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Research Article

# LC-MS/MS-BASED METABOLOMIC CHARACTERIZATION AND ANTIDIABETIC POTENTIAL OF ETHANOLIC EXTRACT OF THE *IPOMOEA MOMBASSANA* VATKE STEM

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**ABSTRACT**

**Objectives:** *Ipomoea mombassana Vatke* is a lesser-known species of the genus Ipomoea, with no prior reports on its phytochemical or pharmacological properties. This study investigated the phytochemical profile, enzyme inhibition, antioxidant activity, and antidiabetic potential of its stem extracts.

**Methods:** Stems were successively extracted with solvents of increasing polarity, and yields were determined. The ethanolic *I. mombassana* stem extract (IMSE) was selected for detailed evaluation. Phytochemical screening was performed, and *in vitro* α-amylase and α-glucosidase inhibition assays (100–500 µg/mL) were carried out. Liquid chromatography-mass spectrometry (LC-MS/MS) analysis was conducted using an Agilent 1290 Infinity II LC system with 6550 iFunnel Q-TOF in positive electrospray ionization mode. *In vivo* activity was assessed in Streptozotocin-induced diabetic rats treated with IMSE (100 and 200 mg/kg) for 21 days, compared with normal, diabetic control (DC), and Metformin groups.

**Results:** IMSE exhibited strong α-amylase ($IC_{50}$=45.78±0.18 µg/mL) and α-glucosidase inhibition (42.26±0.97 µg/mL) compared to acarbose (34.4±0.56 and 32.95±0.45 µg/mL, respectively). LC-MS/MS identified 42 compounds, including pilocarpine, maritimetin, and ganoderic acid F. *In vivo*, IMSE significantly reduced fasting blood glucose from 344±1.6 mg/dL in DCs to 198±2.1 mg/dL at 200 mg/kg ($p<0.001$). It also improved lipid profiles by lowering total cholesterol, triglycerides, and low-density lipoprotein cholesterol while elevating high-density lipoprotein cholesterol ($p<0.01$–$0.001$). Liver enzyme levels were normalized (serum glutamate oxaloacetate transaminase: 71±1.6→52±1.1 U/L; serum glutamate pyruvate transaminase: 87±2.2→55±1.5 U/L; $p<0.001$). Antioxidant markers were restored, including superoxide dismutase (1.3±0.03→5.4±0.30 U/mL) and glutathione (0.32±0.056 → 1.7±0.069 mg/dL; $p<0.001$).

**Conclusion:** The ethanolic extract of *I. mombassana* demonstrated significant enzyme inhibition and hypoglycemic effects, accompanied by improvements in lipid metabolism, hepatic function, and antioxidant defense, supporting its potential as a natural therapeutic for diabetes management.

**Keywords:** Antioxidant enzymes, Diabetes mellitus, High-resolution liquid chromatography-mass spectrometry, *Ipomoea mombassana* Vatke stem, Metformin and streptozotocin.

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## INTRODUCTION

*Ipomoea mombassana* Vatke, a member of the *Convolvulaceae* family, belongs to the diverse genus *Ipomoea*, which is predominantly found in tropical and subtropical climates worldwide [1]. Originally discovered in 1882 in Mombasa, Kenya, where it was first described, this species is commonly found along Kenya's coastal regions. It was later reported in the dry deciduous forests of Chinnar, Kerala, by Biju [2]. According to Pullaiah [3] *et al*., there are 18 species of the genus Ipomoea in Telangana State, but Reddy [4] *et al*. found just 14. Conventionally, various *Ipomoea* species have been used in folk medicine for managing numerous health conditions. The identification of *I. mombassana* in Telangana marks a significant addition to the region's floral diversity, representing a new distributional record for the state [5].

Despite its taxonomic documentation, the pharmacological and phytochemical properties of *I. mombassana* Vatke remain scientifically uncharacterized. This study aims to bridge that knowledge gap by evaluating the bioactive potential of its stem extracts. The selection of appropriate extraction solvents is critical in phytochemical investigations [6], as it directly influences the composition and efficacy of the isolated constituents. *In vitro* assays, particularly those targeting α-amylase inhibition play a pivotal role in the preliminary screening of plant-derived compounds for therapeutic relevance [7]. These assays offer a controlled and efficient method for assessing the potential of natural products to modulate specific biological targets, such as those implicated in hyperglycemia and other metabolic disorders.

Inhibitors of carbohydrate-digesting enzymes, particularly α-amylase and α-glucosidase, play a pivotal role in managing postprandial hyperglycemia by slowing the breakdown of dietary starch and delaying glucose absorption from the gut [8]. Clinically used enzyme inhibitors such as acarbose, voglibose, and miglitol target both pancreatic α-amylase and intestinal α-glucosidase but are frequently associated with gastrointestinal side effects, including flatulence, bloating, and diarrhea [9]. Due to these adverse effects, there is growing interest in safer, plant-derived enzyme inhibitors. Several botanical sourcesincluding *Salacia reticulata*, [10] *Salacia oblonga*, [11] *Syzygium cumini*, *Momordica charantia*, and *Trigonella foenum graecum* have demonstrated promising α-amylase and α-glucosidase inhibitory activities, primarily attributed to their polyphenolic and flavonoid constituents. Investigating *I. mombassana* within this context may offer a novel, natural source of dual enzyme inhibitors with therapeutic potential for diabetes management.

Plants synthesize a wide range of specialized metabolites that are crucial for their protection, communication, and adaptation to external stresses. These non-primary compounds, such as terpenes,

phenolic acids, alkaloids, and flavonoids, are highly valued in both medicinal and commercial contexts due to their diverse bioactivities. To explore and characterize these metabolites, liquid chromatography–mass spectrometry (LC-MS) has become an essential analytical technique. LC-MS integrates the effective separation capabilities of liquid chromatography with the high sensitivity and specificity of mass spectrometry, enabling the identification of complex chemical profiles in plant extracts, even at trace concentrations. Through detailed mass analysis, including fragmentation behavior and spectral database matching, this method facilitates accurate structural characterization [11]. Moreover, LC-MS-driven metabolomic approaches allow researchers to monitor shifts in plant metabolite production under different environmental or physiological conditions, supporting the identification of novel therapeutic candidates [12]. The precision and depth of data provided by LC-MS have made it a cornerstone in natural products research and the expanding field of plant-based pharmaceutical development.

Diabetes is a disease in which blood sugar levels stay high for a long time, and insulin is not made or used properly. As of 2019, global diabetes prevalence was estimated at 9.3% of adults, or 463 million people, and is projected to rise to 10.9% by 2045 in parallel with global population growth. According to official data, [13] diabetes affects 4% of adults in Nepal and is responsible for 696,900 deaths and 696,900 cases of disability. Diabetes is a disease that affects hormones and metabolism. Type 2 diabetes is present if the fasting blood glucose level is 7 mmol/L or higher, or if anyone takes medicine for high blood glucose. This complicated disease causes problems with the breakdown of carbohydrates, fats, and proteins, which leads to changes and problems in both small and large blood vessels [14]. Long-term consequences include cardiovascular disease, stroke, nephropathy, limb amputation, retinopathy, and neuropathy [15]. It is possible to stop carbohydrate-hydrolyzing enzymes in the digestive system, such as α-glucosidase and α-amylase, from working. This slows down the absorption of glucose and keeps blood sugar levels stable and low [16].

