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Research Article

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Research article

South African *Momordica balsamina Linn.* Chemical Characterization, In-vitro Antidiabetic Evaluation and In-Extracts Compounds Identification

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Abstract

Purpose *Momordica balsamina* is used traditionally for the treatment and management of diabetes. This study aimed to evaluate the *in-vitro* antidiabetic and cytotoxic effects of *Momordica balsamina* leaf extracts and to identify the bioactive compounds potentially responsible for its antidiabetic properties.

Methods Antidiabetic activity was assessed using α-amylase, α-glucosidase, and β-glucosidase inhibition assays with acarbose as reference. GC-MS analyses was employed to identify compounds present in the extracts and MTT assay was used to assess the cytotoxicity of extracts exhibiting antidiabetic properties.

Results All extracts demonstrated a concentration-dependent increase in enzyme inhibition. Notably, DCM leaves exhibited a lower IC_{50} (0.156) for α-amylase compared to acarbose (0.209), indicating stronger inhibitory activity. For α-glucosidase inhibition, acarbose had the lowest IC_{50} (0.129), however, EA leaves and DCM leaves extracts followed closely with IC_{50} values of 0.301 and 0.367. Compared to acarbose's IC_{50} (0.464) for β-glucosidase, EA leaves and DCM leaves extracts exhibited stronger activity with IC50's of 0.301 and 0.367 respectively. GC-MS analysis revealed several antidiabetic-associated bioactive compounds, including vanillin and 2,4-di-tert-butylphenol in the DCM leaves, and propylene glycol, 2-methoxy-4-vinylphenol, hexadecenoic acid, oleic acid, phytol, and octadecanoic acid in the ethyl acetate leaves extract. The MTT assay results confirmed the safety of DCM and ethyl acetate leaves extracts, as they exhibited no significant cytotoxicity at concentrations of 500 µg/mL and above.

Conclusion These findings validate the traditional use of *Momordica balsamina* as a herbal remedy for diabetes and also highlight its potential as a source of safe and effective antidiabetic agents for further development.

KEYWORDS: Diabetes mellitus, *Momordica balsamina*, Antidiabetic activity, Enzyme inhibition, Bioactive compounds, Cytotoxicity,

Introduction

Diabetes mellitus is a prevalent metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1,2]. Persistent hyperglycemia is associated with the development of

both microvascular and macrovascular complications, significantly increasing the risk of morbidity and mortality [3]. Type 2 diabetes mellitus (T2DM) is the most common type of diabetes, accounting for approximately 90–95% of all cases, and its rapid global increase has presented a significant challenge to the healthcare systems. [4]. An estimated 451 million people between the ages of 18 and 99 worldwide have diabetes, with a notably high prevalence in low- and middle-income nations [5]. According to predictions from the World Health Organisation, 15.5 million adults in Africa have diabetes, and 69.2% of them are not aware of their condition [6]. Between 2016 and 2017, diabetes mellitus was the second most common cause of death in South Africa, with mortality rates rising from 5.4% in 2015 to 5.7% in 2017. The available antidiabetic drugs does not cure diabetes and they are associated with adverse effects, high costs, and limited availability, particularly in low-resource settings [7,8].

The growing incidence and associated complications of diabetes highlight the urgent need for effective prevention, management, and treatment strategies. As a result, there is growing interest in exploring alternative antidiabetic therapies derived from natural sources, particularly medicinal plants, which offer potential advantages in terms of affordability, accessibility, and safety [9,10]. To date, approximately 1,200 plant species have been documented for their traditional use in diabetes management, yet only 30% have been subjected to rigorous biological evaluation [11]. Many of these plants exhibit promising antidiabetic properties, with mechanisms of action that include the inhibition of key carbohydrate-hydrolysing enzymes such as α-amylase**,** α-glucosidase, and β-glucosidase [12]. While α-glucosidase aids in the final hydrolysis of disaccharides into monosaccharides, primarily glucose, and β-glucosidase is involved in the hydrolyses of cellulose into glucose to allow absorption in the small intestine, α-amylase catalyses the early breakdown of polysaccharides into disaccharides. The activity of these enzymes significantly contributes to postprandial hyperglycemia, a major factor in the progression of diabetes and its complications [13,14]. Given their crucial role in carbohydrates metabolism, they have emerged as key targets for therapeutic intervention, therefore, inhibiting these enzymes can effectively slow down carbohydrate digestion, thereby reducing postprandial glucose spikes and improving glycaemic control in diabetic individuals [15]. Consequently, the development of innovative plant-based antidiabetic treatments with improved safety and effectiveness profiles may be facilitated by medicinal plants that have the ability to block these enzymes.

Momordica balsamina Linn (*Momordica balsamina*) is a medicinal plant widely utilized in traditional medicine for the treatment and management of various ailments, including diabetes [16,17]. Despite its widespread ethnomedical use, its antidiabetic properties, especially the effectiveness of its isolated bioactive components, have not received any scientific validation. Furthermore, to guarantee these bioactive components' safety for therapeutic uses, their possible cytotoxic effects need to be assessed. Gas Chromatography-Mass Spectrometry (GC-MS) analysis has been employed to identify and characterize the phytochemical constituents of *M. balsamina*, revealing a diverse array of bioactive compounds that may contribute to its antidiabetic activity. The purpose of this study is to evaluate the in-vitro antidiabetic potential of *Momordica balsamina* extracts through enzyme inhibition assays, assess their cytotoxic effects using the MTT assay, and correlate these biological activities with the bioactive compounds identified via GC-MS analysis. The findings of this study will add to the expanding corpus of research on antidiabetic drugs derived from plants and offer important new information on the possible pharmacological uses of *Momordica balsamina*.

Materials and methods

Materials and reagents

All the chemicals and reagents used in this study were of analytical grade. Alpha-glucosidase, alpha-amylase, beta-glucosidase, 2-napthyl-beta-D-glucopyranoside, acarbose, glucose, Vero cell lines, DMEM culture medium and fast blue salt were obtained from Sigma-Aldrich (Germany).

Plant material

Fresh leaves of *Momordica balsamina* were collected from Phake ya Ratlhagane village (25°08'51.1"S, 28°30'28.2"E) in Mpumalanga Province, South Africa with the assistance of an indigenous health practitioner Mr Boynet Khosana. Plant specimens were taken to the South African National Biodiversity Institute (SANBI) for botanical identification. A voucher specimen MA001 was then deposited in the Herbarium of the Pharmaceutical Sciences Unit of the School of Pharmacy, Sefako Makgatho Health Sciences University. The plant materials were air-dried in a well-ventilated area away from direct sunlight to prevent degradation of bioactive compounds. During and after the drying process, seeds were carefully removed from the fruit pulp. The dried samples were then finely ground using a Polymix Laboratory Dry Mill Drive Unit (Polymix™ PX-MFC 90 D, Kinematica AG, Luzern, Switzerland) and stored in airtight containers at room temperature in a laboratory cupboard until further analysis.

