPa; *vs.* 4.73, 2.62, 1.06, and 0.50 at 35 MPa, respectively. Totals of saponin, flavonoid, phenolic, tannins, and alkaloid were recorded in maximum yield at 25 MPa. *Moringa oleifera* extracted at 35 MPa reflected highest inhibition zones of 27 ± 0.1, 30 ± 0.2, and 30 ± 0.1 mm against *C. glabrata, C. tropicalis*, and *C. albicans*, correspondingly. α-Amylase and α-glucosidase activities were greatly suppressed by the *M. oleifera* extract at 25 MPa with less IC_{50} (12.97 µg/mL and 6.0 µg/mL), than the IC_{50} (53.46 and 22.02 µg/mL) at 15 MPa, compared with acarbose IC_{50} (5.52 and 2.64 µg/mL), correspondingly.

DOI: 10.15376/biores.19.3.5961-5977

Keywords: Moringa oleifera; SFE-CO_2; Anti-yeast; α-Amylase; α-Glucosidase; Wound healing

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INTRODUCTION

Natural products of plant origin perform a critical job in the management of several diseases in humans as a harmless substitute to drugs from chemical industries (Al-Rajhi *et*

al. 2022; Qanash *et al*. 2022; Al-Rajhi and Abdelghany 2023a,b; Qanash *et al*. 2023). Moringaceae is a family of one genus, *Moringa* that has 13 species indigenous to numerous countries (Stephenson and Fahey 2004). Among the 13 species, *Moringa oleifera* is the most studied and utilized species. *Moringa oleifera* Lam. is a fast-developing tree that is native to India and is well known for its therapeutic and dietary properties for centuries (Rani *et al*. 2028). Scientists have recently learned that *M. oleifera* has rich bioactive potential and phytochemical content (Dzuvo *et al*. 2021; Khalid *et al*. 2023), leading to increased usage of its extracts and oils in merchantable properties, such as therapeutical, nutraceutical, pharmaceutical, and cosmetic formulations (Muhammad *et al*. 2013). Preliminary investigations have shown that the various parts of plant; leaves, roots and seeds reveal characteristic compositions and properties (Alawlaqi *et al*. 2023; Al-Rajhi *et al*. 2023a,b).

Studies show that several polyphenols, primarily phenolic acids, flavonoids, and tannins were included in *M. oleifera*; and to detect these compounds, advanced techniques of chromatography comprising gas chromatography-mass spectrometry, high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry are applied. For example, leaves of *M. oleifera* contained significant quantities of myricetin, rutin, kaempferol, lutein, and quercetin constituents, which perform vital functions like antioxidant, inflammatory, and anti-diabetic activities that improve the viability and propagation of the cellular response to injuries of tissue (Baldisserotto *et al*. 2018). Profiling *M. oleifera* leaf extract by HPLC showed that it contains *p*-coumaric, gallic, ferulic, chlorigenic acid, and sinapic acids that offer antioxidant functions to stabilize free radicals (Khalid *et al*. 2023). Many revisions have reported the hypoglycemic influence of *M. oleifera*, which might be because of the occurrence of fiber and quercetin-3-glucoside components, possibly by stopping glucose application and reducing the rate of gastric emptying (Ndong *et al*. 2017); improving secretion of insulin, and enhancing glycogen production, besides glucose uptake in the liver as well in muscles (Olayaki *et al*. 2015; Muhammad *et al*. 2016). *Moringa oleifera* leaves are acknowledged to decline blood glucose concentration, modeled in type 2 diabetics control (Ndong *et al*. 2017). Another study reported that uptake of *M. oleifera* leaf extract depresses levels of sugar in blood within 3 h once consumption (Mittal *et al*. 2007).

Diverse pharmacological utilizations were linked with *M. oleifera* extracts as mentioned in several scientific reports; for instance, *Moringa oleifera* methanolic extract has shown wound healing properties in infected wounds *via* methicillin-resistant bacteria in rats infected with diabetes (Al-Ghanayem *et al*. 2022). In a wound healing investigation according to Rathi *et al*. (2006), the healing process of the aqueous extract of *M. oleifera* leaves was documented and showed a strong improvement in skin and granuloma breaking strength, wound occlusion rate, hydroxylproline content, and decrease in scar zone, besides granuloma dry weight. Different compounds present in the *M. oleifera* extract were determined and applied locally as an ointment that enhanced the contraction of the wound and reduced the time of epithelization. Its efficacy was attributed to the rise in collagen formation, activities of antioxidant enzyme, and density of capillary (Al-Ghanayem *et al*. 2022).

Candida species are pathogenic microorganisms of yeasts developing mucocutaneous and systemic illnesses in humans, mostly in diabetic patients, low oral hygiene (immunocompromised patients), xerostomia, transplant recipients, malnutrition, and malignancy (Ndong *et al*. 2017). According to Rathi *et al*. (2006) four yeasts namely *Cryptococcus neoformans*, with three species of *Candida* including C. *tropicalis, C.*

