

# Bioassay-Guided Fractionation, Characterization and DPP-IV Inhibitory Potential of Small Bioactive Molecules from *Aloe vera* (L.) Burm. F. Extract

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**Abstract:** To evaluate the anti-diabetic potential of water-soluble active molecules from ethanolic *Aloe vera* (L.) Burm. f. (*Aloe vera*) extract through inhibition of dipeptidyl peptidase-IV, an important drug target for the management of diabetes mellitus- in vitro studies. *Aloe vera* gel ethanolic extract, having Dipeptidyl peptidase-IV inhibitory potential, was subjected to purification through a three-step activity-guided fractionation involving Semi-Preparative RP-HPLC as a first step, followed by LH20 Sephadex gel filtration and a final polishing step using Analytical RP-HPLC. The purification was guided by the inhibition of human DPP-IV activity in the fractions at each step. The molecules were further characterized using FTIR and Mass spectrometry to determine the presence of functional groups and their intact masses, respectively. *Aloe vera* extract demonstrated a dose-dependent increase in % inhibition of DPP-IV. Based on the mass spectrometry data, two compounds with DPP-IV inhibitory potential were identified from fractions A and B with m/z values of 226.94 Da and 543.15 Da, respectively. FTIR studies demonstrated structural differences between the two molecules, reflected in different intensities in the 1600-1700 cm<sup>-1</sup> and 900-1000 cm<sup>-1</sup> regions, which are in agreement with the mass spectrometry data. The identified phyto compound (s) from *A. vera* with m/z values of 226.94 Da and 543.15 Da may find use in pharmacological applications, with additional research on safety and scientific validity.

**Keywords:** *Aloe vera*; Diabetes mellitus; DPP-IV inhibitors; C-18 RP-HPLC; LH20 Sephadex; FTIR; mass spectrometer.

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## 1. Introduction

Type 2 Diabetes mellitus is a complex metabolic disorder characterized by chronic hyperglycemia and a progressive decline in  $\beta$ -cell function, with insulin resistance [1]. The prevalence of diabetes mellitus and associated complications is rising and is an alarming concern worldwide [2]. In addition to loss of  $\beta$ -cell function, research has shown that patients with T2DM have decreased  $\beta$ -cell mass compared with non-diabetic individuals of the same age and BMI [3]. Thus, it is plausible that both the reduced number of  $\beta$ -cells and impaired  $\beta$ -cell function, resulting in diminished functional islet mass, contribute to the development and subsequent progression of T2DM.

A major challenge in the treatment of T2DM is identifying a therapeutic agent that can alter the course of the disease by preventing the gradual decline in pancreatic  $\beta$ -cell function and the diminution of  $\beta$ -cell mass [4]. In this regard, incretin-based glucose-lowering medications, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP), have gained significant importance in the management of T2DM [5]. Dipeptidyl peptidase-IV (DPP-IV, also known as CD26) is a serine protease that cleaves incretin hormones such as GLP-1 and GIP, thereby reducing their serum levels, which otherwise augment glucose-stimulated insulin secretion from pancreatic  $\beta$ -cells [6]. Therefore, this enzyme is an important pharmacological target for the management of T2DM. People with diabetes have decreased circulating GLP-1 levels, but retain the capacity to respond to this hormone. Thus, glycaemic control can be achieved via exogenous GLP-1 or DPP-IV inhibitors [7]. Thus, DPP-IV inhibitors are recognized as a popular anti-diabetic medication that prevents the degradation of incretin hormones, thereby increasing their bio-availability and enhancing the so-called incretin effect, resulting in glucose-stimulated insulin secretion [8]. GLP-1 and its congeners are likely candidates for use as anti-hyperglycemic agents because they stimulate insulin secretion under only hyperglycaemic conditions, which minimizes the risk of hypoglycaemia [9].