Among them, α-amylase (EC 3.2.1.1), also known as α-1,4-glucan-4-glucanohydrolase, is a widely distributed hydrolase present in microbes, plants, and animals [17,18]. It is a metalloenzyme that requires at least one $Ca^{2+}$ ion for catalytic activity and structural stability [19]. Porcine pancreatic α-amylase, composed of 496 amino acid residues, shows 83% sequence similarity with human pancreatic α-amylase, making it a reliable experimental model. In humans, α-amylase is secreted in both saliva and pancreatic juice, where it hydrolyzes α-1,4-glycosidic bonds in starch and glycogen, producing maltose and glucose that are rapidly absorbed into the bloodstream [20,21]. Due to this central role in postprandial glucose metabolism, inhibition of α-amylase is considered an effective therapeutic approach for controlling hyperglycemia, as it slows starch digestion and attenuates rapid increases in blood glucose levels."

Therefore, the present study was designed to evaluate the phytochemical composition of *I. mombassana* stem extracts using LC-MS and to investigate there *in vivo* antidiabetic and antioxidant potential, alongside *in vitro* inhibitory activities against α-amylase and α-glucosidase, thereby exploring their therapeutic relevance in diabetes management.

## METHODS

### Collection of plant material, chemicals, and drugs

In August 2023, specimens of *I. mombassana* Vatke (Convolvulaceae) were collected from uncultivated land in Palamkula village, Ranga Reddy district, Telangana, India. The species was identified using standard taxonomic literature and authenticated by Dr. A. Vijaya Bhasker Reddy, Assistant Professor, Department of Botany, University College of Science, Osmania University, Hyderabad, Telangana. A voucher specimen (OUAS-148) was deposited in the departmental herbarium. Stems were shade-dried, powdered, and stored in airtight containers at room temperature. Ethanol (Bio Liqua Research Pvt Ltd, Bangalore, India), n-hexane, ethyl acetate, and chloroform (Molychem, Mumbai, India) were used for successive extraction. Streptozotocin (STZ), saccharomyces cerevisiae, p-Nitrophenyl-α-D-glucopyranoside (pNPG), acarbose, 0.1M phosphate buffer (pH 6.9), 1M sodium carbonate, methanol, and metformin (Sigma-Aldrich, Bangalore, India) were used for pharmacological studies. UV-Visible spectrophotometer (Shimadzu) and colorimeter (Elico) instruments were used. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) under approval number CCSEA/IAEC/JLS/21/04/017. Male Wistar rats (n=6 per group), aged 7 weeks and weighing 160–200 g, were housed under standard laboratory conditions (22–24°C, 12 h light/dark cycle).

### General procedure

*Extraction*

The stem powder (150 g) of *I. mombassana* was extracted using a Soxhlet apparatus successively with n-hexane, chloroform, ethyl acetate, and ethanol, at temperatures close to the boiling point of each solvent. All extracts were filtered and evaporated to dryness in a rotary evaporator and stored at 4°C [22].

*Phytochemical analysis*

Preliminary qualitative analysis of the stem ethanolic extract was used for the preliminary screening of phytochemicals such as alkaloids, carbohydrates, glycosides, saponins, proteins, phytosterols, terpenoids, fixed oils, phenolic compounds, flavonoids, and tannins [23].

### *In vitro* studies

*α-amylase inhibitory activity*

Successive solvent extracts of *I. mombassana* stem (hexane, chloroform, ethyl acetate, and ethanol) were prepared and evaluated for α-amylase inhibitory activity. Each extract was tested at concentrations ranging from 100 to 500 µg/mL. The assay was performed using the 3,5-dinitrosalicylic acid colorimetric method, which quantifies the amount of reducing sugars released from starch hydrolysis. Acarbose was used as the standard positive control, while appropriate solvent blanks served as negative controls. The percentage inhibition was calculated, and $IC_{50}$ values were determined for each extract using dose-response curves generated from absorbance readings [24].

*α-glucosidase inhibitory activity*

Using pNPG as the substrate, the α-glucosidase inhibitory activity was measured using a slightly modified version of the method [25]. In short, 50 µL of α-glucosidase (1 U/mL in phosphate buffer) and 250 µL of 0.1 M phosphate buffer (pH 6.9) were combined with 100 µL of the plant extract or acarbose (made in methanol; 20–100 µg/mL) to get the final concentration. For 20 min, the mixture was pre-incubated at 37°C. Following pre-incubation, the reaction was started by adding 10 µL of 10 mM pNPG, which had been made in the same buffer. For half an hour, the mixture was incubated at 37°C. The addition of 650 µL of 1 M $Na_2CO_3$ stopped the process. The absorbance of the released p-nitrophenol was measured at 405 nm using a spectrophotometer. All experiments were conducted in triplicate. Detailed inhibition values at each concentration for all extracts are presented in Supplementary Tables S1-S4.

$$\text{Inhibition } (\%) = \frac{(Abs_{\{control\}} - Abs_{\{sample\}})}{Abs_{\{control\}}} \times 100$$

### Detection method

*LC/MS/MS metabolite profiling of ethanolic extract of I. mombassana stem extract (IMSE)*

High-resolution LC-MS analysis of the ethanolic extract was conducted at the sophisticated analytical instrument facility, Indian Institute of Technology Bombay, for metabolite profiling and impurity characterization. The analysis was performed using an Agilent 1290 Infinity II UHPLC system coupled with an Agilent 6550 iFunnel Q-TOF mass spectrometer equipped with an electrospray ionization (ESI) source operating in positive ion mode. Chromatographic separation

was achieved on an Agilent InfinityLab Poroshell 120 EC-C18 column (3.0×100 mm, 2.7 μm) maintained at 40°C in a thermostated column compartment (G1316C). The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B), delivered using a gradient program: 5% B (0 min) to 100% B (25 min), held for 5 min, and re-equilibrated to 5% B by 35 min, at a flow rate of 0.3 mL/min. The injection volume was 5 μL, and the system pressure was capped at 1200 bar.

Mass spectrometric detection was performed in AutoMS/MS mode across a mass range of m/z 120–1,200. MS and MS/MS spectra were acquired at a scan rate of 1 spectrum/sec, with collision energies ramped between 15 and 50 eV. ESI source conditions included a drying gas temperature of 250°C, sheath gas temperature of 300°C, drying gas flow of 13 L/min, sheath gas flow of 11 L/min, and nebulizer pressure of 35 psi. The capillary and nozzle voltages were set at 3500 V and 1000 V, respectively. Precursor ions were selected based on intensity, with active exclusion enabled to enhance detection of less abundant compounds. Compound identification was performed using METLIN and Agilent PCDL libraries within the MassHunter platform. Identification confidence was assigned following the Metabolomics Standards Initiative [26].

*In vivo studies*

Dose selection was guided by acute oral toxicity data (Organization for Economic Co-operation and Development [OECD] 423) demonstrating safety up to 2,000 mg/kg. Accordingly, doses of 100 and 200 mg/kg (1/20 and 1/10 of the maximal non-lethal dose) were selected for efficacy evaluation, with a higher 500 mg/kg dose added to probe dose-response effects within the non-toxic range. Related studies such as the use of 400–600 mg/kg of *I. batatas* extract in hypercholesterolemic rats [27] and even higher doses for anti-atherosclerosis models up to 2,500 mg/kg [28], support these choices as well within the pharmacologically relevant spectrum. Animal behavior was closely monitored during the 1st h and periodically over the following 14 days, with continuous observation for the first 4 h and subsequent checks at 6, 24, and 48-h post-administration [29]. For diabetes induction, all rats fasted for 6–8 h (with free access to water) before receiving an intraperitoneal (i.p.) injection of STZ at 65 mg/kg body weight, prepared in 50 mM sodium citrate buffer (pH 4.5). Control rats received an equivalent volume of citrate buffer. Post-injection, rats were returned to their cages with access to 10% sucrose water, which was replaced with regular water on the 2nd day [30]. Rats were divided into seven groups: Group I (normal control), Group II (diabetic control [DC]), Groups III and IV (IMSE-treated at 100 and 200 mg/kg, respectively), and Group V (metformin-treated at 100 mg/kg). Treatments were administered daily for 21 days, during which fasting blood glucose levels were monitored through tail vein sampling using a One Touch glucometer. Body weights were also regularly recorded. On day 21, blood was collected under ether anesthesia from the retro-orbital sinus, and serum was separated by centrifugation (10,000 g, 10 min). Biochemical analysis included triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), with low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) calculated using Friedewald's formula [31]. Liver enzyme markers glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase were also assessed. Antioxidant status was evaluated by measuring serum levels of superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), and malondialdehyde (MDA). On the final day, a histopathological examination of pancreatic tissues was conducted. Tissues were fixed in 10% formaldehyde, embedded in paraffin, sectioned at 3–5 μm using a microtome (Finesse 325, Thermo Scientific, Runcorn, UK), stained with hematoxylin and eosin (H&E), and examined under ×200 and ×400 magnification, following the procedure of Kumar and Mitra [32].