parapsilosis, and *C. glabrata*, are considered the greatest principal pathogenic yeasts for humans. Enormous species of *Candida* show as unaffected by antifungal constituents (Mittal *et al*. 2007). Thus, there is a demand for new constituents to inhibit pathogens of yeasts plus better effectiveness and less poisonousness. *Moringa oleifera* extracts showed fungistatic traits that elevate the metabolism change of numerous *Candida* isolates comprising *C. albicans*; *M. oleifera* has improved metabolism alterations (stress response) of cells of *C. albicans*, which get together with the capability to prevent growth and formation of its biofilm up to 72 h (Gani *et al*. 2023). Moreover, *in vivo* employment of *M. oleifera* extracts showed high fungitoxic impact that completely prevented the growth of *G. candidum* at 100 mg/mL. The effectiveness of *M. oleifera* extract was due to the occurrence of saponins, phenols, tannins, flavonoids, and terpenoids, besides glycosides (Chiejina and Onaebi 2016). With regard to *Candida tropicalis*, flavonoids, steroids, tannins, alkaloids *etc*., present in the ethanolic and aqueous extracts leaves of *M. oleifera* exhibited highly antifungal properties (Patel *et al*. 2014). Al-Ghanayem *et al*. (2022) documented the application of *M. oleifera* extract in the healing of diabetic rat wounds diseased with methicillin-resistant bacteria. Merits of *M. oleifera* can be attained by utilizing the fresh part of plant or *via* implying extraction methods. Ordinary techniques of extraction, including maceration, Soxhlet, and hydrodistillation entail different drawbacks like long extraction time, extracts containing residual toxic solvents, and thermal disintegration of target constituents (Chemat *et al*. 2019). New safe and more environmentally friendly alternatives have increasingly been a demand that have made scientists suggest green tools as a solution to dominate the environmental and quality matters. These technologies, such as extraction with pressurized hot-H_2O (Matshediso *et al*. 2015), microwave-assisted (Rodríguez-Pérez *et al*. 2016a), and ultrasound (Zhong *et al*. 2018), usually provide high yields of the total extraction.

Supercritical fluid extraction (SFE) with carbon dioxide (SFE-CO_2) has been applied on a large-scale as a substitute green technology. It offers extracts with developed compound selectivity that can be improved by checking the temperature and pressure. The SFE-CO_2 shows dispersal coefficients alike to gases and slight viscosities alike to solutions, these properties causing a decline in solvent surface tension and rise in the solvent diffusion into the tissues (Zorić *et al*. 2022). SFE-CO_2 has been earlier utilized to get extracts of *M. oleifera* leaves (Rodríguez-Pérez *et al.* 2016b; da Silva *et al.* 2022). The goals of this paper were to estimate SFE-CO_2 of the obtained extracts from *M. oleifera* leaves and profile them utilizing HPLC assay to define the present bioactive ingredients to stand up for their potential use as anti-yeast, anti-diabetic, antimicrobial, and healing efficacy**.**

EXPERIMENTAL

Plant Samples

Moringa oleifera leaves were brought from the National Research Center, Cairo, Egypt during October 2023. The revised species has been identified *via* the deceased Prof. Dr. Abo El-Fetoh Mohamed Abd-Allah at National Research Center, Egypt. Plant leaves were air-dried for 48 h, and subsequently ground to powder, sieved, and kept in paper bags at room temperature in dark until further use.

Plant Extract *via* Supercritical CO_2 Extraction (SFE-CO_2)

The SFE-CO_2 method was used to obtain plant extracts by utilizing the unique properties of supercritical fluids. In this case, carbon dioxide is pressurized and heated to its supercritical state, where it exhibits both gas and liquid-like properties. This allows it to effectively extract desired compounds from plants without leaving behind any residual solvents. The first step is to set up the supercritical CO_2 extraction equipment, which typically consists of a high-pressure vessel, a pump, a heat exchanger, and a collection vessel. The equipment must be designed to handle the high pressures and temperatures included in the procedures. The powder of *M. oleifera* leaves is loaded into the container of extraction. The vessel is then sealed, ensuring that it is airtight to prevent any CO_2 leaks during the extraction process. CO_2 is introduced into the system through the pump, and pressure is gradually increased to reach the supercritical state. The temperature is also carefully controlled to maintain the desired supercritical conditions. Supercritical CO_2 acts as a solvent and penetrates the plant material, dissolving and carrying away the desired compounds so adjustments can be made to optimize the extraction process. After the extraction process, the SFE-CO_2 containing the dissolved compounds is depressurized and directed to a collection vessel. As the pressure decreases, the SFE-CO_2 reverts to a gaseous state, leaving behind the extracted compounds. The collected extract may undergo further processing steps to remove any residual CO_2 or impurities, such as filtration, evaporation, or winterization, depending on the specific requirements of the extract**.**

Assessment of Total Contents of Flavonoid, Phenolic, Tannin, Saponins, and Alkaloid of Plant Extract

Total phenolic content was estimated *via* a colorimetric technique using Folin-Ciocalteu reagent (Sembiring *et al.* 2018). The $AlCl_3$ calorimetric technique was utilized to detect the total content of flavonoid (Sembiring *et al.* 2018). Total content of alkaloid was assessed according to approach of Shamsa *et al*. (2008). Total saponins content (TSC) was estimated using Vanillin-Sulphuric acid method (Anh *et al.* 2018). Tannin content was estimated *via* acidified vanillin method according to Broadhurst and Jones (Broadhurst and Jones 1978).

Analysis of Plant Extract *via* HPLC

The HPLC investigation of *M. oleifera* leaves was performed utilizing an instrument (Agilent 1260 series). Separation was achieved using an Eclipse C18 column (4.6 mm × 250 mm in diameter, 5 µm). The mobile phase consisted of both (A) water and (B) 0.05% trifluoroacetic acid in acetonitrile at constant movement rate of 0.9 mL/min. The mobile phase was sequentially programmed with a linear gradient as follows: 0 min for 82% A; 0 to 5 min for 80% A; 5 to 8 min for 60% A; 8-12 min for 60% A; 12-15 min for 82% A; 15 to 16 minutes for 82% A and 16 to 20 min for 82% A. The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µL for each solution of tested sample. The column temperature was preserved at 40°C.