Natural products offer a plethora of opportunities to develop compounds that act as anti-hyperglycemic agents by regulating the activity of incretin hormones to upregulate insulin secretion and  $\beta$ -cell mass, and lower blood glucose levels without side effects. *Aloe barbadensis*, also known as *Aloe vera*, is a scrumptious plant belonging to the Asphodelaceae family and has been part of traditional medicine [10]. It has been used by different cultures for more than 5000 years and has been used as a therapeutic molecule by various pharmacopeias due to its extraordinary medicinal properties. It contains more than 70 active compounds, including vitamins, minerals, enzymes, polysaccharides, phenolic compounds, and organic acids. It has been reported that the polysaccharides present in *A. vera* gel exhibit therapeutic properties, including anti-inflammatory, healing, antibacterial, antioxidant, anticarcinogenic, antidiabetic, and antiaging [11]. Further, its effectiveness in the treatment of wound healing, ageing, immunomodulation, and diabetes mellitus is attributed to its antimicrobial, anti-inflammatory, and antioxidant activities [12]. The presence of bioactive components in *A. vera* has been suggested to be responsible for its therapeutic effects [13].

The anti-hyperglycemic potential of *A. vera* has been well documented both *in vivo* and in clinical studies [14]. A plethora of studies have suggested that the glucose-lowering activity of *A. vera* is mediated through various mechanisms and may act as a safe anti-hyperglycemic agent [15,16]. Further, the polypeptide-enriched fraction from *A. vera* gel extract effectively inhibited DPP-IV in STZ-induced diabetic rats [17]. Prior research from our group showed that DPP-IV levels were increased and GLP-1 levels were decreased in STZ-induced WNIN/GR-OB rats, and that treatment with *A. vera* extract (300 mg/kg bw) reduced DPP-IV levels, increasing GLP-1 levels. Additionally, beta cell function was restored, with improvements in insulin sensitivity, demonstrating that inhibition of DPP-IV is one of the mechanisms by which *A. vera* exerts its beneficial effects [18], even though numerous researchers have demonstrated the DPP-IV-inhibitory potential of *A. vera*. However, identification and purification of small molecules from *A. vera* as DPP-IV inhibitors are very scanty. Earlier research demonstrated that *A. vera* effectively inhibits DPP-IV, and a dipyrrole derivative with a molecular weight of 225.175 Da was identified from *A. vera*, which inhibited DPP-IV in a non-competitive manner [19]. However, the beneficial effects of *A. vera* are attributed to numerous components that are

known to work synergistically and/or alone in imparting their therapeutic effects [14]. Thus, the present study is an attempt to gain deeper insights into the mechanisms and the role of molecules with DPP-IV inhibitory potential derived from *A. vera* extract, which can be further developed as a potential anti-hyperglycemic agent.

## 2. Materials and Methods

### 2.1. Chemicals.

All chemicals, including solvents, were of analytical grade. Glycine-proline-p-nitroaniline-DPP-IV substrate (GLY-PRO-pNA) was purchased from Sigma Chemicals; Tris-HCl from SRL Chemicals, India; and acetonitrile and methanol from Sisco Research Laboratories, Mumbai, India.

### 2.2. Plant material.

Fresh *A. vera* plant was purchased from Krishnagiri, Tamil Nadu, India in June 2018. It was authenticated by Dr C. Murgan (Scientist E, Botanical Survey of India, Southern Regional Centre, Coimbatore, India (BSI/SRC/5/23/2018/Tech/729). A voucher specimen was deposited at the Centre for Bio-Separation Technology, Vellore Institute of Technology, Vellore.

### 2.3. Extract preparation.

The extraction process for *A. vera* gel was strictly carried out according to the previously established procedures [19]. A succinct description of the extraction procedure is provided: washing the leaves thoroughly, air-drying, and removing the epidermis to reveal the colourless, mucilaginous gel. The gel was then cut into 2–3 cm pieces and lyophilized using a Christ Alpha 1-4 lyophilizer to remove water. The lyophilized material was collected, ground, and used for extraction using the Soxhlet apparatus. For the extraction process, 50 g of gel powder was refluxed with 600 mL of 90% ethanol for 4 hours (3 cycles) at 90°C. This extract was then concentrated to powder under vacuum using a Buchi R-210 rotary evaporator and stored at 4°C until further use.