**Statistical analysis**

GraphGraphPad Prism was used to do statistical analyses. The statistically significant differences across all groups were examined using an analysis of variance test. Differences were deemed significant when the $p<0.05$, and the Tukey's test was utilized for this purpose.

## RESULTS

**Extraction yield**

Successive extraction of 150 g of *I. mombassana* stem powder was carried out sequentially with n-hexane, chloroform, ethyl acetate, and ethanol. The percentage yield (% w/w) of each solvent extract is presented in Table 1. As expected in successive extraction, the yields differed depending on solvent polarity, with non-polar solvents producing lower yields compared to polar solvents. Among the extracts, the chloroform extract gave the maximum yield, whereas the hexane extract showed the least, reflecting the differential solubility of phytoconstituents across solvents of increasing polarity.

**Preliminary phytochemical analysis**

Preliminary phytochemical screening of the IMSE indicated the presence of a diverse array of bioactive constituents, including alkaloids, carbohydrates, glycosides, saponins, terpenoids, phenolic compounds, flavonoids, and tannins.

**Enzyme inhibition assays**

*α-Amylase inhibition activity of Ipomoea mombasana stem*

Out of all the solvent extracts of *I. mombassana* stem exhibited concentration-dependent inhibition of α-amylase activity, the ethanolic extract showed the most potent effect, with an $IC_{50}$ of 45.78±0.18 μg/mL, followed by ethyl acetate (50.05±1.4 μg/mL), hexane (61.32±0.88 μg/mL), and chloroform (67.06±2.1 μg/mL). In comparison, the standard inhibitor acarbose displayed the lowest $IC_{50}$ (34.4±0.56 μg/mL). These results are summarized in Table 2. The assay was validated using appropriate positive and negative controls, and the reduction in absorbance in extract-treated samples confirmed effective α-amylase inhibition.

*α-glucosidase inhibitory activity of Ipomoea mombasana stem*

The ethanolic extract exhibited the strongest activity ($IC_{50}$=42.26±0.97 μg/mL), followed by ethyl acetate (47.26±0.16 μg/mL), hexane (57.76±0.08 μg/mL), and chloroform (61.4±0.76 μg/mL). Acarbose again showed the highest potency ($IC_{50}$=32.95±0.45 μg/mL). The maximum inhibition at the tested concentration of 500 μg/mL reached ~100% for all extracts

**Table 1: Successive extraction yields (% w/w) of *Ipomoea mombassana* stem powder using solvents of increasing polarity**

| S. No. | Fractions | Yield of extract (g) | Yield of extract (%w/w) |
|---|---|---|---|
| 1 | Hexane | 0.86 | 1.72 |
| 2 | Chloroform | 1.81 | 3.62 |
| 3 | Ethyl acetate | 1.62 | 3.24 |
| 4 | Ethanol | 1.2 | 2.4 |

Values represent the mean of three independent experiments (n=3). Data are expressed as actual yields without statistical analysis

**Table 2: Inhibitory activity of plant extracts against α-amylase and α-glucosidase ($IC_{50}$ values, μg/mL)**

| Extract | α-amylase $IC_{50}$ (μg/mL, mean±SD) | Max inhibition at 500 μg/ mL (%) | α-glucosidase $IC_{50}$ (μg/mL, mean±SD) | Max inhibition at 500 μg/ mL (%) |
|---|---|---|---|---|
| Hexane | 61.32±0.88 | 100 | 57.76±0.08 | 100 |
| Chloroform | 67.06±2.1 | 100 | 61.4±0.76 | 100 |
| Ethyl acetate | 50.05±1.4 | 100 | 47.26±0.16 | 100 |
| Ethanol | 45.78±0.18 | 100 | 42.26±0.97 | 100 |
| Acarbose | 34.4±0.56 | 100 | 32.95±0.45 | 100 |

Values are expressed as mean±SD of three independent experiments (n=3).
SD: Standard deviation

after blank correction and normalization. The comparative $IC_{50}$ values for both enzymes are presented in Table 2, highlighting the strong inhibitory potential of ethanolic and ethyl acetate extracts of *I. mombassana*.

The full inhibition datasets are given in Supplementary Tables S1-S4.

*Metabolite profiling of the ethanolic extract using LC-MS/MS*

Forty-two unique molecules with $[M+H]^+$ values ranging from m/z 123.08 to 607.28, indicating a structurally varied metabolite pool, were identified using a thorough LC-MS/MS study, and the identified compounds are given in Table 3. The LC/MS/MS chromatogram is given in Fig. 1.

Alkaloids, flavonoids, terpenoids, peptides, phenolic derivatives, and nitrogen-containing aromatics were among the chemical classes to which the detected substances belonged and given in Fig. 2, with their MS spectrum and structure, respectively.

The ability to neutralize reactive oxygen species and reduce oxidative stress was demonstrated by a number of flavonoids and phenolic compounds, including maritimetin (m/z 287.05), hesperetin 7-O-glucuronide (m/z 499.12), maculosin (m/z 283.10), perlolyrine (m/z 265.09), and mahaleboside (m/z 325.08). The main components in the antidiabetic category were quinolin-2-ol (m/z 146.05), which was reported to have both antidiabetic and anticancer properties; fabianine (m/z 220.16), which was linked to insulin-mimetic and antioxidant activity; and ganoderic acid F (m/z 593.27), a triterpenoid with hepatoprotective and glucose-lowering effects. Pilocarpine (m/z 231.11) and lysergic acid (m/z 269.12), two neuroactive substances found in the alkaloid group, contributed to the extract's cholinergic and serotonergic effects. Furthermore, phytosphingosine (m/z 318.29) showed both antibacterial and anti-inflammatory qualities, and dihydrocapsaicin (m/z 308.11) and euphornin (m/z 607.28) were found to be strong anticancer agents. The extract's medicinal potential is highlighted by its varied chemical composition and numerous bioactive profiles, which are especially useful in the treatment of oxidative stress, metabolic diseases, and neurological illnesses.