Anti-yeast Activity of Plant Extract

Using the cup-plate agar diffusion technique, the anti-yeast potential of the *M. oleifera* extracts was evaluated against *Candida albicans* with code number ATCC 10231, *C. glabrata* with code number RCMB 027016, and *C. tropicalis* with code number ATCC 10243. After standardising the used yeasts to the 0.5 McFarland scale, they were poured into petri dishes and sown in a molten, sterile Sabouraud dextrose medium. Four cups were

cut from agar layer and taken out once the mixture solidified using a sterile cork borer with a 6-mm radius. Each cup was packed with 100 µL of the extract (20 µg/mL) using an automatic microlitre pipette. The cups were then refrigerated at 2 °C for 20 min to permit the tested extract to permeate over the surface of agar and an overnight incubation period at 25 °C. An accurately calibrated millimetre ruler was used to record the inhibition zones that were visible (Abdelghany *et al.* 2021).

Anti-diabetic Activity *via* Inhibition of α-Amylase and α-Glucosidase Activities *in vitro*

Pistia-Brueggeman and Hollingsworth's procedure (Pistia-Brueggeman and Hollingsworth 2001) was minor change to test the utilized extract for inhibition of α-glucosidase activity. In summary, 0.1 M phosphate buffer (125 µL) adjusted at 6.8 of pH was mixed with 50 µL of the tested *M. oleifera* extract at different doses (from 1.97 up to 1000 µg/mL) and incubated for 20 min at 37 °C along through 10 µL of the α-glucosidase. After the period of incubation, one M *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG) (20 µL) was added as enzyme substrate to start the enzyme reaction, and afterward the mixture of reaction was left for 30 min. Then, it was fortified with 50 µL of Na_2CO_3 (0.1 N), the enzyme reaction was blocked, and the wavelength at 405 nm was measured utilizing a spectrophotometer model Biosystem 310 Plus. The inhibition of α-glucosidase activity was estimated *via* Eq. 1

$$\alpha - \text{Glucosidase (\%) inhibition} = 100 - \frac{A_{un} - A_{tr}}{A_{un}} 100 \quad (1)$$

where A_{un} is the untreated α-glucosidase absorbance, while A_{tr} is treated α-glucosidase absorbance by the extract of *M. oleifera*. One unit of the enzyme (E) was described as the amount of E required for the making of one µmol of *p*-nitrophenol got from the *p*-NPG/ minute.

A modified version of Wickramaratne *et al*. (2016) procedure was employed to test the used extract for α-amylase inhibitory potential employing the 3,5-dinitrosalicylic acid (DNSA) procedure. To dissolve the tested extract, 10% DMSO was used in total. After that, it was further dissolved in a buffer consisting of NaCl (0.006 M) adapted to pH 6.9 and Na_2HPO_4 (0.02 M)/NaH_2PO_4 (0.02 M), yielding doses started from 1.9 up to 1000 µg/mL. A total of 200 µL of the tested sample and α-amylase (2 units/mL) were joined to create the reaction mixture, which was subsequently incubated at 30 °C for 10 min. Next, the reaction mixture was added to suspended starch (200 µL) in water (1%), which was left for 3 min. To halt the reaction, 200 µL of DNSA reagent was inserted to the mixture and allowed to boil at 90 °C in a water bath for 10 min. After cooling of the reaction mixture up to 25 °C, it diluted utilizing 5 mL of H_2O. At 540 nm, the wavelength was then determined. The tested extract was mixed with 200 µL of buffer to create activity (100%) of α-amylase control, while the tested extract was used to make the blank reaction without the α-amylase solution. Equation 2 was utilized to compute the α-amylase inhibition,

$$\alpha - \text{Amylase (\%)} = 100 - \frac{A_{un} - A_{tr}}{A_{un}} 100 \quad (2)$$

where A_{tr} is the absorbance of enzyme treated with the *M. oleifera* extract, and A_{un} is the absorbance of α-amylase without treatment (control).

Using a regression equation, the extract dose required to inhibit 50% of the activity of enzymes (IC_{50}, µg/mL) was determined by plotting doses (1.95 to 1000 µg/mL) and developing a percentage of inhibition.

Scratch Wound Closure Assay for Measuring the Healing Properties of Plant Extract

Examination of the scratch wound was performed using a multi-well plate. Ten µg/mL of fibronectin extracellular matrix substrate was coated. The plate was then kept at 37°C for a 2-hour incubation period. After release of unbound extracellular matrix, phosphate-buffered saline (PBS) was used as a wash. Trypsin was employed to remove the developed cells from the plate containing the tissue culture. Cells were grown on a scratched research plate and then incubated to allow the cells to proliferate and form a uniform monolayer. A monolayer cell containing a uniform monolayer was rubbed with the pipette tip. Following the scratching, the cell layer was gently rinsed to remove any detached cells. Next, a fresh medium containing verified cells was employed. The plate was placed in a cell culture incubator at 37 °C for 24 up to 48 hours. Post the incubation period, the cell layer was washed with PBS. The cells were then fixed using 3.7% paraformaldehyde for 15 minutes. Subsequently, the cells were stained with 1% crystal violet in ethanol for 10 minutes. A phase-contrast microscope was utilized to examine the cell culture (Martinotti and Ranzato 2020).The subsequent Eqs. 3 to 5 were utilized to calculate the analysis as follows:

$$\text{Migration rate (MR)} = \frac{\text{Initial wound width (um)} - \text{Final wound width (um)}}{\text{Time span of the assay (hours)}} 100 \quad (3)$$

$$\text{Wound closure (\%)} = \frac{\text{Initial wound area} - \text{Wound area after n hours}}{\text{Initial wound area}} \times 100 \quad (4)$$

$$Area\ difference\ (\%) = Initial\ area - Final\ area \quad (5)$$

Statistical Analysis of Findings

The data were collected three times, and the outcomes are expressed as mean ± SD (standard error). CoStat software (version 14, IBM Corp., Armonk, NY, USA) as One-way investigation of variance (ANOVA) was employed to statistical investigations