### 2.4. DPP-IV inhibitory activity of *A. vera* extract.

The DPP-IV inhibitory activity was determined following a previously established method with some modifications [19]. Human plasma from healthy individuals was used as an enzyme source (University Human Ethical Committee Reference: VIT/ UHEC-5/No.2; dated 27 August 2012). The assay was performed in a 96-well flat-bottom plate, measuring the increase in absorbance at 405 nm using Gly-Pro-p-nitroanilide as substrate. The enzyme (20 µl) 0.1 U) was pre-incubated with various concentrations of *A. vera* extract (0, 0.6, 1.5, 3.0, and 4.5 mg) for 15 min at 37°C in 100 mM Tris-HCl, pH 8.0. The reaction was initiated by adding substrate (1 mM prepared in Tris-HCl buffer). Following a 30 min incubation at 37°C, released p-Nitroaniline was measured at 405 nm using a Perkin Elmer multi-detection microplate reader. The assay was performed in triplicate with appropriate blanks.

## 2.5. Isolation of the active molecule.

Three activity-guided chromatographic steps were used to isolate the active molecule (s), which included semi-preparative C-18 RP-HPLC, followed by LH20 Sephadex (gel filtration) column purification, and a polishing step was carried out using an analytical C-18 RP-HPLC column [19]. The purification was steered based on the DPP-IV inhibitory potential of the fractions.

### 2.5.1. Semi-preparative RP-HPLC.

The first step involves semi-preparative RP-HPLC. 100mg/mL of ethanolic *A. vera* extract was injected (500  $\mu$ L) into an HPLC equipped with a C18 column (10 $\times$ 250 mm i.d., 5  $\mu$ m) and a Photodiode Array (PDA) detector (Waters, India). The elution was carried out at a flow rate of 5 mL/min, using 0.1% acetic acid (A) and acetonitrile (B) as mobile phases. Gradient separation was used for purification, with 88% A from 0-10 min, 70% A from 10-25 min, 45% A from 25-40 min, 40% A from 41-48 min, and 10% A from 48-54 min. The data were acquired and processed using Empower software. Each fraction was collected and concentrated using the Eppendorf concentrator and the SpeedVac (Model No.5305). The fractions were then reconstituted in deionized water at 5 mg/mL and tested for the *in vitro* DPP-IV inhibition as described above.

### 2.5.2. Gel filtration using LH-20 Sephadex.

The RP-HPLC fraction exhibiting DPP-IV inhibitory activity was further subjected to gel filtration using LH-20 Sephadex, which was packed manually into an Amersham column (75 $\times$ 130mm) and connected to the Econo pump chromatography system (Bio-Rad, USA). The separation was performed with 20% methanol at a flow rate of 70 $\mu$ L/min at room temperature. 10 mg/mL (200  $\mu$ L) of the active peak showing DPP-IV inhibition was injected into the column pre-equilibrated with 20% methanol, and the data were recorded at a detection wavelength of 265 nm. The fractions were collected, concentrated, and then reconstituted in deionized water to 5 mg/mL and tested for *in vitro* DPP-IV inhibition as described above.

### 2.5.3. Analytical RP-HPLC.

The polishing step involved separation using an analytical C18 RP-HPLC column (150  $\times$  4.6 mm i.d., 5 $\mu$ m) connected to a 2707 autosampler and a 2489 UV/Visible detector. The elution was carried out using 95% water (Solvent A) and 5% acetonitrile (Solvent B) as a mobile phase in an isocratic mode at a flow rate of 1 mL/min. 5 mg/mL (25  $\mu$ L) of active peak demonstrating DPP-IV inhibition was injected into the column at a detection wavelength of 265 nm. The data were acquired and processed using Breeze software. The fractions were collected, concentrated, and then reconstituted in deionized water to 1 mg/mL and tested for *in vitro* DPP-IV inhibition as described above.

## 2.6. Fourier transform infrared spectroscopy (FT-IR).

The functional groups present in the fractions were determined by FT-IR on a powdered sample. The correction was made at baseline using KBr plates, and 2 mg of the pelleted sample was used to record the spectrum.

### 2.7. Mass spectrometry.

The data on the intact mass of bioactive molecule (s) was acquired on Acquity UPLC coupled to Quattro Premier XE (Waters), which is a triple quadrupole ESI mass spectrometer. C-18 (100 × 2.1 mm) column was used on LC with water (solvent A) and methanol (solvent B) as mobile phases. A linear gradient, 5% - 95% solvent B over 20 minutes, was followed at a flow rate of 0.1 mL/min. The sample manager (Acquity) was set to partial loop mode, and 5 µL of the sample was injected onto the column. The data were acquired in negative-ion ESI polarity, and the detection range was set to m/z 100-700. The capillary voltage was 3.5 kilovolts (kV); the Cone voltage was 25 V; the extractor voltage was 5 V; and the source temperature was set to 100°C. The desolvation temperature of 300°C was used. Desolvation gas (nitrogen; N<sub>2</sub>) Flow: 700 L/hr; cone gas (nitrogen; N<sub>2</sub>) Flow: 75 L/hr. The data were processed and analysed in Mass Lynx.