**Pharmacological evaluation of IMSE**

*Acute toxicity study*

Acute oral toxicity was assessed according to OECD Guideline 423. The ethanolic extract of *I. mombassana* stem (IMSE) was administered to Wistar rats in increasing doses ranging from 50 to 2,000 mg/kg body weight. Animals were observed continuously for the first 4 h

**Table 3: Representative LC–MS/MS–identified metabolites (10–15 most abundant or biologically relevant) from *Ipomoea mombassana* extracts. The complete list of 42 annotated compounds is provided in Supplementary Table S5**

| S. No. | Retention time (min) | [M+H] *(m/z)* | Error (ppm) | Molecular formula | MS/MS fragment ions *(m/z)* | Identification |
|---|---|---|---|---|---|---|
| 1 | 5.721 | 231.11 | −3.4 | $C_{11}H_{16}N_2O_2$ | 158.09 | Pilocarpine |
| 2 | 6.024 | 287.13 | −0.72 | $C_{14}H_{20}N_2O_3$ | 269.12 | Feruloyl putrescine |
| 3 | 6.029 | 273.12 | −1.31 | $C_{13}H_{18}N_2O_3$ | 195.09 | Caffeoyl putrescine |
| 4 | 6.788 | 269.12 | 5.23 | $C_{16}H_{16}N_2O_2$ | 197.06 | Lysergic acid |
| 5 | 6.928 | 283.10 | 0.57 | $C_{14}H_{16}N_2O_3$ | 195.09 | Maculosin |
| 6 | 7.501 | 227.11 | −4.32 | $C_{12}H_{16}N_2O$ | 209.10 | Bufotenine |
| 7 | 7.942 | 265.09 | 7.36 | $C_{16}H_{12}N_2O_2$ | 206.08 | Perlolyrine |
| 8 | 8.178 | 499.12 | 1.36 | $C_{23}H_{24}O_{11}$ | 163.03 | Hesperetin7-O-glucuronide |
| 9 | 8.588 | 287.05 | 8.93 | $C_{15}H_{10}O_6$ | 258.11 | Maritimetin |
| 10 | 9.408 | 284.12 | 1 | $C_{15}H_{19}NO_3$ | 147.04 | Cochlearine |
| 11 | 9.903 | 309.09 | 2.71 | $C_{13}H_{18}O_7$ | 290.20 | Isosalicin |
| 12 | 9.977 | 308.21 | 10.85 | $C_{18}H_{29}NO_3$ | 179.12 | Dihydrocapsaicin |
| 13 | 14.47 | 318.29 | 8.48 | $C_{18}H_{39}NO_3$ | 282.27 | Phytosphingosine |
| 14 | 24.172 | 593.27 | −1.24 | $C_{32}H_{42}O_9$ | 533.25 | Ganoderic acid F |

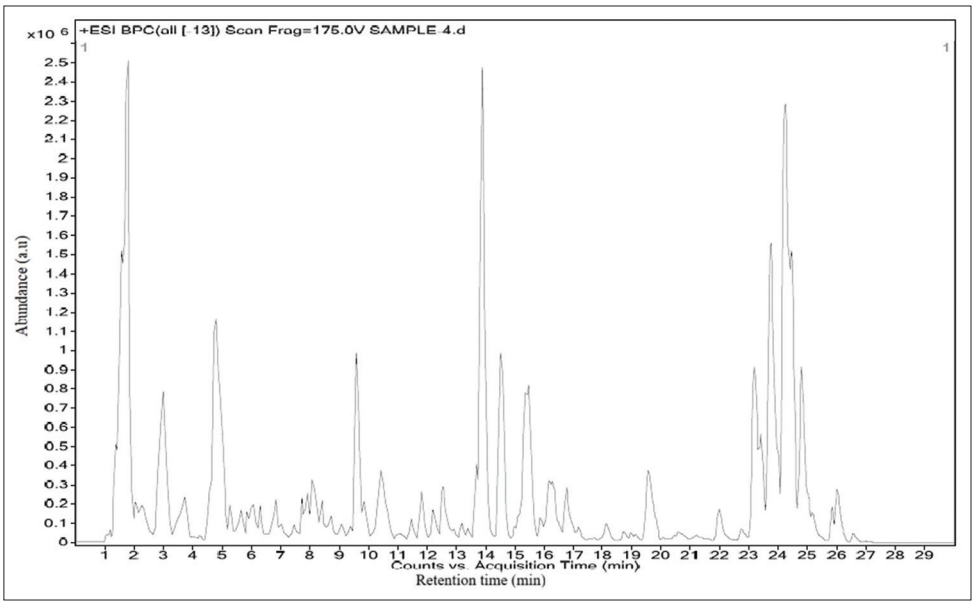


**Fig. 1: Liquid chromatography-mass spectrometry (MS)/MS chromatogram of ethanolic extract *Ipomoea mombassana* Stem**

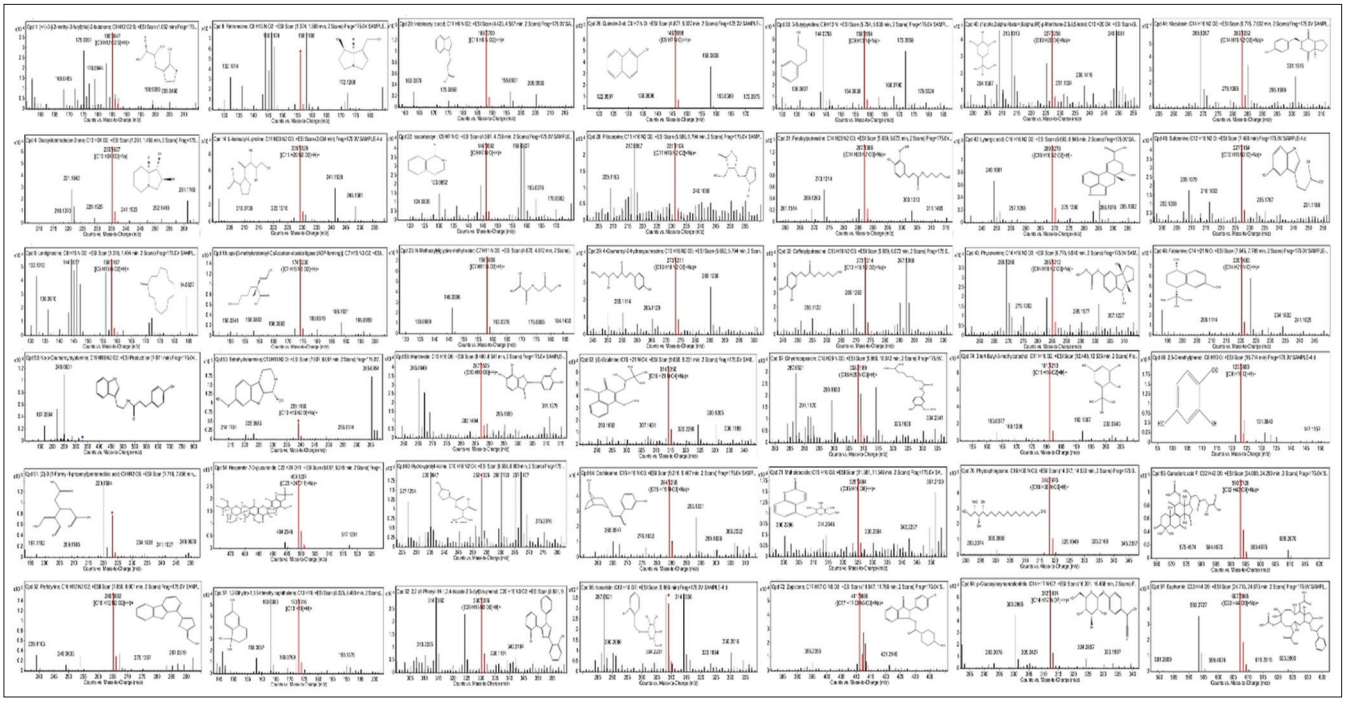

**Fig. 2: Analysis of ethanolic extract *Ipomoea mombassana* stem (IMSE) phytochemicals based on mass spectrometry data and molecular structure interpretation**

post-administration and periodically for up to 21 days. Observational endpoints included signs of toxicity such as changes in skin, fur, eyes, mucous membranes, respiratory and circulatory patterns, autonomic and central nervous system activity, behavioral changes (e.g., tremors, convulsions, salivation, lethargy), and mortality. Body weight was also monitored throughout the study. No deaths or significant toxic signs were observed, indicating that the extract was safe at the tested doses.