Preparation of the extracts

The powdered leaf samples were subjected to sequential extraction using hexane, dichloromethane (DCM), ethyl acetate (EA), and methanol in order of increasing polarity. The extraction procedure was carried out using an orbital platform shaker (Model 261, 8 kg, 230 V – 50 Hz, 120 W, Labotec, South Africa) in 24-hour cycles, with two repetitions for each solvent and 2L of solvent was used in each extraction cycle. The resulting supernatant was filtered using filter discs (Filter Discs Qual. 3 hw, 125 mm, BOECO, Germany) to remove particulate matter. Excess solvent was evaporated using a Stuart rotary evaporator (RE400, COLE-PARMER LTD., Stone, United Kingdom) at 37°C with a rotation speed of 120 cycles per minute. The concentrated extracts were then air-dried at room temperature until further use.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The phytochemical constituents of *Momordica balsamina* extracts were analysed using GC-MS. The analysis was conducted using a Shimadzu QP2010 SE (Shimadzu Corporation, Kyoto, Japan) gas chromatograph-mass spectrometer equipped with an inert cap 5MS/SIL silica capillary column (30 m × 0.25 mm ID × 0.25 µm df), composed of 100% dimethylpolysiloxane. Helium (99.99%) was used as the carrier gas at a constant flow rate of 1 mL/min. A 2 µL sample was injected with a split ratio of 1:10. The injector temperature was set to 290°C, and the ion-source temperature was maintained at 230°C.

The oven temperature program was as follows: isothermal at 50°C for 1 minute, increased to 180°C at 20°C/min, held at 180°C for 5 minutes, then increased to 240°C at 5°C/min, and finally increased to 280°C at 20°C/min. The mass spectra were recorded at 70 eV with a scan interval of 0.3 seconds over a mass range of 50–700 m/z. Data acquisition and analysis were performed using GC-MS Solutions software, version 2.6. Compound identification was achieved by comparing the obtained mass spectra with those in the National Institute of Standards and Technology (NIST) library and by analysing retention indices. The relative percentage of each compound was determined by calculating the peak area normalization.

Antidiabetic activity

Inhibition assay for α-amylase activity

The α-amylase inhibitory activity of *Momordica balsamina* extracts and compounds was determined using a modified version of the standard method described by Poovitha and Parani, 2016 [18]. Dried extracts were reconstituted in their respective extraction solvents to obtain a stock concentration of 1 mg/mL in a 96-well microplate, 100 µL of potassium phosphate buffer (pH 7.0) was added to all wells, followed by 100 µL of each extract in the test wells, which were serially diluted across the plate. As the positive control, 100 µL of acarbose was serially diluted using the same method. Subsequently, 20 µL of α-amylase enzyme solution was added to each well, and the plate was incubated at 37°C for 5 minutes. After incubation, 50 µL of the KATKIT substrate was introduced into each well, and the reaction mixture was incubated for an additional 20 minutes. The absorbance was recorded at 405 nm using a spectrophotometer. Equation 1 below was utilised to get the α-amylase activity inhibition %.

Equation 1:

$$\%\ alpha - amylase\ inhibition\ = \frac{A_0 - A_S}{A_0}\ x\ 100$$

Where A_s is the absorbance in the presence of the test substance and A_0 is absorbance of the control.

Inhibition assay for α-glucosidase activity

A modified α-glucosidase assay was used to assess the α-glucosidase inhibitory activity of *Momordica balsamina* extracts and compounds. The produced *Momordica balsamina* extracts were serially diluted throughout a 96-well microplate after 50 µL of potassium phosphate buffer (pH 7.0) was added to each well. Each well was then filled with 100 µL of a commercial glucose test reagent and 50 µL of sucrose solution. An aliquote30 µL of intestinal acetone rat powder was added to each well to start the reaction, and the plate was then incubated for 30 minutes at 37°C. The same process was used to make acarbose, which was utilised as a positive control. A spectrophotometer was used to detect absorbance at 505 nm [19]. The percentage inhibition of α-glucosidase activity was calculated using Equation 1.

Inhibition assay for β-glucosidase activity

The β-glucosidase inhibitory activity of *Momordica balsamina* extracts was assessed at varying concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL). A 120 µL of each concentration was added to a 96-well microplate, followed by the addition of β-glucosidase enzyme. The plate was incubated at 37°C for 15 minutes. Subsequently, 20 µL of 2-Naphthyl-β-D-glucopyranoside was added to each well, and the plate was incubated for an additional 15 minutes at 37°C. The reaction was terminated by adding 80 µL of sodium carbonate solution to each well. Absorbance was measured at 405 nm using a spectrophotometer. Acarbose, prepared under identical conditions, served as the positive control. The percentage inhibition of β-glucosidase activity was calculated using Equation 1.

Cytotoxicity

Preparation of the cell lines

The Vero cell lines (American Type Culture Collection [ATCC], Manassas, VA, USA) were maintained in Dulbecco's Modified Eagle Medium (DMEM) (Sigma, South Africa) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. The culture medium was replaced with fresh medium every 2–3 days until the cells reached 70–80% confluency. Using a light microscope (Nikon TS100, Germany), cell morphology was regularly observed in order to evaluate cell viability, attachment, mycoplasma contamination, and any morphological alterations.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Cytotoxicity Assay

The MTT test was used to evaluate the cytotoxicity of *Momordica balsamina* extracts and isolated compounds in-vitro, following the guidelines provided by Van Meerloo and co-workers [20]. For the purpose of allowing cell adhesion and achieving confluency, a cell suspension (2,500 cells/mL) was seeded into 96-well microplates and incubated for 24 hours at 37°C in a humidified environment with 5% CO_2. and a TC20 cell counter (Bio-Rad, USA) was used to calculate the number of cells needed for the experiment. Following attachment, cells were treated with different concentrations of *Momordica balsamina* extracts (1, 0.5, 0.25, and 0.125 mg/mL) and further incubated for 24 hours under the same conditions. After incubation, 20 µL of MTT reagent (5 mg/mL in PBS) (Sigma-Aldrich, South Africa) was added to each well, and the plates were incubated for an additional 4 hours. The resulting yellow formazan crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO). The negative control was untreated cells, whereas the positive control was hydrogen peroxide (0.1, 0.05, 0.025, and 0.0125% v/ v). The absorbance of the formazan product was measured at 570 nm using a microplate spectrophotometer to determine cell viability.

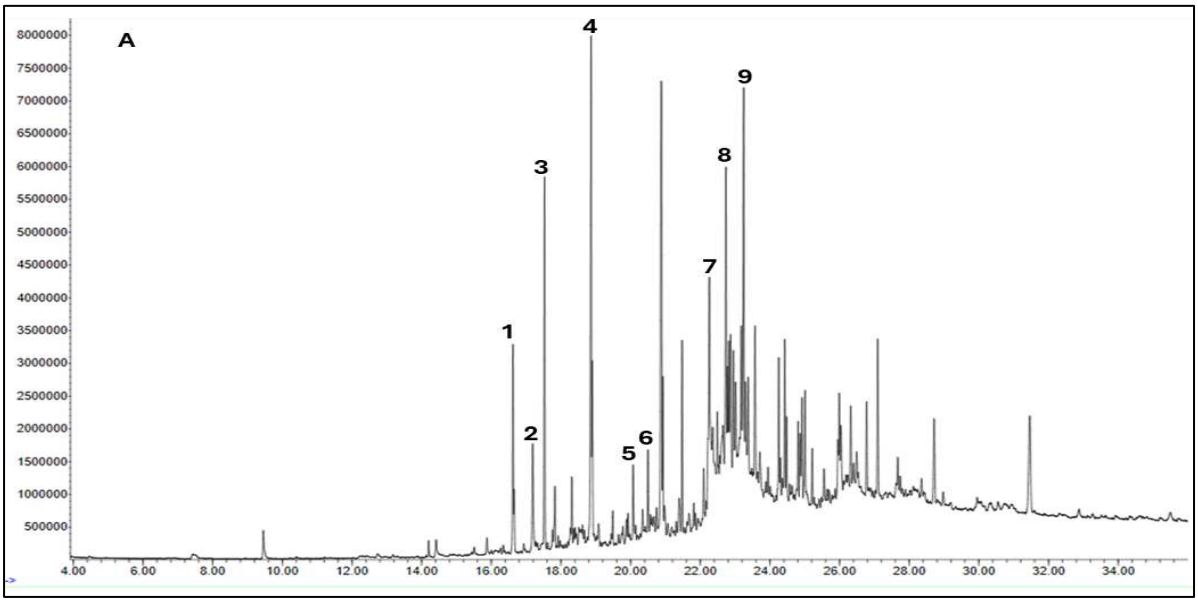
Results and discussion

Phytochemical characterization Momordica balsamina leaf extracts by GC-MS analysis.