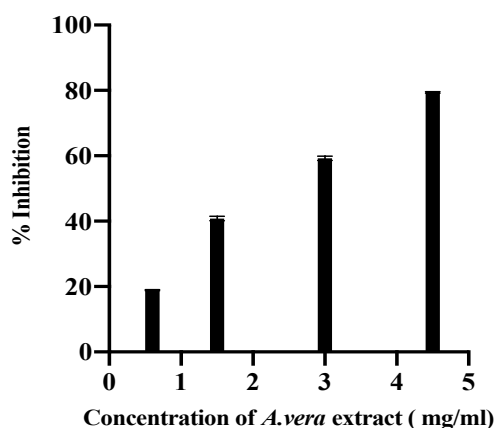
### 2.8. Statistical analysis.

The data were expressed as mean ± SD and were analysed using GraphPad Prism 8.0 (GraphPad Software, Inc., La Jolla, CA, USA).

## 3. Results and Discussion

### 3.1. DPP-IV inhibitory potential of *A. vera* extract.

*A. vera* extract demonstrated a dose-dependent increase in % inhibition of DPP-IV enzyme at various concentrations, 0, 0.6, 1.5, 3.0, and 4.5 mg/mL Figure 1. This extract was also used to isolate active molecules.

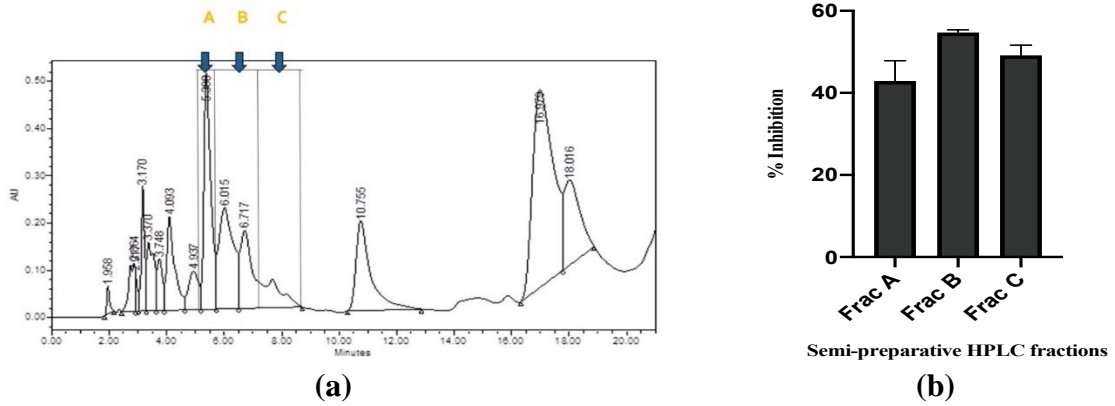


**Figure 1.** DPP-IV inhibitory potential of *A. vera* extract at different concentrations. Values expressed as Mean±SD.

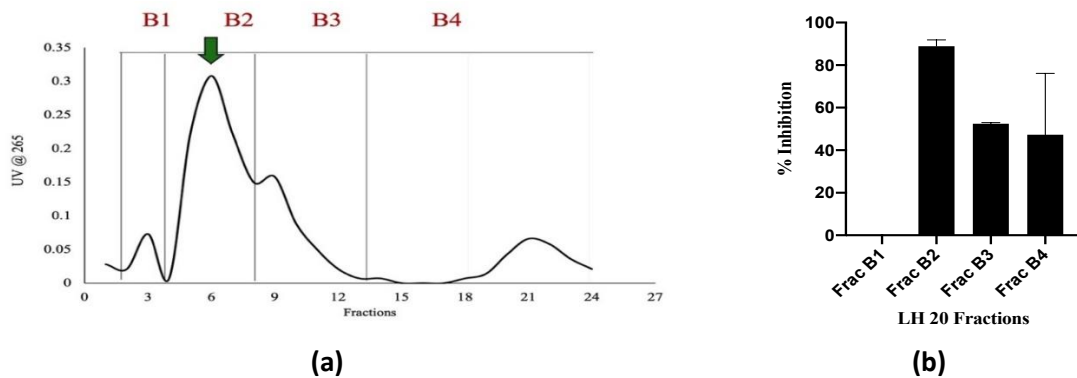
### 3.2. Purification of the bioactive molecule (s).