RESULTS AND DISCUSSION

Solvent extraction is the foundation of the most popular procedures for the extraction of significant constituents of plants. The main drawback of this extraction, though, is that the extracts contain solvent residue. To recover the extract without solvent residue, SFE-CO_2 has recently drawn rising consideration relative to traditional ways such as solvent extraction and hydro-distillation. The SFE-CO_2 operates at low temperatures, preserving the thermally labile constituents in the extracts, which is another advantage it has over traditional extraction methods. It can be seen from Table 1, maximum yield (0.850 g) of the *M. oleifera* extract was obtained at 25 MPa, followed by 35 MPa (0.448 g), and 15 MPa (0.272 g) pressure (Table 1). Through SFE, an earlier study (Zhao and Zhang 2013) reported that extract yield from the oil of *M. oleifera* leaves increased with pressure ranged from 30 to 50 MPa, where the maximum yield (6.34%) was recorded at 50 MPa at 60 °C.

The current results were also in parallel with other findings (Chen *et al*. 2022) but with extraction of *Moringa* seed oil.

Table 1. Extraction Yield from 5.0 g Dry Powder of *M. oleifera* Leaves Exposed to Different Conditions of Pressure Bar

Temperature (°C)	Pressure (MPa)	Extraction Time (min)	Leaves Quantity (g)	Extract Quantity (g)
50	15	50 min	5.0	0.272
50	25	50 min	5.0	0.850
50	35	50 min	5.0	0.448

Table 2. Phenols and Flavonoid Compounds of *M. oleifera* Extract at Different Levels of Pressure Values of Extraction

Compound Name	15 MPa			25 MPa			35 MPa		
	RT	Area mAU*s	Conc. (µg/mL)	RT	Area (mAU*s)	Conc. (µg/mL)	RT	Area (mAU*s)	Conc. (µg/mL)
Gallic acid	3.51	17.35	1.53	3.61	1016.38	89.90	3.59	29.67	2.62
Chlorogenic acid	4.27	5.65	0.73	4.21	1160.51	150.59	4.27	36.47	4.73
Catechin	4.45	0.00	0.00	4.45	0.00	0.00	4.57	3.00	0.65
Methyl gallate	5.44	0.00	0.00	5.55	30.08	1.52	5.42	1.03	0.05
Caffeic acid	5.86	0.00	0.00	5.76	64.63	5.00	5.79	31.80	2.46
Syringic acid	6.37	1.31	0.10	6.21	41.32	3.02	6.35	11.73	0.86
Pyro catechol	6.58	0.00	0.00	6.58	0.00	0.00	6.58	0.00	0.00
Rutin	6.83	6.85	1.01	6.81	199.29	29.40	6.84	304.03	44.85
Ellagic acid	7.42	1.71	0.17	7.13	118.18	11.80	7.16	37.73	3.77
Coumaric acid	8.72	1.25	0.04	8.73	826.43	29.41	8.59	14.15	0.50
Vanillin	8.98	3.30	0.12	9.12	58.23	2.16	9.15	44.30	1.65
Ferulic acid	9.61	0.00	0.00	9.57	27.96	1.62	9.59	3.42	0.20
Naringenin	9.97	1.44	0.13	10.38	39.59	3.62	10.42	6.31	0.58
Rosmarinic acid	11.52	2.25	0.24	11.49	417.38	44.75	11.55	9.89	1.06
Daidzein	15.60	1.27	0.07	15.94	8.30	0.47	15.60	11.85	0.66
Quercetin	17.47	3.97	0.54	17.15	13.28	1.79	17.20	1.99	0.27
Cinnamic acid	19.13	28.15	0.50	19.12	96.01	1.72	19.15	64.83	1.16
Kaempferol	20.52	0.00	0.00	20.49	9.44	0.60	20.69	8.32	0.53
Hesperetin	21.13	0.00	0.00	21.17	10.06	0.49	21.13	0.00	0.00
Unknown (1)	-	-	-	7.84	1561.2	-	-	-	-
Unknown (2)	-	-	-	9.95	488.38	-	-	-	-

The HPLC analysis of extract showed that extraction at 25 MPa increased the releasing numbers and concentration of phenolic and flavonoid constituents (Table 2 and Fig. 1). For example, the concentrations of gallic acid, chlorogenic acid, coumaric acid, and rosmarinic acid were 89.90, 150.59, 29.41, and 44.75 µg/mL with increasing percentages of 98.3%, 99.52%, 99.86%, and 99.46%, respectively, when compared with their concentrations at 15 MPa (Table 2 and Fig. 2). Moreover, besides hesperetin, two unknown compounds with retention times (RT) 7.84 and 9.95 appeared only at 25 MPa. Remarkable detection of rutin (44.85 µg/mL) was observed at 35 MPa compared with their concentrations 29.40 and 1.01 µg/mL at 25 and 15 MPa, respectively (Table 2 and Fig. 3). The obtained outcomes showed that extraction at 25 MPa is a promising condition to liberate active compounds. Methyl gallate, caffeic acid, syringic acid, and ferulic acid were not detected at 15 MPa, but they appeared at high pressures of 25 and 35 MPa. These results were consistent with some other reports. Verma *et al*. (2009) *via* HPLC showed existence