GC-MS was employed to identify the phytochemical constituents potentially responsible for the antidiabetic properties of the plant extracts. The results of GC-MS analysis revealed nine distinct compounds in the DCM extract and nine in hexane extract (figure 1 and table 1), fourteen in the EA extract and four in methanol extracts respectively (figure 2 and table 2), . Notably, several of these compounds have been previously reported to exhibit antidiabetic properties, suggesting their potential contribution to the observed bioactivity of *Momordica balsamina*. However, several other peaks in all the extracts could not be identified due to the zero matching from the GC-MS library.

GC-MS analysis of DCM and Hexane leaf extracts

Amongst the compounds in the DCM extracts are hexane, vanillin, 2,4-di-tert-butylphenol, octadecane, eicosane, hexadecenoic acid ethyl ester, hexadecenoic acid, hexadecenoic acid butyl ester and decanol-2-hexyl. As for the hexane extract, all including the identified compounds were saturated hydrocarbons hexadecane, octadecane, eicosane, docasne, tetracosane, heptacosane, octacosane, tetrtricotane and heptane.



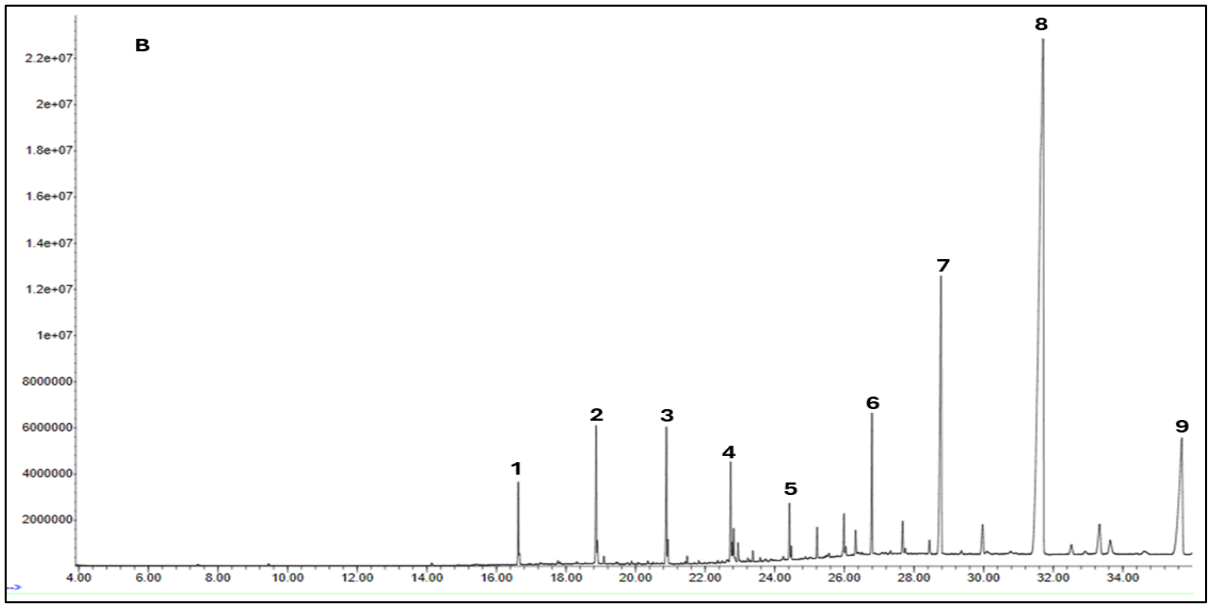
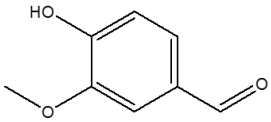
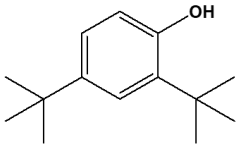
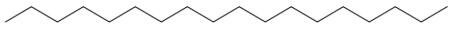
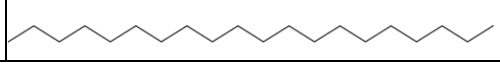
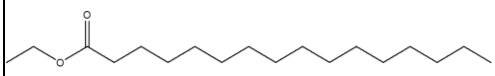
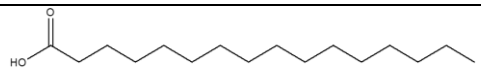
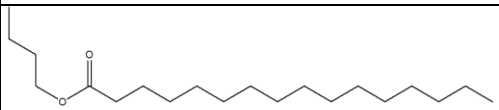
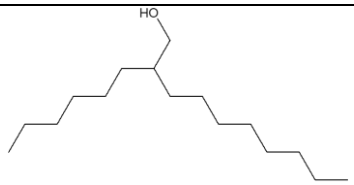
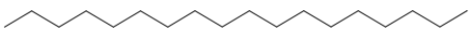
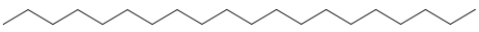


Fig. 1 GC-MS chromatogram of DCM (A) and hexane (B) extracted from *Momordica. balsamina* leaves.

Table 1. GC-MS phytochemical profiling of the DCM and hexane extracts of the *Momordica balsamina* leaves. The structures in the table were drawn by the authors using ChemDraw Ultra 8.0 (Chemistry Software Ltd Gateways, Surrey, UK).

Peak No	RT (min)	Compound Name	Compounds Structure	Composition %Area
DCM extract chemical characterization				
1	16.624	Hexadecane		2.551
2	17.191	Vanillin		1.873
3	17.528	2,4-Di-tert-butylphenol		3.859
4	18.865	Octadecane		7.831
5	20.878	Eicosane		9.559

6	21.479	Hexadecanoic acid, ethyl ester		3.461
7	22.260	n-Hexadecanoic acid		14.304
8	23.245	Hexadecanoic acid, butyl ester		42.822
9	2372	1-Decanol, 2- hexyl		5.430
Hexane extract chemical characterization				
1	16.633	Hexadecane		1.673
2	18.865	Octadecane		2.893
3	20.883	Eicosane		3.246
4	22.732	Docosane		3.093
5	24.422	Tetracosane		1.700
6	26.790	Heptacosane		4.176
7	28.764	Octacosane		13.018
8	31.666	Tetratriacotane		52.129
9	35.669	Heptacosane		9.201

GC-MS analysis of EA and MeOH leaf extract

The EA L extract consisted of a combination of straight, branched, ring alcohols as phenols, amines and certain alkanoic acid. Amongst these compounds were propylene glycol, glycerin, 2,3-dihydro-3,5-dihydro-6-methyl-4-pyrone. Others were 1,2,3-Propanetriol, 1- acetate, 2-Methoxy-4- vinylphenol, 2-Methyl-4-amino-6-dimethylamino-s- triazine, 1-Nonadecene, Neophytadiene, 3,7,11,15-Tetramethyl-2- hexadecen-1-ol, Hexadecanoic acid ethyl ester, n-Hexadecanoic acid, Oleic Acid, phytol and octadecanoic acid.