The purification of active molecules involved a three-step activity-guided fractionation [20]. In the first step, *A. vera* extract (100 mg/mL) was injected onto a semi-preparative RP-HPLC column, and the peaks were collected (Figure 2a), reconstituted, and analysed for DPP-IV inhibitory potential as previously described. The three fractions were assayed for DPP-IV inhibition. Fraction B exhibited the highest percentage of inhibition (Figure 2b) and was selected for the subsequent purification step. In the second step, fraction B (10mg/mL) was injected into the LH-20 Sephadex column, and fractions were collected Figure 3a,

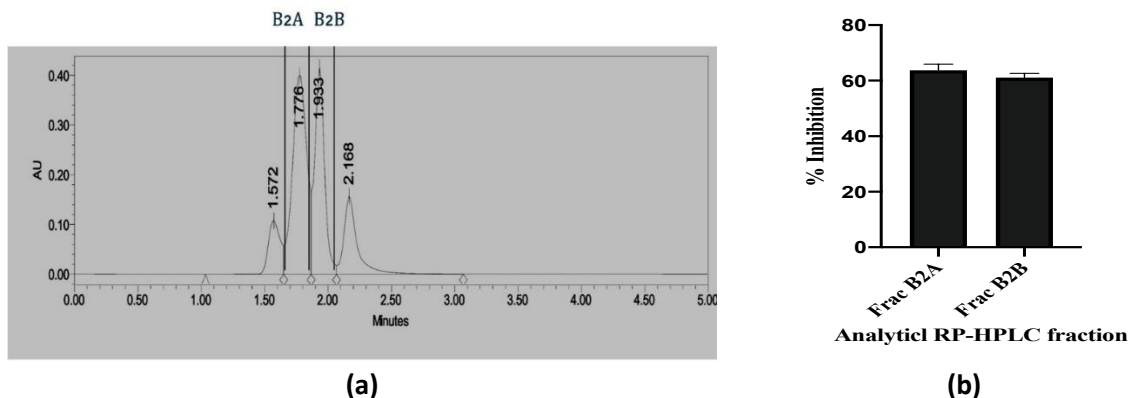
reconstituted, and analyzed for DPP-IV inhibitory potential. The three fractions were assayed for DPP-IV inhibition. Fraction B2 exhibited the highest percentage of inhibition (Figure 3b) and was selected for the subsequent purification step. In the third step, fraction B2 (5 mg/mL) was injected onto the C18 analytical RP-HPLC column, and fractions were collected (Figure 4a), reconstituted, and analyzed for DPP-IV inhibitory potential. The two fractions B2A and B2B were assayed for DPP-IV inhibitory potential. Both fractions exhibited DPP-IV inhibition Figure 4b and were taken for further characterization.



**Figure 2.** (a) RP-HPLC profile of ethanolic *A. vera* extract (Semi-preparative); (b) DPP-IV inhibition of semi-preparative HPLC fractions. Values are expressed as Mean±SD.



**Figure 3.** (a) Absorbance profile of fraction B on LH-20 Sephadex column; (b) DPP-IV inhibition of LH-20 fractions. Values are expressed as Mean±SD.