of flavonoids, such as rutin, kaempferol, and quercetin, in addition to phenolic acids such as chlorogenic, gallic, ferulic, and ellagic acids, in *M. oleifera* extract. According to Braham *et al*. (2020), chlorogenic acid and quercetin represented the most detected compounds in the hydroacetonic and hydroethanolic extracts of *M. oleifera*. Moreover, Qadir *et al*. (2022) reported that the RP-HPLC investigation of *M. oleifera* leaves extract reflected that the major compounds were quercetin followed by ferulic acid, *p*-coumaric acid, and gallic acid. The differences in the number and concentrations of detected constituents may be dependent on the cultivation conditions, extraction methods, and used solvents. *Moringa oleifera* leaves were extracted at varying pressures (15 to 25 MPa) and temperatures (35 to 80 °C) (da Silva *et al*. 2022), where 20 MPa and 60 °C were the best conditions for obtaining the maximum quantity of extract yield, total phenolic compounds, and bioactive constituents. Further, tannins (TanE)/gm, alkaloid (µg/g), saponin (mg AE/g), flavonoid (mg QuE/g), and phenolic (mg Ga)/g) of *M. oleifera* were detected in highest concentrations at 25 MPa. Moreover, their concentrations at 35 MPa were more than that at 20 MPa (Table 3).

Table 3. Total Phytochemical Content of *M. oleifera* Extract at Different Pressure Values

Total Phytochemical Content	Pressure (MPa)		
	15	25	35
Tannins (TanE)/g	39.76 ± 0.40	66.87 ± 0.87	54.73 ± 0.65
Alkaloid (µg/g)	3.6 ± 0.49	12.52 ± 0.44	8.65 ± 0.25
Saponin mg(AE)/g	2.4 ± 0.33	16.45 ± 0.75	10.52 ± 0.33
Flavonoid mg(QuE)/g	42.4 ± 0.80	83.40 ± 0.66	55.65 ± 0.33
Phenolic mg(gal)/g	59.7 ± 0.50	176.5 ± 0.90	119.35 ± 0.25

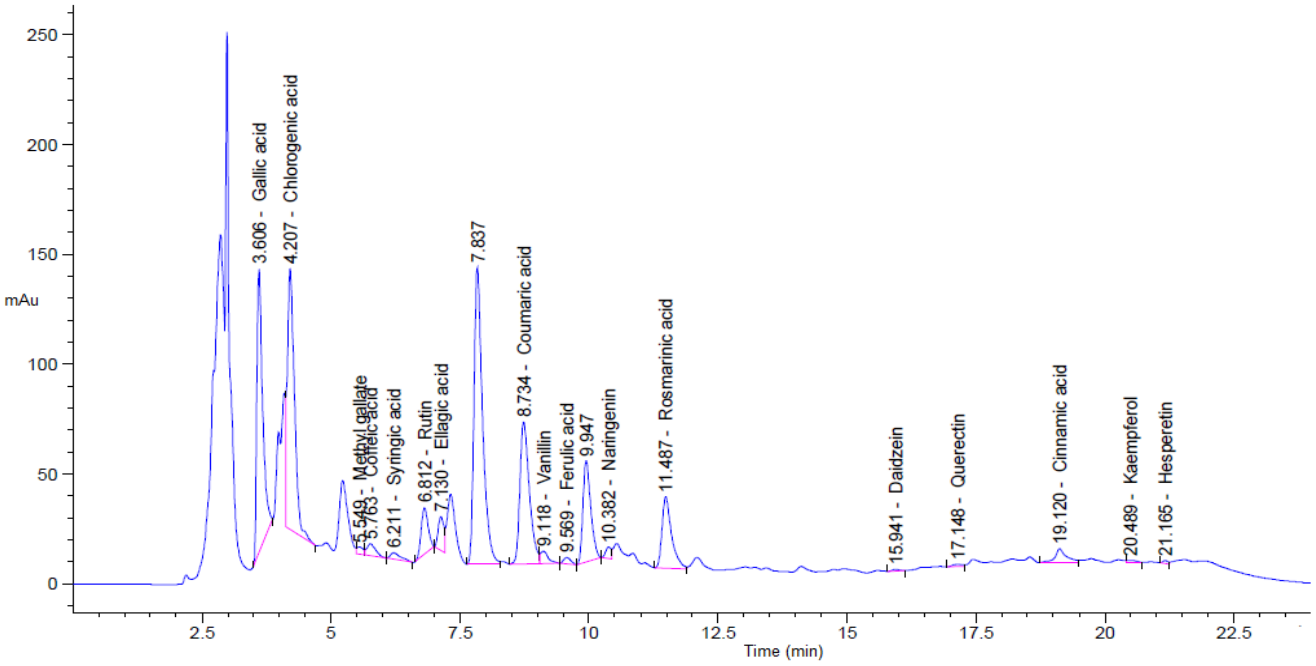

Fig. 1. HPLC chromatograms of identified phenols and flavonoids in the extracts at 25 MPa