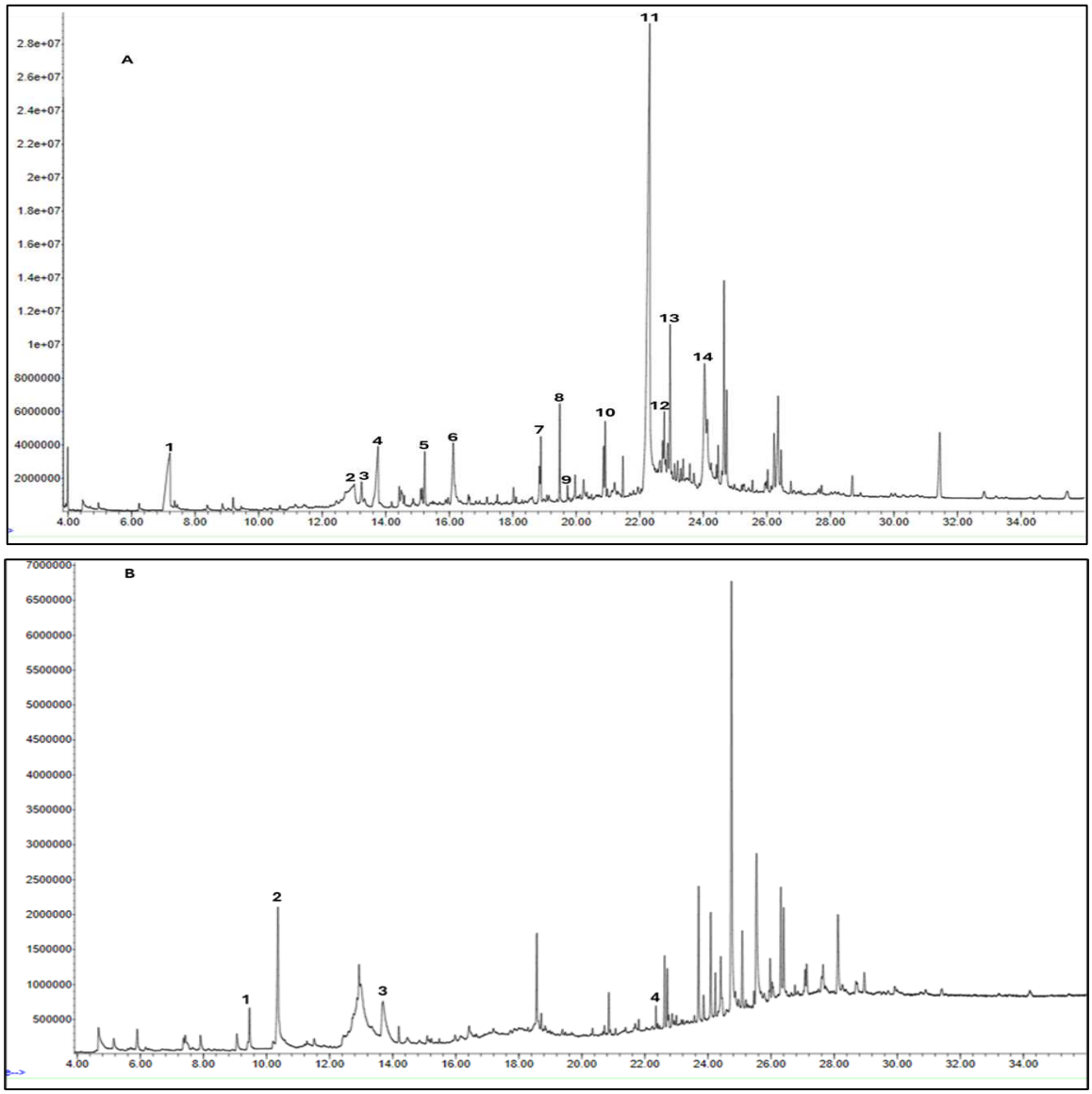
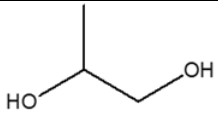
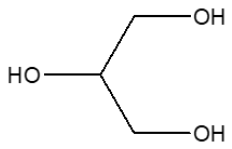
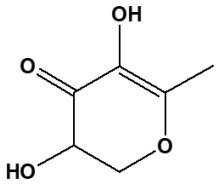
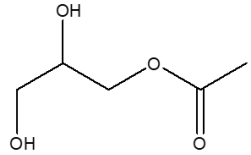
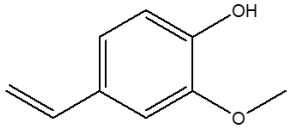
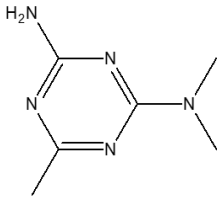
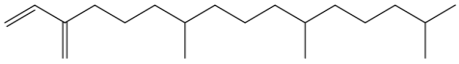
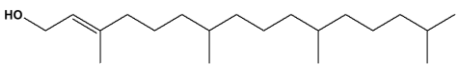
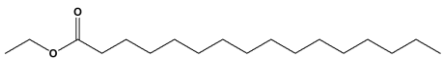
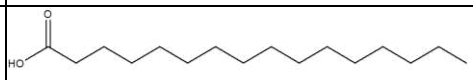
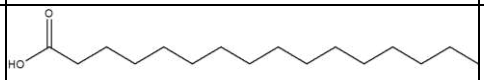
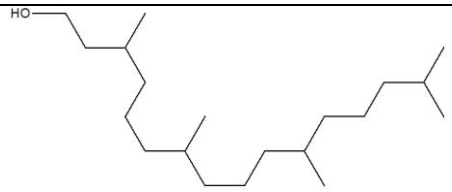
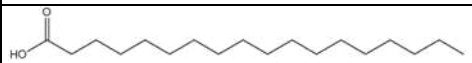
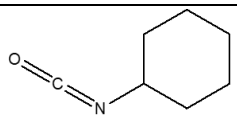
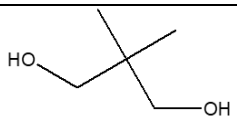
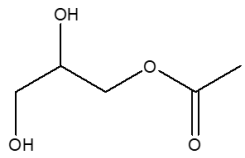
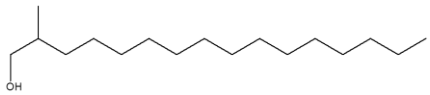


Fig. 2 GC-MS chromatogram of EA (A) and MeOH (B) extracted from the leaves of *M. balsamina*.

Table 2 GC-MS phytochemical profiles of EA and MeOH extracts of *M. balsamina* leaves. The structures in the table were drawn by the authors using ChemDraw Ultra 8.0 (Chemistry Software Ltd Gateways, Surrey, UK).