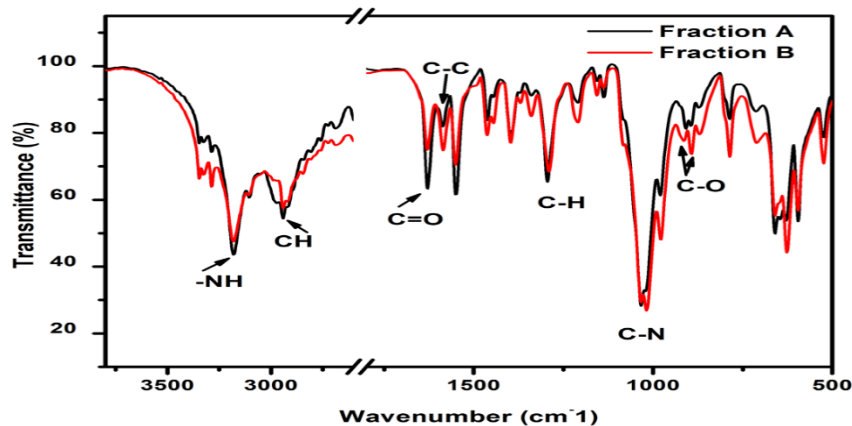


**Figure 4.** (a) RP-HPLC profile of fraction B (Analytical RP-HPLC); (b) DPP-IV inhibition of analytical RP-HPLC fractions. Values are expressed as Mean±SD.

### 3.3. FTIR.

The IR spectrum (Figure 5) provides information about the functional groups present in the molecules obtained in fractions A and B. In both cases, several major intense peaks were observed: 3200 cm<sup>-1</sup>, 2950 cm<sup>-1</sup>, 1715 cm<sup>-1</sup>, 1650 cm<sup>-1</sup>, 1250-1020 cm<sup>-1</sup>, and 1000-900 cm<sup>-1</sup> <https://nanobioletters.com/>

1, which correspond to N-H (amine), C-H, C=O (carbonyl), C-C, C-N, and C-O functional groups, respectively. Compared to fraction A, the peak intensities of fraction B for N-H, C-H, C=O, and C-C were significantly lower. Compared to fraction B, the C-O peak at  $1000\text{ cm}^{-1}$  for fraction A disappeared, and the region has some changes in peak shapes. All these structural changes indicate that the molecule (s) in fractions A and B may differ structurally.

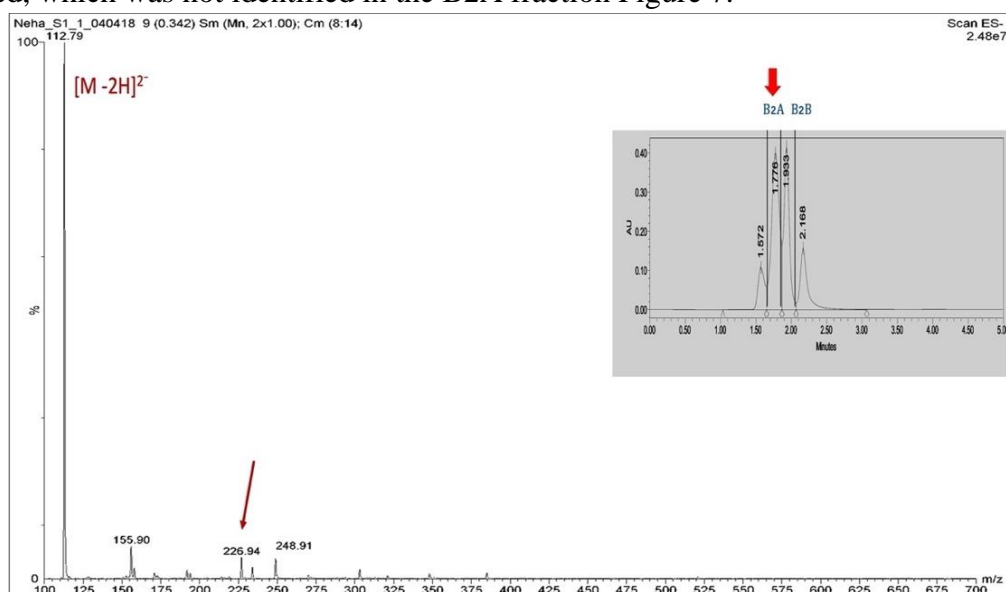


**Figure 5.** IR Spectrum of B2A and B2B fraction.

The spectrum showed the presence of multiple functional groups, including a carbonyl and an amine. There is a sharp peak corresponding to the C-C bond in  $1600\text{-}1700\text{ cm}^{-1}$ , and there is a peak for the C-H bond stretch appearing around  $3000\text{ cm}^{-1}$ , which might indicate the presence of an alkene. There is a C-N stretch at  $1020\text{-}1250\text{ cm}^{-1}$ , which may correspond to the presence of amines. There are structural differences between fractions A and B corresponding to different intensities in the  $1600\text{-}1700\text{ cm}^{-1}$  and  $900\text{-}1000\text{ cm}^{-1}$  region.