*Body weight and serum glucose levels*

Diabetes induction led to a significant reduction in body weight and elevation in serum glucose levels. The results were shown in Figs. 3 and 4. The DC group showed a body weight drop from 335±2.4 g to 228±2.5 g and a rise in glucose levels from 122±1.3 mg/dL to 344±1.6 mg/dL ($p<0.001$). IMSE treatment significantly improved both parameters in comparison to the DC group ($p<0.001$).

*Oral glucose tolerance test (OGTT)*

The OGTT levels were significantly elevated in the DC group (383±2.5 mg-h/dL) compared to the normal control (NC: 261±6.5 mg-h/dL). IMSE-treated groups showed a significant reduction in glucose levels ($p<0.001$). The results are shown in Fig. 5.

*Lipid profile*

DC rats exhibited marked dyslipidemia, characterized by significantly elevated TC, TG, LDL-c, and VLDL-c, along with a marked reduction in HDL-c ($p<0.001$ vs. NC) Fig. 6. Treatment with IMSE (100 and 200 mg/kg) significantly reversed these alterations in a dose-dependent manner ($p<0.01–0.001$). Notably, IMSE at 200 mg/kg and metformin produced the greatest improvement, with a pronounced increase in HDL-c ($p<0.001$) and a significant reduction in VLDL-c ($p<0.01$). These findings demonstrate the potential of IMSE to restore lipid homeostasis in diabetic conditions.

*Liver function enzymes (serum glutamate oxaloacetate transaminase [SGOT] and serum glutamate pyruvate transaminase [SGPT])*

Diabetes significantly elevated SGOT and SGPT levels ($p<0.001$), which were significantly reduced by IMSE treatment ($p<0.01$ to $p<0.001$). The results are shown in Table 4.

**Table 4: Effect of IMSE on SGOT and SGPT levels**

| Group | Treatment | SGOT (U/L) (Mean±SD) | SGPT (U/L) (Mean±SD) |
|---|---|---|---|
| I | NC | 37±1.9 | 48±3.2[a] |
| II | DC | 71±1.6[α] | 87±2.2[α] |
| III | IMSE 100 | 61±1.5[c] | 73±2.8[a] |
| IV | IMSE 200 | 52±1.1[a] | 55±1.5[a] |
| V | Standard group | 48±3.2[a] | 51±2.5[b] |

NC: Normal control, DC: Diabetic control, IMSE 100: *Ipomoea mombassana* stem extract 100 mg/kg; IMSE 200=*I. mombassana* stem extract 200 mg/kg; Values are expressed as Mean±SD (n=6). Statistical significance was determined using one-way ANOVA followed by Tukey's test. [α]$p<0.001$, [β]$p<0.01$, when compared to the NC group; [a]$p<0.001$, [b]$p<0.01$, [c]$p<0.05$ when compared to DC group. SGOT: Serum glutamate oxaloacetate transaminase, SD: Standard deviation, SGPT: Serum glutamate pyruvate transaminase, ANOVA: Analysis of variance

*Antioxidant parameters*

IMSE significantly restored antioxidant enzyme levels affected by diabetes. SOD and CAT levels, which were decreased in the DC group, were significantly elevated post-treatment ($p<0.01–0.001$). GSH levels were also significantly restored ($p<0.001$). Conversely, MDA levels were elevated in the DC group (9.2±0.32 μM) and significantly reduced in the IMSE-treated groups ($p<0.001$), indicating reduced lipid peroxidation. The results are shown in Table 5.

*Histopathological studies*

Control group: When the pancreas of control rats was examined under a microscope after H&E staining, normal cells and the rounded proportions of the Langerhans islets were seen. Prominent nuclei with well-organized lobules are shown in Fig. 7a. In pancreatic slices of untreated diabetic rats, the diabetic group showed small, shrunken islets associated with severe degenerative changes, karyolysis, reduced islet cells, congestion, and inflammatory cell infiltration (Fig. 7b). Diabetes IMLE 100–200 mg, as well as the metformin-treated group. The reversal of STZ-induced tissue damage is demonstrated in Fig. 7c-e, wherein the pancreatic architecture returns to normal, exhibiting β-cell vacuolations, mild islet cell hyperplasia, and pancreatic parenchymal congestion. In the

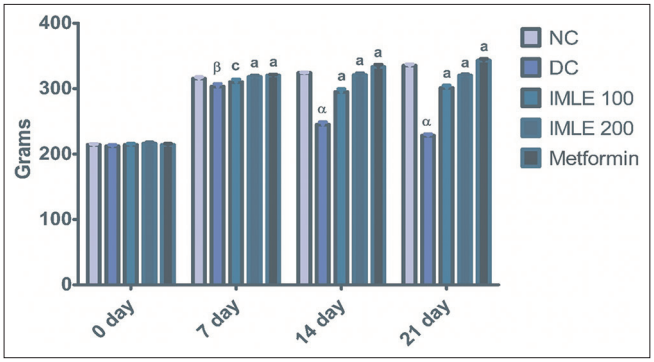


**Fig. 3: Effect of *Ipomoea mombassana* stem ethanolic extract (IMSE) on body weight changes. Value means±standard error of mean; n=6 in each group. Statistical significance was determined using one-way analysis of variance followed by Tukey's test. $^{\alpha}p<0.001$, $^{\beta}p<0.01$, when compared to the NC group; $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$, when compared to DC group**

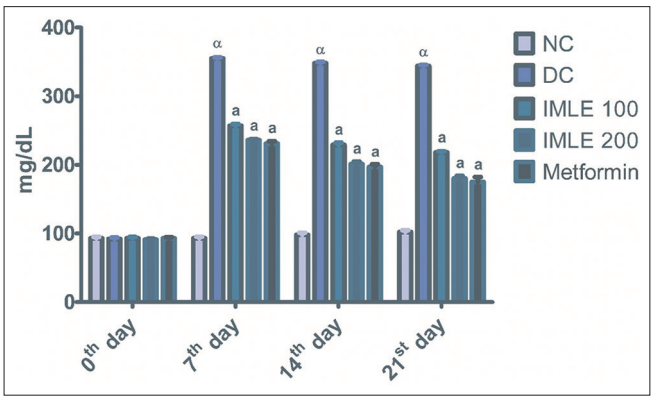


**Fig. 4: Effect of *Ipomoea mombassana* stem ethanolic extract (IMSE) on serum glucose levels in streptozotocin-induced diabetic rats over 21 days. Glucose levels (mg/dL) were measured on days 0, 7, 14, and 21. Values are presented as mean±standard error of mean (n=6). NC: Normal control; DC: Diabetic control; IMSE 100/200: Groups treated with IMSE at 100 and 200 mg/kg; Metformin: Standard drug-treated group (100 mg/kg). α indicates significant difference versus NC group (p<0.001); a indicates significant difference versus DC group (p<0.001). Statistical analysis was performed using one-way analysis of variance followed by Tukey's multiple comparison test**

pancreatic tissue, lobules, islets of Langerhans, and conventional vascular and arterial architecture were seen.

## DISCUSSION

The present study offers a comprehensive evaluation of *I. mombassana* through phytochemical screening, *in vitro* assays, LC-MS/MS-based metabolite profiling, *in vivo* antidiabetic evaluations, and histopathological investigations. This multifaceted approach highlights the therapeutic potential of plants, particularly in the treatment of diabetes (DM) and related complications.

Preliminary phytochemical analysis confirms the presence of major secondary metabolites such as flavonoids, alkaloids, phenolics, terpenoids, and peptides, all of which are known to contribute to a range of bioactivities, particularly antioxidant and antidiabetic effects [33]. These compounds are recognized as their ability to regulate metabolic tracks and prevent oxidative damage, and cast the foundations for subsequent biological research.