Peak No	RT [min]	Compound Name	Compound Structure	Composition % Area
EA extract chemical characterization				
	7.180	Propylene glycol		4.679

	12.991	Glycerin		5.022
	13.240	2,3-Dihydro-3,5- dihydroxy-6-methyl-4- pyrone		1.368
	13.741	1,2,3-Propanetriol, 1- acetate		3.097
	15.227	2-Methoxy-4- vinylphenol		1.627
	16.123	2-Methyl-4-amino-6-dimethylamino-s- triazine		2.906
	18.877	1-Nonadecene		2.33
	19.480	Neophytadiene		1.53
	19.962	3,7,11,15-Tetramethyl-2- hexadecen-1-ol		1.057
	21.469	Hexadecanoic acid ethyl ester		2.211
	22.293	n-Hexadecanoic acid		28.554
	22.764	Oleic Acid		5.567

	22.953	Phytol		5.741
	24.043	Octadecanoic acid		11.823
Methanol extract phytochemical profile				
	9.461	Cyclohexyl isocyanate		1.625
	10.365	Neopentyl glycol		6.648
	13.696	1,2,3-Propanetriol, 1- acetate		5.813
	22.622	2-Methylhexadecan- 1-ol		3.16

Antidiabetic activity

The potential of *Momordica balsamina* extracts to regulate blood glucose levels was assessed through α-amylase, α-glucosidase, and β-glucosidase inhibitory assays. The inhibitory activity of *Momordica balsamina* extracts was compared to that of acarbose, a standard antidiabetic drug used in the management of type 2 diabetes. Acarbose was selected as a reference inhibitor due to its well-established role in targeting key carbohydrate-metabolizing enzymes [21].

α-amylase activity

The enzyme α-amylase facilitates the breakdown of polysaccharides into simple sugars, enabling their absorption in the intestine. Inhibiting α-amylase reduces carbohydrate digestion and absorption, thereby helping to regulate

blood glucose levels [22]. As a result, α-amylase inhibition is considered a promising approach for managing postprandial hyperglycemia.

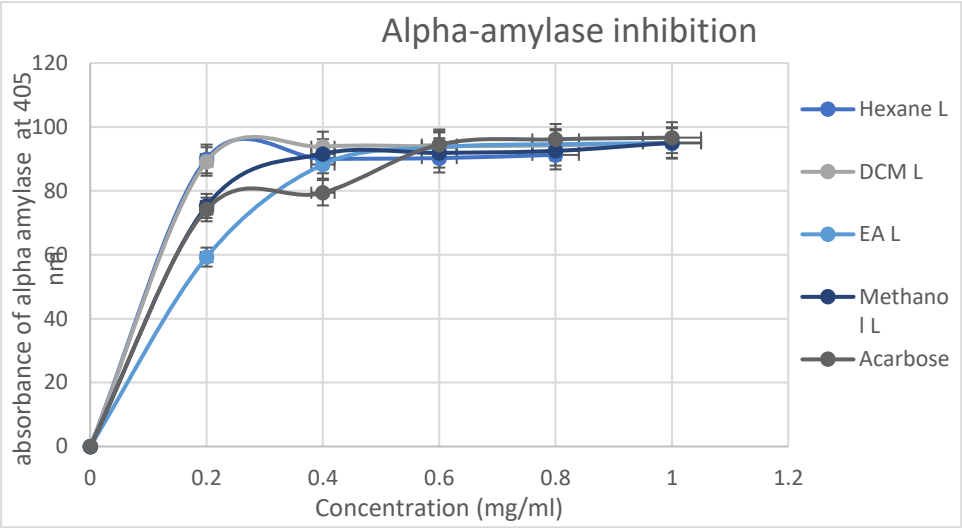


Fig. 3 The α-amylase inhibitory activity of *Momordica balsamina* leaf extracts at different concentrations. L: Leaves.

All of the *Momordica balsamina* extracts had an inhibitory pattern resembling that of the common antidiabetic medication acarbose, as seen in figure 3. It shows that absorbance increases with rising drug concentrations, with the DCM L extract demonstrating the highest inhibition percentage (91.4–98.99%). This implies that *Momordica balsamina* extracts may be able to control blood glucose levels and efficiently suppress α-amylase activity.

α-glucosidase activity

α-Glucosidase is an enzyme responsible for catalysing the breakdown of disaccharides into monosaccharides, facilitating their absorption in the small intestine. Inhibitors of α-glucosidase slow the final stages of carbohydrate digestion, thereby preventing the rapid entry of glucose into the bloodstream [23,24]. Inhibition of this enzyme delays glucose absorption, which helps to stabilize blood glucose levels and can contribute to the management of diabetes progression.

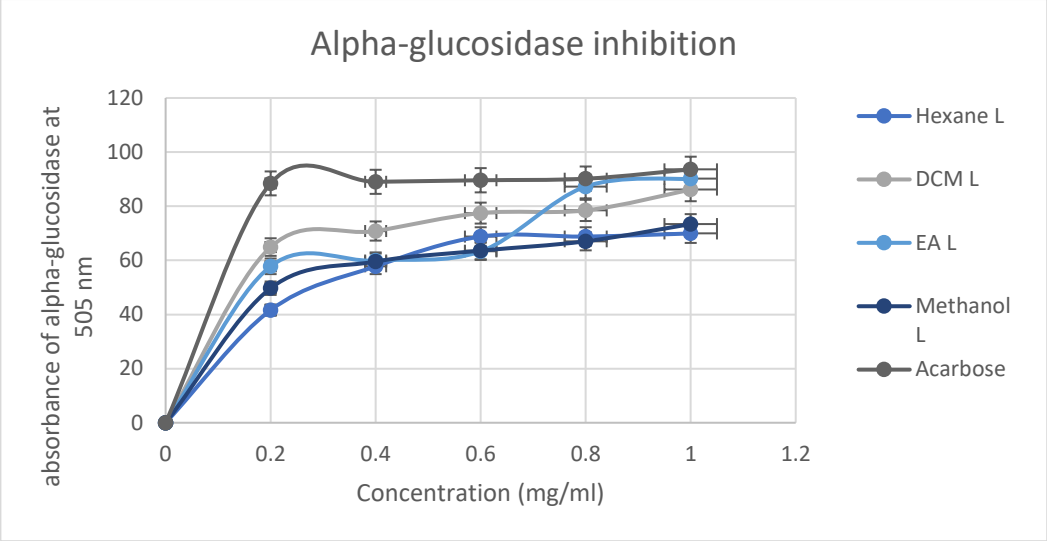


Fig. 4 The α-glucosidase inhibitory activity of *Momordica balsamina* leaf and fruit extracts of different concentrations. L: Leaves

As shown in Figure 4, the inhibitory effects of *Momordica balsamina* extracts mirrored the trend observed with the standard drug, acarbose. Absorbance increased in a concentration-dependent manner for both acarbose and the extracts; however, acarbose demonstrated higher absorption percentages (88.44–93.63%) at lower concentrations, maintaining this level of inhibition up to the highest concentration (1 mg/mL).

β-glucosidase activity

The inhibitory potential for β-glucosidase was assessed because its suppression is important in a number of situations, including the control of type 2 diabetes (by lowering postprandial hyperglycemia) and the alteration of specific metabolic processes. The inhibitory potential of plant extracts is assessed using pharmaceutical drugs such as acarbose as standard. The results (figure 5) demonstrated that all the extracts, except hexane, exhibited stronger antidiabetic activity than the standard acarbose.