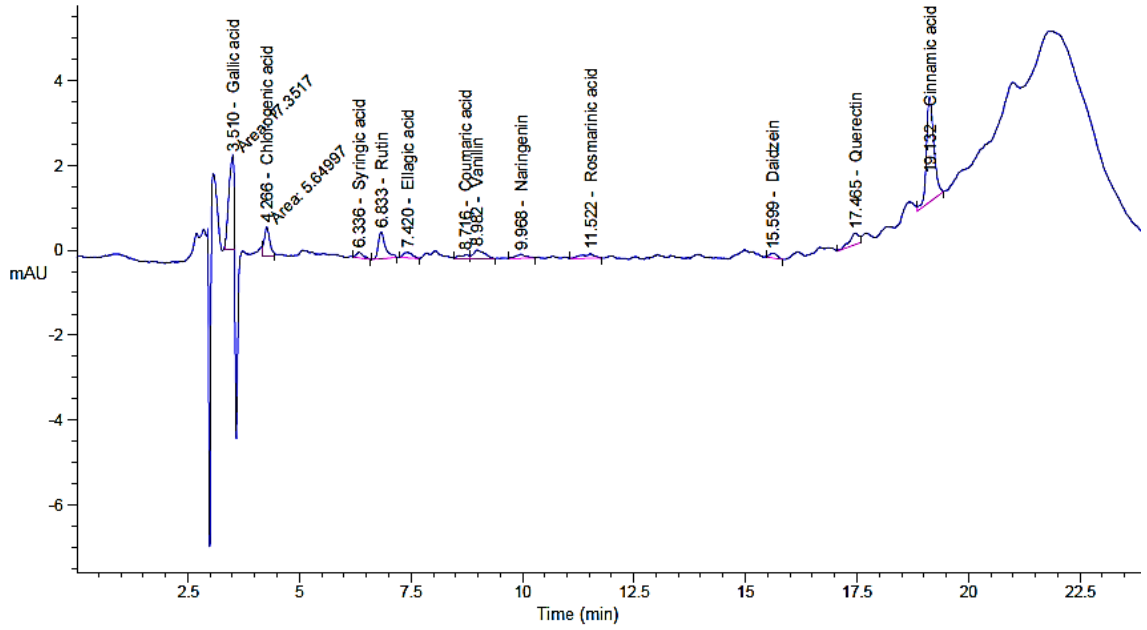

Fig. 2. HPLC chromatograms of identified phenols and flavonoids in the extract at 15 MPa

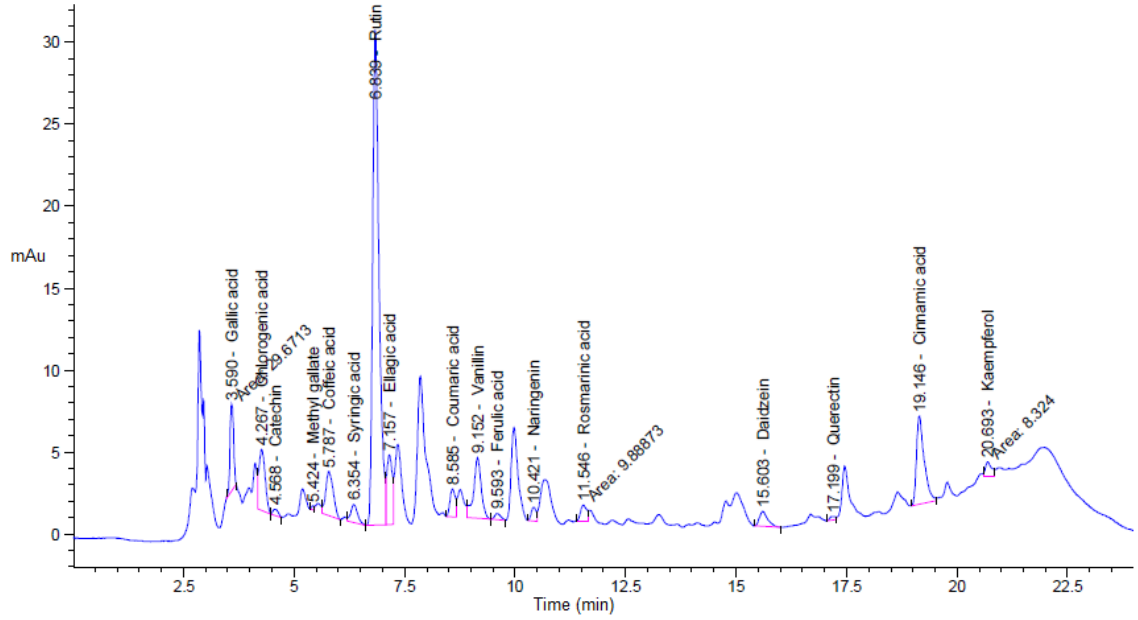

Fig. 3. HPLC chromatograms of identified phenols and flavonoids in the extract at 35 MPa

Moringa oleifera extract possesses anti-yeast activity at all extraction conditions; however, extract at 25 MPa exhibited more inhibition zones of 27 ± 0.1, 30 ± 0.2, and 30 ± 0.1 mm against *C. glabrata*, *C. tropicalis*, and *C. albicans*, respectively, than the inhibition zones for extracts obtained at 15 or 35 MPa. Moreover, the effect of positive control was less than the effect of extract at 25 MPa (Table 4 and Fig. 4). According to Aisha *et al*. (2016) various fungi, primarily *C. albicans*, *Rhizopus stolonifer*, and *Aspergillus niger*, were repressed *via* ethanolic extracts of *M. oleifera*, but *C. albicans* was the most susceptible to the extract. The inhibition of fungi was dependent on the used solvent, for example the extracted *M. oleifera* by petroleum ether did not reflect prevention

of *C. albicans* and *A. niger* growth unlike its reaction to a polar solvent (Moyo *et al*. 2012). Strong antifungal efficacy of *M. oleifera* extracted by methanol was observed on mycelial development, spore germination, and *B. cinerea* ultrastructure at MIC concentration (Ahmadu *et al*. 2020). Chlorogenic acid that was detected with high concentration in the extract of *M. oleifera* at 25 MPa in the present study played a vital role as an anti-yeast (*Geotrichum candidum* and *C. albicans*) agent in a recent investigation (Alsalamah *et al*. 2023).