The ethanol extract of *I. mombassana* (IMSE) showed dual inhibition of α-amylase and α-glucosidase, supporting its therapeutic relevance in

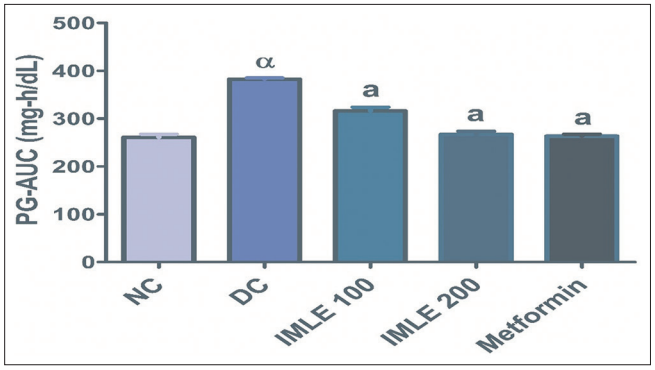


**Fig. 5: Effect of *Ipomoea mombassana* stem ethanolic extract (IMSE) on postprandial glucose area under the curve during the oral glucose tolerance test (OGTT) in Streptozotocin -induced diabetic rats. Values are presented as mean±standard error of mean (n=6). NC: Normal control; DC: Diabetic control; IMSE 100/200: Groups treated with IMSE at 100 and 200 mg/kg; Metformin: Standard drug-treated group (100 mg/kg). α indicates significant difference versus NC group (p<0.001); a indicates significant difference versus DC group (p<0.001). Statistical analysis was performed using one-way analysis of variance followed by Tukey's multiple comparison test**

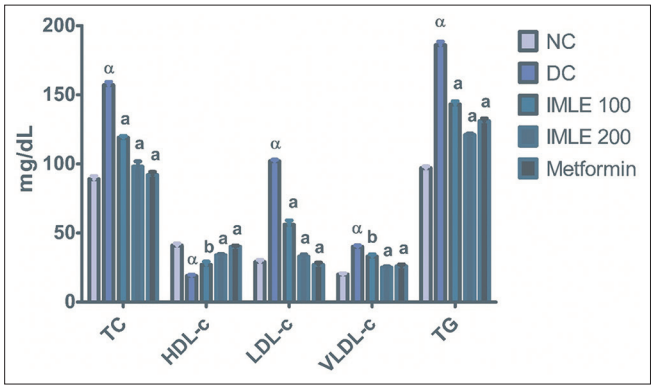


**Fig. 6: Effect of *Ipomoea mombassana* stem ethanolic extract (IMSE) on serum lipid profile parameters (total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and very low-density lipoprotein cholesterol) in Streptozotocin -induced diabetic rats after 21 days of treatment. Values are expressed as mean±standard error of mean (n=6). NC: Normal control; DC: Diabetic control; IMSE 100/200: IMSE-treated groups at 100 and 200 mg/kg; Metformin: Standard drug-treated group (100 mg/kg). α indicates significant difference versus NC group (p<0.001); a indicates significant difference versus DC group (p<0.001). Statistical analysis was performed using one-way analysis of variance followed by Tukey's multiple comparison test**

type 2 diabetes by lowering postprandial glucose. Its superior activity justified selection for LC-MS/MS characterization and *in vivo* evaluation. The activity can be attributed to flavonoids (e.g., maritimetin, hesperetin glucuronide) and alkaloids (e.g., pilocarpine, bufotenine), which inhibit enzymes via hydrogen bonding, π–π stacking, and hydrophobic interactions with catalytic residues. IMSE ($IC_{50}$=0.045 mg/mL) was comparable to or stronger than extracts from *Ipomoea batatas* (0.078 mg/mL) [34] and *Trigonella foenumgraecum* (0.05–0.06 mg/mL) [35]. The presence of multiple metabolites suggests synergistic inhibition, offering an advantage over single-compound therapies.

LC-MS/MS profiling of the ethanol extract revealed a chemically diverse metabolite pool consisting of 42 unique compounds, with $[M+H]^+$ ion values ranging from m/z 123.08 to 607.28. The identified metabolites

**Table 5: Effect of IMSE on antioxidant parameters**

| Parameters | NC | DC | IMSE 100 | IMSE 200 | Metformin group |
|---|---|---|---|---|---|
| SOD (U/mL) | 4.9±0.21 | 1.3±0.037$^{\alpha}$ | 3.3±0.19$^{b}$ | 5.4±0.30$^{a}$ | 6.5±0.42$^{a}$ |
| Catalase (kU) | 8.2±0.44 | 2.4±0.26$^{\alpha}$ | 4.6±0.31$^{a}$ | 6.9±0.39$^{a}$ | 8.4±0.25$^{a}$ |
| MDA (μM) | 3.6±0.20 | 9.2±0.32$^{\alpha}$ | 6.8±0.35$^{a}$ | 4.9±0.26$^{a}$ | 3.3±0.28$^{a}$ |
| GSH (mg/dL) | 2.4±0.19 | 0.32±0.056$^{\alpha}$ | 1.0±0.057$^{a}$ | 1.7±0.069$^{a}$ | 2.7±0.072$^{a}$ |

NC: Normal control, DC: Diabetic control, IMSE 100: *Ipomoea mombassana* stem extract 100 mg/kg, IMSE 200: *I. mombassana* stem extract 200 mg/kg; Values are expressed as mean±SD (n=6). Statistical significance was determined using one-way ANOVA followed by Tukey's test. $^{\alpha}$p<0.001, $^{\beta}$p<0.01, when compared to the NC group; $^{a}$p<0.001, $^{b}$p<0.01, $^{c}$p<0.05 when compared to DC group. GSH: Glutathione, MDA: Malondialdehyde

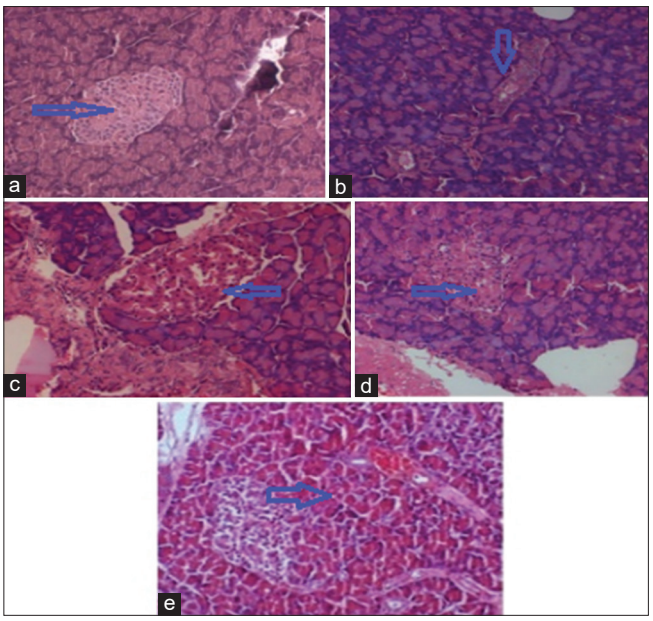


**Fig. 7: Histopathological changes in pancreatic tissues of control and experimental groups (Hematoxylin and Eosin stain, ×200 and ×400 magnification). Representative photomicrographs from six animals per group (n=6). Qualitative data; no statistical analysis. (a) Normal control group showing intact islets of Langerhans and well-organized pancreatic architecture. (b) Diabetic control group showing distorted islet morphology, necrosis, and inflammatory infiltration. (c) *Ipomoea mombassana* stem extract (IMSE) 100 mg/kg group showing partial regeneration and mild islet hyperplasia. (d) IMSE 200 mg/kg group showing notable recovery of islet structure and reduced degeneration. (e) Metformin-treated group showing restoration of β-cell architecture and near-normal morphology**

spanned several chemical classes, including flavonoids, alkaloids, terpenoids, peptides, phenolic derivatives, and nitrogenous aromatics. Compounds such as maritimetin, hesperetin 7-O-glucuronide, maculosin, and perlolyrine demonstrated potent antioxidant activity, indicating the extract's capacity to mitigate oxidative stress key factor in diabetes pathology [36]. Furthermore, bioactive constituents such as quinolin-2-ol, fabianine, and ganoderic acid F showcased antidiabetic, insulin-mimetic, and hepatoprotective effects, respectively [35,37,38]. The presence of neuroactive alkaloids (e.g., pilocarpine and lysergic acid) and potent anticancer agents (e.g., dihydrocapsaicin and euphornin) further extends the plant's pharmacological spectrum [39,40].