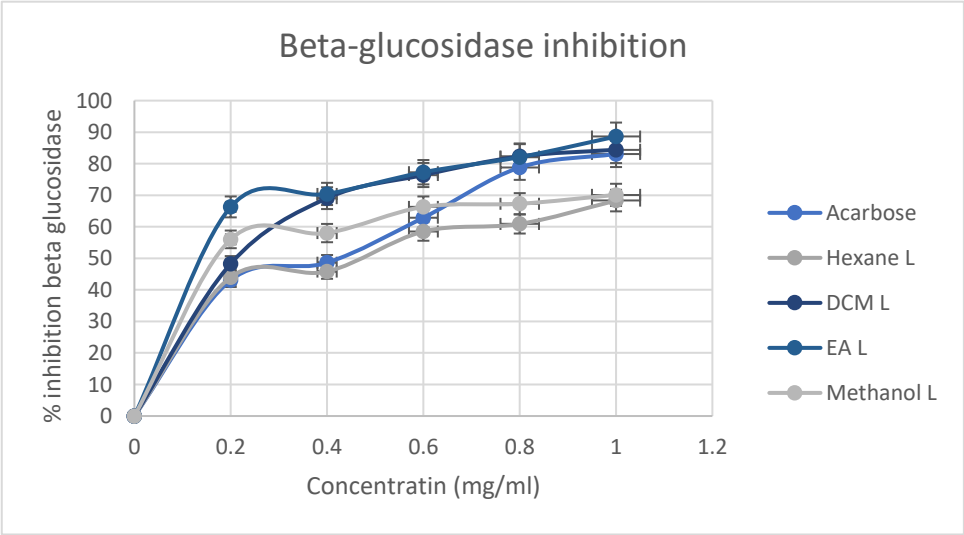


Fig. 5 The α-glucosidase inhibitory activity of *Momordica balsamina* leaf and fruit extracts of different concentrations. L: Leaves

IC50 values of enzymes against standard *Momordica balsamina Linn*. extracts

The half-maximal inhibitory concentration (IC_{50}) values of the extracts and acarbose were determined (table 3). All extracts displayed lower IC_{50} values (0.106–0.204 mg/mL) compared to acarbose (IC_{50} = 0.209 mg/mL), with the DCM L extract exhibiting the lowest IC_{50} (0.106 mg/mL). The lower IC_{50} values indicate that *Momordica balsamina* extracts possess strong α-amylase inhibitory activity, suggesting their potential as effective antidiabetic agents that may perform comparably or even superior to existing pharmaceutical treatments.

The half-maximal inhibitory concentration (IC_{50}) values, calculated and presented in Table 2, revealed that acarbose exhibited the lowest IC_{50} value of 0.129, compared to *Momordica balsamina* extracts, which ranged from 0.31 to 0.533. Among the extracts, the DCM fruit and leaf extracts showed the lowest IC_{50} values (0.31), followed by the EA L extract with an IC_{50} of 0.374. While the differences in IC_{50} values between acarbose and the extracts may not be statistically significant, the results suggest promising inhibitory activity. Further isolation of active compounds is warranted, as the antidiabetic effects of *Momordica balsamina* may be attributable to a specific compound or a synergistic combination of bioactive constituents.

The extracts' varying IC_{50} values are indicative of variations in their phytochemical makeup. Being a somewhat polar solvent, EA probably removes a variety of bioactive substances, including tannins, phenolic acids, and flavonoids, which are especially good in blocking β-glucosidase. Despite being polar, the methanol extract is marginally less effective than the DCM extract, which is similarly moderately polar and exhibits strong inhibitory potential. On the other hand, the hexane extract, which was made with a non-polar solvent, might not have enough of these active polar components, which would raise the IC_{50} value.

The IC_{50} of acarbose, a well-known pharmacological inhibitor of glucosidase enzymes, is 0.464 mg/mL. Both the DCM L (0.367 mg/mL) and EA L (0.301 mg/mL) extracts exhibit lower IC_{50} values than acarbose, which implies that they may have similar or perhaps better inhibitory effects. This suggests that the plant extracts may have therapeutic value, especially if they also have a good safety record.

Table 3 The IC50 values of the standard and various *Momordica balsamina* extracts

Standard and samples	**IC_{50}**		
	a-amylase	**a-glucosidase**	**b-glucosidase**
Acarbose	0.209	0.129	0.464
Hexane L	0.156	0.475	0.565
DCM L	0.106	0.31	0.367
EA L	0.204	0.374	0.301

Methanol L	0.176	0.463	0.447

*IC50, Half maximal Inhibitory Concentration

In-dichloromethane and hexane extracts proposed antidiabetic compounds

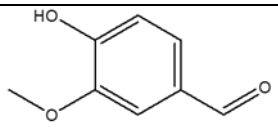
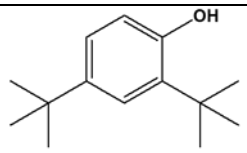
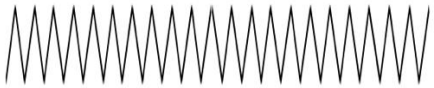
Table 4 below reports the proposed antidiabetic compounds for In-dichloromethane and hexane extracts. A study by [25] has demonstrated vanillin to inhibit the α-glucosidase enzyme activity, with the inhibitory effect positively correlated with the concentration of vanillin. Vanillin successfully bound to α-glucosidase with a binding free energy of -8.42 Kcal/mol, indicating good binding (-7 to -9 Kcal/mol), and this suggests a good potential for managing postprandial hyperglycemia, a key factor in controlling type 2 diabetes. These results provide credence to vanillin's function of inhibiting the digestion and absorption of carbohydrates, thereby lowering postprandial blood glucose level.

Compound 2,4-Di-tert-butylphenol has been reported to inhibit α-amylase activity, making it a potential contributor to the antidiabetic properties of the DCM extract. This compound has been proven to possess high binding affinity for the human pancreatic α-amylase, suggesting its role in inhibiting starch breakdown and further glucose absorption. The compound further displayed improved metabolism, a skin permeability of −3.87 cm/s, gastrointestinal absorption of 95.48 %, and a total clearance of 0.984 log ml min^{-1} kg^{-1} indicating its pharmacokinetic profile and promising bioavailability [26].

As per the discussion above, vanillin helps reduce postprandial glucose levels by inhibiting α-glucosidase, whereas 2,4-Di-tert-butylphenol modifies starch digestion by decreasing α-amylase. These findings therefor suggests a synergistic interaction of the two compounds identified in DCM extract. The dual action of the two compounds support the promising potential of DCM extract as a successful therapeutic strategy for the management of diabetes and hyperglycemia. In addition, vanillin's antioxidant qualities and 2,4-Di-tert-butylphenol's anti-inflammatory effects may enhance their antidiabetic action by reducing inflammation and oxidative stress, which worsen insulin resistance in diabetics.

The GC-MS analysis of hexane leaf extract revealed the presence of various long chain alkanes as mentioned on section 3.2.1 in table 1 of the article above. Of all the alkanes identified, is only one compound has been reported to directly influence the antidiabetic properties of a plant extract. A study by Fayez and co-workers has demonstrated that the compound Tetra tetracontane has the ability to inhibit the activity of α-amylase and α-glucosidase supported by molecular docking with the binding energy of -7kca/mol for α-amylase and -12.1kcal/mol for α-glucosidase, indicating moderate to good binding affinity [27]. The two enzymes play separate roles in lowering the postprandial hyperglycemia, a therapeutic target in the management of diabetes. The absence of antidiabetic activity in other identified compounds might suggest that they are not yet studied/reported or tetra tetracontane contributes largely to the antidiabetic potential of hexane extract or rather some minor constituents were not detected.

Table 4 In-dichloromethane and hexane extracts proposed antidiabetic compounds. The structures in the table were drawn by the authors using ChemDraw Ultra 8.0 (Chemistry Software Ltd Gateways, Surrey, UK).