Table 4. Activity of *M. oleifera* Extract Obtained at Different Levels of Pressure

Tested Yeast	Inhibition Zones (mm)			
	15 MPa	25 MPa	35 MPa	Positive Control (Nystatin)
C. glabrata	23 ± 0.2	27 ± 0.1	24 ± 0.2	22 ± 0.1
C. tropicalis	25 ± 0.3	30 ± 0.2	23 ± 0.1	29 ± 0.1
C. albicans	22 ± 0.2	30 ± 0.1	21 ± 0.1	25 ± 0.3

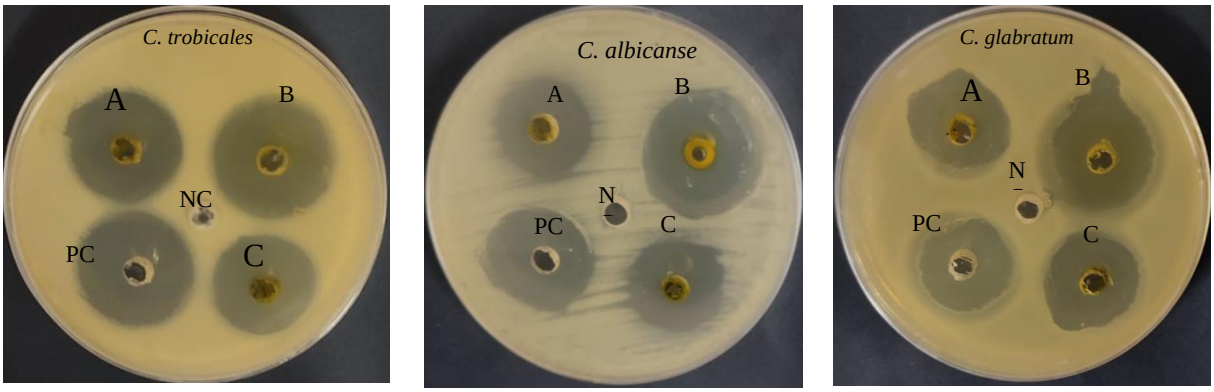

Fig. 4. Impact of various pressures 15 (A), 25 (B), and 35 (C) MPa on the anti-yeast activity of *M. oleifera* extracted (PC, Nystatin as positive control; NC, DMSO as negative control)

The need to investigate natural and biodegradable agents for wound healing *in lieu* of traditional therapies is growing. Because of the existence of bioactive components, like flavonoids, phenols, triterpenes, and alkaloids, medicinal plants are used to treat wounds. In a recent study, extracts of *M. oleifera* reflected inhibitory potential of α-glucosidase (Muzammil *et al*. 2023). As mentioned by Spampinato *et al* (2020), α-glucosidase inhibition *via* phytochemical use is an effective strategy to management of hyperglycemia leading to promotion of wound healing in diabetic patients. The results in Table 5 indicate that *M. oleifera* extract obtained at 30 MPa exhibited excellent suppression of amylase and α-glucosidase activities particularly the α-glucosidase enzyme. Moreover, extraction at 35 MPa was better than at 15 MPa at all tested doses. The extract activity toward enzyme inhibition was confirmed *via* calculation of IC_{50}, where less IC_{50} value of 12.97 µg/mL than IC_{50} (23.83 µg/mL and 53.46 µg/mL) was recorded using the extract at 25, 35, and 15 MPa, respectively compared to the acarbose IC_{50} (5.52 µg/mL) for amylase inhibition. The same observation was recorded for α-glucosidase inhibition with low IC_{50} (6.0 µg/mL), followed by 22.02 and 78.91 µg/mL using the extract at 25, 35, and 15 MPa, correspondingly compared to the acarbose IC_{50} (2.64 µg/mL). High inhibition of amylase and α-glucosidase activities at 30 MPa may be due to the existence of several compounds as identified by HPLC. Magaji *et al*. (2020) revealed that *M. oleifera* extract by different solvents possess an inhibitory potential to α-amylase and α-glucosidase but with different

levels, where hexane and methanol leaves extract established proper inhibition of α-amylase with IC_{50} values of 9.40 and 8.217 mg/mL, respectively. Correspondingly, however, their inhibitory action was less than that of acarbose with IC_{50} value of 0.04 mg/mL, matching approximately with the current findings. Furthermore, root extract by hexane displayed more α-glucosidase inhibition with IC_{50} value of 0.38 mg/mL than that of acarbose with IC_{50} value of 0.88 mg/mL. *Moringa oleifera* extract was effective for several associated-diabetic factors, such as levels of insulin or blood glucose regulation, decreasing of insulin tissue resistance, and blood lipid level enhancement (Mthiyane *et al*. 2022). The activity of certain oxidative enzymes, such as α-glucosidase and α-amylase, has a major impact on glucose levels in blood and hyperglycemia. The metabolic processes that break down complex sugars into simple monomers such as glucose and its level of metabolism are aided by these enzymes (Al-Rajhi *et al*. 2023a&b).