In the present study, metformin was used as the standard reference drug for the *in vivo* antidiabetic model due to its well-established clinical efficacy in managing Type 2 diabetes. Metformin primarily exerts its action by enhancing insulin sensitivity, reducing hepatic glucose production, and improving peripheral glucose uptake. In contrast, acarbose, which was used as the positive control in the *in vitro* α-amylase inhibition assay, acts by inhibiting carbohydrate-digesting enzymes in the gut, such as α-amylase. Although both agents contribute to glycemic control, they target different aspects of glucose metabolism. The inclusion of metformin in the *in vivo* study provided a clinically relevant comparator, while acarbose served as a mechanistically appropriate control for the enzyme inhibition assay. This dual-control approach strengthens the pharmacological validation of the plant extract by allowing both functional and mechanistic comparisons [41].

Elevated liver enzymes (SGOT and SGPT), indicative of hepatocellular damage in diabetic rats, were significantly restored to normal levels following extract administration. This hepatoprotective effect is likely due to the antioxidant potential of phenolic constituents that stabilize cellular membranes and enhance endogenous defense systems. The extract also significantly improved oxidative stress markers, such as SOD, CAT, and GSH, which were previously diminished in diabetic conditions. This aligns with reports linking chronic hyperglycemia to increased reactive oxygen species (ROS), lipid peroxidation, and oxidative tissue injury. Several investigations have shown that hyperglycemia aggravates peroxy and hydroxyl radicals, carbonyl stress, and oxidative stress, leading to cellular damage [42].

Histopathological analyses corroborated these biochemical findings. Liver sections from extract-treated diabetic rats showed regeneration of hepatocytes, reduced cellular vacuolization, and normalized architecture compared to untreated DCs. Pancreatic tissue exhibited preservation of islet structure and improved β-cell integrity, suggesting a protective or restorative effect of the extract on insulin-producing cells.

The cumulative findings underscore the therapeutic promise of *I. mombassana* stem extracts. By integrating antioxidant, α-amylase inhibitory, and insulin-sensitizing properties rooted in a rich phytochemical profile, the extract demonstrates strong potential in the holistic management of type II diabetes. Furthermore, the LC-MS/MS-based metabolite mapping provides a molecular basis for the observed pharmacological effects, paving the way for bioassay-guided fractionation and isolation of lead compounds.

Given the limitations and side effects of conventional antidiabetic therapies, herbal alternatives such as *I. mombassana* offer a safer and potentially more sustainable option. Currently, over 1200 plant species are traditionally used in diabetes treatment, with nearly 50% having undergone scientific evaluation for hypoglycemic activity. In alignment with the World Health Organization recommendations, regular exercise and a balanced diet augmented with antioxidant-rich and antidiabetic plants could serve as a cost-effective strategy to curb the growing prevalence of metabolic disorders, particularly in urban populations [43].

## CONCLUSION

The ethanolic extract of *I. mombassana* stem (IMSE) exhibits significant antidiabetic, antioxidant, and antihyperlipidemic activities, supported by comprehensive *in vitro*, *in vivo*, and metabolomic analyses. The extract effectively inhibited both α-amylase and α-glucosidase activities, indicating its potential to delay carbohydrate digestion and glucose absorption strategies for controlling postprandial hyperglycemia. In addition, IMSE improved glucose tolerance, restored lipid and liver function parameters, and enhanced antioxidant defenses in STZ-induced diabetic rats.

LC-MS/MS profiling revealed a rich diversity of bioactive compounds, including flavonoids, alkaloids, terpenoids, and phenolics, many of which are known for their antidiabetic, hepatoprotective, and neuroactive properties. Histopathological evaluations confirmed pancreatic tissue regeneration and β-cell protection, further underscoring the therapeutic potential of IMSE.

These findings suggest that *I. mombassana* stem extract could serve as a valuable source of phytotherapeutic agents for managing diabetes and its associated complications, particularly through dual enzyme inhibition and antioxidant modulation. Further studies, including molecular mechanism exploration and clinical validation, are recommended to support its development into a standardized herbal formulation.

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**AUTHOR'S CONTRIBUTION**

The author carried out the experiments, analyzed the data, and wrote the manuscript. The work was done under the guidance of a supervisor, who reviewed and approved the final version.

**CONFLICT OF INTEREST**

None to declare.

**FUNDING**

None to declare.

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**SUPPLEMENTARY DATA**

**Table S1: Percentage inhibition of α-amylase and α-glucosidase by the hexane stem extract of *Ipomoea mombassana* (IMSE) at different concentrations (100–500 µg/mL). Normalized values are presented**

| Extract | Concentration (µg/mL) | α-Amylase Inhibition (%) ± SD | α-Glucosidase Inhibition (%) ± SD |
|---|---|---|---|
| Hexane | 100 | 62.45±0.12 | 54.76±0.21 |
| | 200 | 71.09±0.18 | 61.9±0.18 |
| | 300 | 83.21±0.21 | 74.29±0.19 |
| | 400 | 100.00±0.21 | 81.43±0.13 |
| | 500 | 100.00±0.12 | 92.38±0.32 |
| $IC_{50}$ | | 61.32±0.88 | 57.76±0.08 |

Values are expressed as mean±SD (n=3). Percentage inhibition was calculated using the formula [($Abs_{control}$–Abs_sample)/Abs_control] × 100. Data were normalized to the theoretical range of 0–100%; values above 100% were capped at 100%

**Table S2: Percentage inhibition of α-amylase and α-glucosidase by the chloroform stemextract of *Ipomoea mombassana* (IMSE) at different concentrations (100–500 µg/mL). Normalized values are presented**

| Extract | Concentration (µg/mL) | α-Amylase Inhibition (%) ± SD | α-Glucosidase Inhibition (%) ± SD |
|---|---|---|---|
| Chloroform | 100 | 61.78±0.18 | 50.95±0.34 |
| | 200 | 69.56±0.62 | 66.19±0.23 |
| | 300 | 82.07±0.42 | 71.43±0.6 |
| | 400 | 100.76±0.30 | 80.48±0.43 |
| | 500 | 100.64±0.4 | 89.05±0.21 |
| $IC_{50}$ | | 67.06±2.1 | 61.4±0.76 |

Values are expressed as mean±SD (n=3). Percentage inhibition was calculated using the formula [($Abs_{control}$–Abs_sample)/Abs_control] × 100. Data were normalized to the theoretical range of 0–100%; values above 100% were capped at 100%