Proposed antidiabetic compounds in DCM extract			
Rt (min)	**Compound name**	**Structure**	**Antidiabetic mechanism and Ref**
17.191	Vanillin		α-glucosidase activity inhibition [25].
17.528	2,4-Di-tert-butylphenol		α-amylase activity inhibition [26]
Proposed antidiabetic compounds in hexane extract			
31.666	Tetra tetracontane		α-amylase and α-glucosidase activity inhibition [27].

In-ethyl acetate and methanol extracts proposed antidiabetic compounds

For EA L extract, as reported on table 5 below, the identified compound propylene glycol has been reported to inhibit the activity of enzyme α-glucosidase. Inhibition by propylene glycol was concentration dependent with a highest inhibition of 87.79 ± 1.86% at a concentration of 800µg/mL [28]. A study by Mhya and co-workers, (2021), demonstrated that compound 2-Methoxy-4-vinylphenol, a phenolic compound, can inhibit both the activity of α-amylase and α-glucosidase. The study further performed molecular docking against these two enzymes and the compound exhibited binding energies of -4.4 kcal/mol (α-amylase) and -4.2 kcal/mol (α-glucosidase) against standard drug acarbose with -3.9 (α-amylase) and -4.8kcal/mol (α-glucosidase), indicating moderate binding affinity [29]. Another compound, phytol, may be a contributor to the antidiabetic effect of EA extract, showing good binding interaction with protein PPAR- γ (binding energy of -7.27kca/mol) that its upregulation has been reported to alleviate insulin sensitivity and glucose uptake, therefore, reducing blood glucose levels [30].

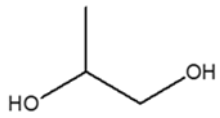
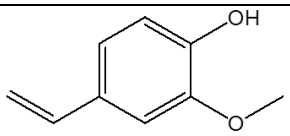
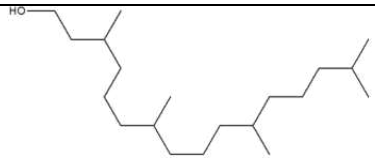
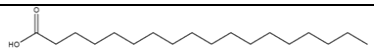
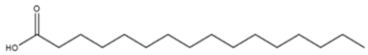
Octadecanoic acid, a fatty acid commonly known as stearic acid, has shown good protein tyrosine phosphatase 1B (PTP1B) activity inhibition in a study reported by Tsuchiya *et al* [31]. As a potential PTP1B inhibitor, stearic acid can cause stimulation of the insulin receptor signalling, resulting in increased glucose uptake into adipocytes. Another fatty acid, oleic acid has been reported to have a positive impact on the glucose uptake as it also improves the insulin receptor signalling by increasing IRS1 expression in a dose-dependent manner [32].

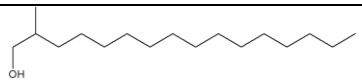
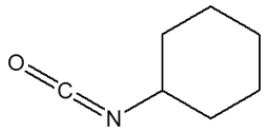
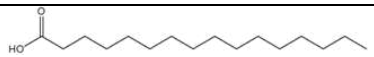
GC-MS results for EA L extract revealed the presence of bioactive compounds with the ability to inhibit key carbohydrates digesting enzymes, α-amylase and α-glucosidase. Inhibition of this enzymes can lead to slow carbohydrate digestion and glucose absorption, thereby, regulating postprandial glucose levels. The results also

revealed the presence of various fatty acids involved in the modulation of proteins responsible for insulin signalling pathway such as insulin receptor substrate (IRS), phosphatidylinositol 3-kinase (PI3K) and Adenosine monophosphate-activated protein kinase (AMPK).

The methanol extract, unexpectedly, demonstrated limited antidiabetic activity across the three assays compared to the other extracts. This suggested that, methanol extract might not possess phytochemical compounds that strongly influence the regulation of glucose. GC-MS results also identified the least number of compounds in methanol (4) than hexane (9), DCM (9) and EA (14). Among the four identified compounds, none of them has been reported to be directly involved in glucose regulation, however, 2-methylhexadecan-1-ol and cyclohexyl isocyanate happens to contribute to the regulation of glucose. According to Adeyemi and co-workers, 2-methylhexadecan-1-ol was isolated as a major compound from an extract that exhibited antidiabetic activity in streptozotocin-induced diabetic rats [33]. Similarly, cyclohexyl isocyanate, is used in the synthesis of sulfonylurea derivatives, a class of antidiabetic drugs [34]. The observed results suggest that methanol extract might contain minor phytochemical constituents with potential activity that may be undetectable by GC-MS.

Table 5 In-ethyl acetate and methanol extracts proposed antidiabetic compounds. The structures in the table were drawn by the authors using ChemDraw Ultra 8.0 (Chemistry Software Ltd Gateways, Surrey, UK). [28-35].

Retention time	**Compound name**	**Compound structure**	**Antidiabetic mechanism and Reference.**
7.180	Propylene glycol		α-glucosidase activity inhibition [28]
15.227	2-Meyhoxy-4-vinylphenol		α-amylase and α-glucosidase activity inhibition [29]
22.953	Phytol		Increase insulin sensitivity [30].
24.043	Octadecanoic acid		Protein tyrosine phosphatase 1B (PTP1B) activity inhibition [31].
22.764	Oleic acid		Enhance insulin receptor protein-1 (IRS1) pathway [32].
Proposed antidiabetic compounds in methanol extract			

22.622	2-methylhexadecan-1-ol		Glucose regulation [33].
9.461	Cyclohexyl isocyanate		Sulfonylureas synthesis precursor [34].
22.293	Hexadecanoic acid		Enhance PI3K/AMPK/Akt and PI3K /ERK1/2 activity [35].

Cytotoxicity

Cytotoxicity testing was performed only on the DCM L and EA L extracts based on their ability to inhibit enzymes involved in hyperglycaemic conditions and the fact that they contained the highest number of antidiabetic compounds as identified by GC-MS analysis. Given limited resources and the need for focused toxicity profiling, it made sense to focus the toxicity test on extracts with promising therapeutic potential. Additionally, these types of extracts contains mid to non-polar bioactives which are usually toxic, warranting early safety assessment.

The MTT assay was based on the ability of mitochondrial dehydrogenases in viable cells to reduce MTT, converting it from a yellow tetrazolium salt into insoluble purple formazan. The cytotoxic effect of the test samples can be classified based on the proportion of viable cell as follows : > 80% = non-cytotoxic, 60% - 80% = weakly cytotoxic, 40% - 60% = moderately cytotoxic, and < 40% = highly cytotoxic [36]. Figure 7 indicates the positive and negative controls for the experiment. The study's negative control consisted of untreated cells (Figure 7, left), and it was assumed that the cells were 100% viable. The positive control demonstrated an inverse relationship between concentration and cytotoxicity, indicating that hydrogen peroxide, a known cytotoxin, is toxic to the Vero cell lines. The cell viability percentages ranged from 38% to 46%. These results demonstrate the importance of concentration in assessing the cytotoxicity of plant extracts. Lower concentrations are frequently less cytotoxic and preserve greater cell viability, whereas higher concentrations can have noticeable cytotoxic effects. Figure 6 below, demonstrates on the left, a negative control whereby only the medium was added to the cells, and cell survival remained at 100% cell viability, meaning no cells died. On the right, figure 6 further demonstrate a positive control, whereby the cells were treated with hydrogen peroxide of different concentrations and cell viability reduced at all concentrations with more reduction of up to 39% at the highest concentration of 1000µg/ml.