Table 5. α-Amylase and α-Glucosidase Inhibitions *by M. oleifera* Extract

Concentration (µg/mL)	α-Amylase Inhibition % At				α-Glucosidase Inhibition % At			
	15 MPa	25 MPa	35 MPa	Acarbose	15 MPa	25 MPa	35 MPa	Acarbose
Control 100%	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
1.95	2.3 ± 0.06	24.1 ± 0.15	18.2 ± 0.06	34.7 ± 0.15	17.4 ± 0.50	41.3 ± 0.20	25.8 ± 0.20	46.6 ± 0.15
3.9	13.3 ± 0.01	34.6 ± 0.20	26.1 ± 0.42	44.7 ± 0.21	22.5 ± 0.50	46.3 ± 0.44	32.4 ± 0.15	53.2 ± 0.53
7.81	22.3 ± 0.31	44.2 ± 0.28	35.5 ± 0.01	52.9 ± 0.5	29.2 ± 0.25	52.4 ± 0.53	39.5 ± 0.53	57.8 ± 0.38
15.62	32.5 ± 0.50	52.8 ± 0.06	46.1 ± 0.53	63.3 ± 0.35	35.7 ± 0.40	57.3 ± 2.00	46.2 ± 0.31	64.4 ± 0.25
31.25	42.4 ± 0.40	62.5 ± 0.20	54.7 ± 0.50	71.4 ± 0.50	42.2 ± 0.53	63.4 ± 0.35	54.3 ± 0.01	70.5 ± 0.50
62.5	52.1 ± 0.50	72.3 ± 0.06	62.4 ± 0.20	78.9 ± 0.06	46.5 ± 0.35	70.0 ± 0.20	60.8 ± 0.25	76.7 ± 0.53
125	62.4 ± 0.42	80.3 ± 0.44	72.4 ± 0.50	84.3 ± 2.00	53.3 ± 0.35	75.2 ± 3.00	67.3 ± 0.35	83.2 ± 0.20
250	72.0 ± 3.00	87.7 ± 0.20	80.3 ± 0.25	89.7 ± 0.20	60.1 ± 0.30	81.9 ± 0.35	74.3 ± 0.44	89.1 ± 2.00
500	83.1 ± 0.50	90.7 ± 0.25	88.5 ± 0.53	92.4 ± 0.25	67.2 ± 0.35	88.4 ± 0.20	81.3 ± 0.35	92.7 ± 0.20
1000	90.9 ± 0.06	92.7 ± 2.00	92.2 ± 0.50	96.7 ± 0.20	73.7 ± 0.38	93.3 ± 0.06	87.6 ± 0.44	94.6 ± 0.38
IC_{50} µg/mL	53.46	12.97	23.83	5.52	78.91	6.00	22.02	2.64

Table 6 demonstrates the results of healing properties of *M. oleifera* extract at different pressures through the scratch wound closure test. The values of wound closure %, rate of migration (RM), and % area difference (75.1 μm^2, 16.6µm, and 786%, respectively) indicated the better efficacy of extract at 25 MPa than that at 35 MPa (Table 6). Generally, the extract of *M. oleifera* showed healing activity, where their extract at 15 MPa provided 59.1, 13.1, and 546 compared to values at control as of 53.9, 10.7, and 522 as wound closure %, RM, and area difference %, respectively. However, the anti-inflammatory of *M. oleifera* extract was not evaluated in the current study, but other studies documented this activity. In this context, lowering the inflammation of tissue may promote the wound healing progression. Moreover, the extract containing high contents of phenols, flavonoids, alkaloids, saponins, and tannins plays a stimulating role in lowering the oxidative stress,

thereby improving the process and accelerating the wound healing time. In an earlier study, *M. oleifera* leaves extract stimulated the migration and proliferation of animal fibroblasts (Gothai *et al*. 2016). Agreeing to Singh *et al*. (2021) the formulated *Moringa* gum with a polyacramide hydrogel in wound dressing's form promoted wound healing *via* fluid absorption, antioxidant action, and mucoadhesion. In a recent investigation, wound contraction and epithelization time were improved by utilization of *M. oleifera* extract, which was linked with the improved antioxidant enzyme potentials, capillary density, and formation of collagen in diabetic rats infected by MRSA (Al-Ghanayem *et al*. 2022).

Table 6. Healing Properties of *M. oleifera* Extract

Treatment	RM (µm)	Wound Closure (% µm²)	Area Difference (%)
Untreated cells (Control)	10.654	53.903	521.871
Treated Extract at 15 MPa	13.065	59.082	545.656
Treated Extract at 25 MPa	16.561	75.053	786.081
Treated Extract at 35 MPa	14.641	70.876	723.901

CONCLUSIONS

1. Supercritical fluid extraction with carbon dioxide (SFE-CO_2) was shown to be an attractive green technique for the extraction of natural materials, which have probable uses in the pharmaceutical and food fields.
2. The maximum extraction yield of *Moringa oleifera* (0.850 g/5 g), was obtained at 25 MPa.
3. The most detected compounds of phenols and flavonoids, such as chlorogenic acid, gallic acid, rosmarinic acid, and coumaric acid, *via* HPLC were recorded at 25 MPa.
4. Moreover, the extract at 25 MPa had the highest anti-yeast activity toward *C. glabrata*, *C. tropicalis*, and *C. albicans* with inhibition zones of 27 ± 0.1, 30 ± 0.2, and 30 ± 0.1 mm, respectively, compared to the activities of extracts at 15 or 35 MPa.
5. Low values of IC_{50} (12.97 µg/mL and 6.0 µg/mL) were obtained from the extracted plant at 25 MPa for the inhibition α-amylase and α-glucosidase activities, respectively.
6. From the wound healing properties test, it promotes the use of SFE-CO_2 for *M. oleifera* extraction at 25 MPa.

FUNDING

This work was funded by the Deanship of Graduate Studies and Scientific Research at Jouf University under grant No. (DGSSR-2024-01-01121).

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Article submitted: April 15, 2024; Peer review completed: June 8, 2024; Revised version received and accepted: July 9, 2024; Published: July 16, 2024.
DOI: 10.15376/biores.19.3.5961-5977