**Table S3: Percentage inhibition of α-amylase and α-glucosidase by the ethyl acetate stem extract of *Ipomoea mombassana* (IMSE) at different concentrations (100–500 µg/mL). Normalized values are presented**

| Extract | Concentration (µg/mL) | α-Amylase Inhibition (%) ± SD | α-Glucosidase Inhibition (%) ± SD |
|---|---|---|---|
| Ethyl acetate | 100 | 64.34±0.29 | 54.29±0.25 |
| | 200 | 73.87±0.35 | 65.24±0.24 |
| | 300 | 85.23±0.30 | 74.29±0.37 |
| | 400 | 100.00±0.38 | 82.38±0.35 |
| | 500 | 100.00±0.58 | 92.76±0.43 |
| $IC_{50}$ | | 50.05±1.4 | 47.26±0.16 |

Values are expressed as mean±SD (n=3). Percentage inhibition was calculated using the formula [($Abs_{control}$–Abs_sample)/Abs_control] × 100. Data were normalized to the theoretical range of 0–100%; values above 100% were capped at 100%

**Table S4: Percentage inhibition of α-amylase and α-glucosidase by the ethanolic stem extract of *Ipomoea mombassana* (IMSE) at different concentrations (100–500 µg/mL). Normalized values are presented**

| Extract | Concentration (µg/mL) | α-Amylase Inhibition (%) ± SD | α-Glucosidase Inhibition (%) ± SD |
|---|---|---|---|
| Ethanol | 100 | 65.09±0.15 | 57.14±0.3 |
| | 200 | 75.78±0.23 | 62.38±0.23 |
| | 300 | 86.34±0.54 | 72.38±0.12 |
| | 400 | 100.00±0.08 | 80±0.54 |
| | 500 | 100.00±0.12 | 92.86±0.63 |
| $IC_{50}$ | | 45.78±0.18 | 42.26±0.97 |

Values are expressed as mean±SD (n=3). Percentage inhibition was calculated using the formula [($Abs_{control}$–$Abs_{sample}$)/$Abs_{control}$] × 100. Data were normalized to the theoretical range of 0–100%; values above 100% were capped at 100%.

**Table S5: Complete LC–MS/MS annotation of 42 compounds identified in *Ipomoea mombassana* ethanolic extract using METLIN and Agilent PCDL with assigned confidence levels (MSI criteria)**

| S.No. | Retention time (min) | [M+H] *(m/z)* | Error (ppm) | Molecular formula | MS/MS Fragment Ions *(m/z)* | Identification |
|---|---|---|---|---|---|---|
| 1 | 1.067 | 185.06 | -8.6 | C9H12O2S | 127.02 | (+/-)-3-[(2-methyl-3- furyl) thio]-2-butanone |
| 2 | 1.374 | 235.16 | 15.84 | C13H24O2 | 235.16 | Oxacyclotetradecan-2- one |
| 3 | 1.428 | 158.11 | 5.42 | C8H15NO2 | 112.11 | Lentiginosine |
| 4 | 1.483 | 156.10 | 5.19 | C8H13NO2 | 110.09 | Retronecine |
| 5 | 2.047 | 229.15 | 7.85 | C11H20N2O3 | 142.08 | L-isoleucyl-L-proline |
| 6 | 3.755 | 174.12 | 4.4 | C7H15N3O2 | 115.05 | apo-[3- methylcrotonoyl- CoA: carbon-dioxide ligase |
| 7 | 4.537 | 188.07 | 3.35 | C11H9NO2 | 146.05, 118.06 | Indoleacrylic acid |
| 8 | 4.686 | 146.05 | 5.11 | C9H7NO | - | Isocarbostyril |
| 9 | 4.753 | 158.08 | 2.64 | C7H11NO3 | 146.05, 147.06 | N-Methacrylylglycine methyl ester |
| 10 | 4.969 | 146.05 | 1.77 | C9H7NO | 118.06 | Quinolin-2-ol |
| 11 | 5.721 | 231.11 | -3.4 | C11H16N2O2 | 158.09 | Pilocarpine |
| 12 | 5.74 | 273.12 | -1 | C13H18N2O3 | 195.09 | 4-Coumaroyl-2- hydroxyputrescine |
| 13 | 5.888 | 158.09 | -12.13 | C9H13N | 143.07 | 3-Butylpyridine |
| 14 | 6.024 | 287.13 | -0.72 | C14H20N2O3 | 269.12 | feruloyl putrescine |
| 15 | 6.029 | 273.12 | -1.31 | C13H18N2O3 | 195.09 | Caffeoyl putrescine |
| 16 | 6.715 | 227.12 | 0.08 | C10H20O4 | 131.08 | 1alpha, 2alpha, 4betaH ,6alpha, 8R)-p-Menthane-2,6,8,9- tetrol |
| 17 | 6.788 | 269.12 | 5.23 | C16H16N2O2 | 197.06 | Lysergic acid |
| 18 | 6.876 | 285.12 | -0.98 | C14H18N2O3 | 195.09 | Physovenine |
| 19 | 6.928 | 283.10 | 0.57 | C14H16N2O3 | 195.09 | Maculosin |
| 20 | 7.501 | 227.11 | -4.32 | C12H16N2O | 209.10 | Bufotenine |
| 21 | 7.747 | 220.16 | 6.2 | C14H21NO | 176.14 | Fabianine |
| 22 | 7.797 | 329.12 | -7.59 | C19H18N2O2 | 249.06 | Nb-p- Coumaroyltryptamine |
| 23 | 7.802 | 223.05 | -4.14 | C9 H12 O5 | 190.02 | (Z)-3-(1-Formyl-1- propenyl) pentanedioic acid |
| 24 | 7.942 | 265.09 | 7.36 | C16H12N2O2 | 206.08 | Perlolyrine |
| 25 | 8.042 | 239.11 | -2.6 | C13H16N2O | 221.10 | Tetrahydroharmine |
| 26 | 8.178 | 499.12 | 1.36 | C23H24O11 | 163.03 | Hesperetin 7-O-glucuronide |
| 27 | 8.425 | 173.13 | 5.57 | C13H16 | 158.10 | 1,2-Dihydro-1,1,6- trimethylnaphthalene |
| 28 | 8.588 | 287.05 | 8.93 | C15H10O6 | 258.11 | Maritimetin |
| 29 | 8.725 | 253.11 | -4.86 | C10H18N2O4 | 235.12 , 206.08 | Hydroxyprolyl-Valine |
| 30 | 9.089 | 330.12 | 9.54 | C20H15N3O2 | 284.11 | 2,2'-(1-Phenyl-1H-1,2,4-triazole-3,5- diyl) bis-phenol |
| 31 | 9.164 | 314.13 | 1.25 | C16H21NO4 | 177.05 | (S)-Edulinine |
| 32 | 9.408 | 284.12 | 1 | C15H19NO3 | 147.04 | Cochlearine |
| 33 | 9.903 | 309.09 | 2.71 | C13H18O7 | 290.20 | Isosalicin |
| 34 | 9.977 | 308.21 | 10.85 | C18H29NO3 | 179.12 | Dihydrocapsaicin |
| 35 | 11.497 | 325.08 | 10.26 | C15H16O8 | 163.03 | Mahaleboside |
| 36 | 11.733 | 411.08 | 14.52 | C17H17CLN6O3 | 118.08, 163.03 | Zopiclone |
| 37 | 12.559 | 181.12 | 6.12 | C11H16O2 | 135.11, 163.11 | 3-tert-Butyl-5- methylcatechol |
| 38 | 14.47 | 318.29 | 8.48 | C18H39NO3 | 282.27 | Phytosphingosine |
| 39 | 16.396 | 312.11 | -7.23 | C14H17NO7 | 167.05 | p- Glucosyloxymandelonit rile |
| 40 | 16.727 | 123.08 | -0.32 | C8H10O | - | 2,5-Dimethylphenol |
| 41 | 24.172 | 593.27 | -1.24 | C32H42O9 | 533.25 | Ganoderic acid F |
| 42 | 24.806 | 607.28 | -0.14 | C33H44O9 | 607.28 | Euphornin |