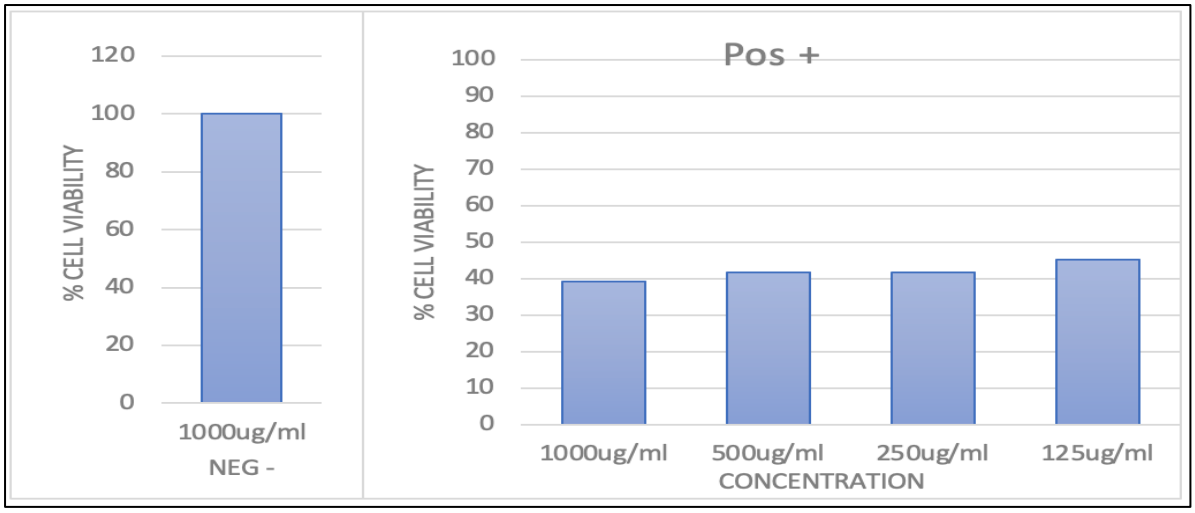


Fig. 6 Cell survival following treatment of Vero cells for 48 h with the positive control and nothing was added on the negative control thus it was assumed that there is 100% cell survival

Cytotoxicity evaluation of DCM and EA leaf extracts

In contrast to the cytotoxic hydrogen peroxide, the cytotoxic assessment of DCM and EA leaf extracts in Figure 7 below revealed that both extracts are non-cytotoxic across all tested concentrations (1000–125 µg/mL), as cell viability remained above 80% in all cases.

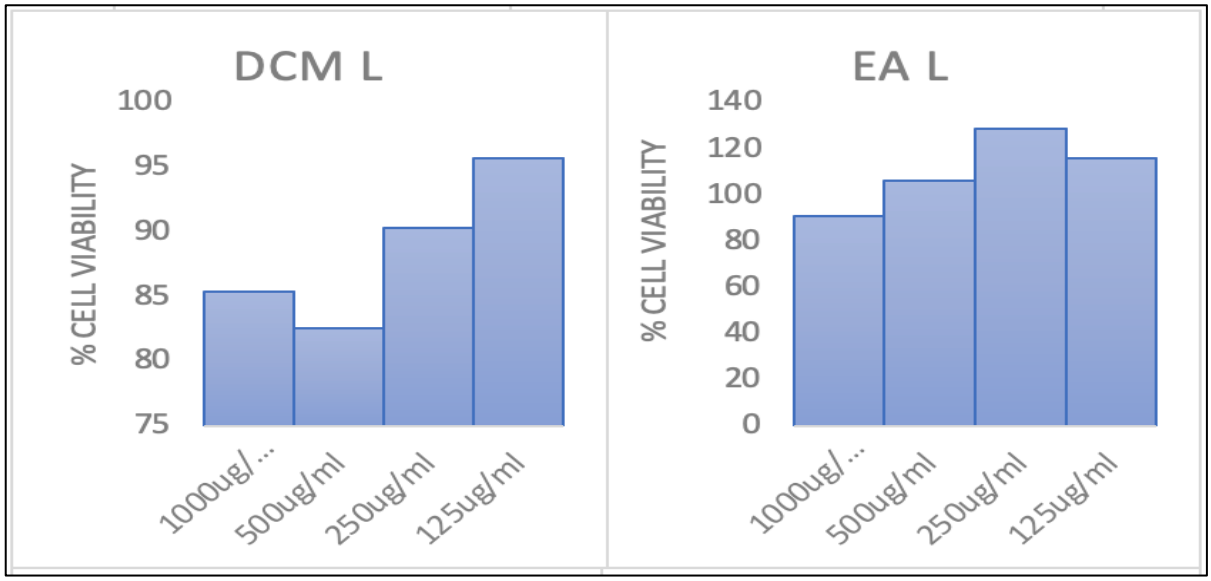


Fig. 7 Cell survival following treatment of Vero cells for 48 h with DCM leaf extract (left) and EA leaf extract (right) as determined by the MTT assay. L: Leaves

According to figure 7 above, the DCM extract demonstrated that it is not toxic to Vero cells at any concentration, supported but cell viability of over 80% even at the highest concentration in. The results follow the conventional trend of cytotoxicity whereby percentage cell viability increases as the test concentration decreases. The concentration dependent increase in cell viability ranges from approximately 82% at 500 µg/mL to 95% at 125

µg/mL, supporting the safety of DCM and suggesting good cellular tolerance. On the other hand, EA L extract results deviate from the conventional trend of cytotoxicity, rather, cell viability is above 90% even at the maximum dose (1000 µg/mL), indicating that EA L is not harmful at any quantity that has been tested. Cell vitality surpasses 100% from 500 µg/mL and below, indicating that the extract may not be harmful but rather have a protective impact on the cells. A high % cell viability might indicate that EA L extract promote cellular activity rather than suppress it. These findings suggest that both the extracts are not harmful to the Vero cells, rather, safe to use.

Conclusion

The current study demonstrated the noteworthy in-vitro antidiabetic activity of *Momordica balsamina Linn.* leaf extracts based on their ability to inhibit α-amylase, α-glucosidase, and β-glucosidase. The observed antidiabetic activity may be attributed to bioactive compounds previously reported to possess such properties, which were identified in the extracts through GC-MS analysis. Both the EA L and DCM L leaf extracts exhibited non-cytotoxic responses in the MTT assay against Vero cells, further supporting their potential safety for therapeutic use.

In South Africa, *Momordica balsamina* is traditionally used by many individuals to manage diabetes and is sometimes even recommended by healthcare professionals. These findings therefore support the traditional use of South African *Momordica balsamina* for the treatment and management of diabetes, and highlight its potential for the development of new, plant-based antidiabetic agents.

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Author contributions Buang Matseke: Conceptualization, Investigation, methodology, analysis, writing, original draft preparation. Tswaledi Daniel: methodology, data analysis. Kokoette Bassey: Conceptualization, methodology, supervision, data curation, review and editing, validation. All authors have read and agreed to the published version of the manuscript.

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Data availability The datasets used and analysed in the current study are contained within the article.

Declarations

Ethical approval This study does not contain any studies involving human and animal subjects, therefor, ethical approval is not applicable.

Consent for publication Not applicable in this study

Clinical trial number Not applicable in this study

Conflict of interest All the authors declare no conflict of interest.

Plant collection permission The land owner gave us permission to collect the pant in his yard**.** No specific procedure was followed.

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