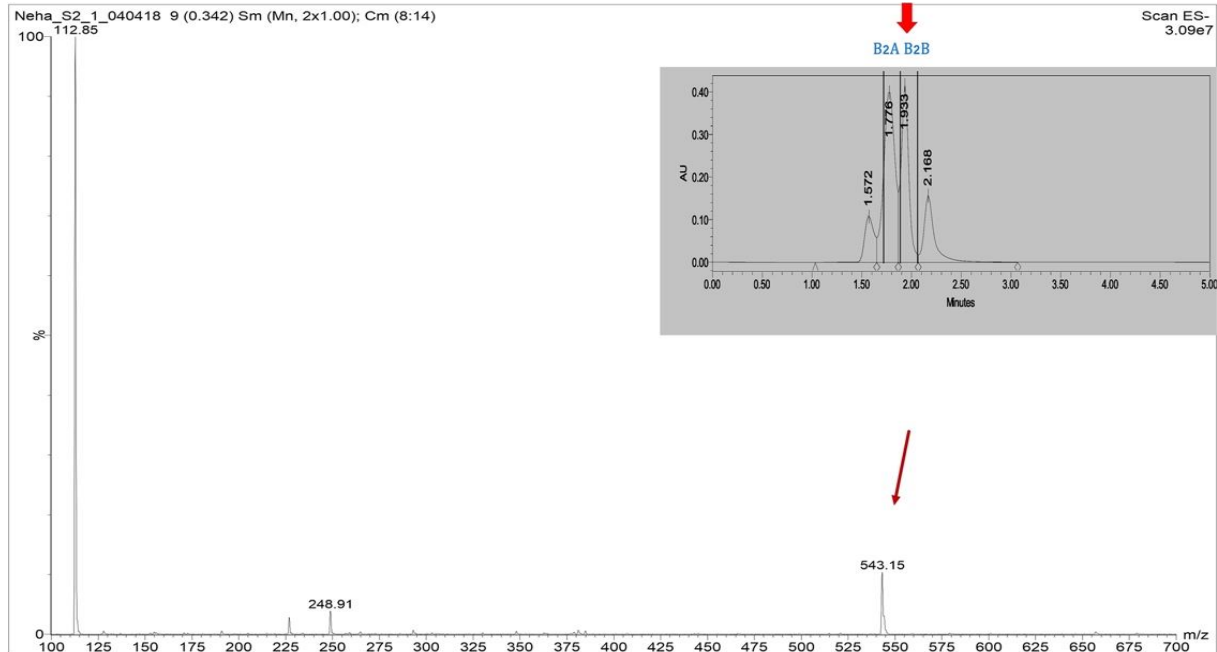
### 3.4. Mass spectrometry.

The molecules having DPP-IV inhibition obtained from analytical RP-HPLC fractions were subjected to MS analysis. The total ion chromatogram (TIC) of the analyte showed an intense peak at an acquisition time of 8.14 min. The spectrum was recorded in negative ion mode in the range of  $100\text{-}700$ . In the B2A fraction, the major peak appears at  $m/z$  of 226.9 and is doubly charged at  $m/z$  of 112.79 (Figure 6). In the B2B fraction, a peak at  $m/z$  of 543.15 was observed, which was not identified in the B2A fraction (Figure 7).



**Figure 6.** Mass spec analysis of B2A fraction

Mass of the fraction was acquired using Acquity UPLC coupled to Quattro Premier XE (Waters), which is a triple quadrupole ESI mass spectrometer. The spectrum was recorded in negative ion mode, and the  $m/z$  of 226.94 was identified, which means the neutral mass of the molecule is 227.94. There is a peak at  $m/z$  of 112.79, which is a doubly charged peak of 226.94.



**Figure 7.** Mass analysis of the B2B fraction.

The mass of the fraction was acquired using an Acquity UPLC coupled to a Quattro Premier XE (Waters), a triple quadrupole ESI mass spectrometer. The spectrum was recorded in negative-ion mode, and the  $m/z$  of 543.15 was observed, indicating a neutral mass of 544.15 Da.

### 3.5. Discussion.

Research into the role of gut hormones in regulating pancreatic beta-cell function has led to new targets for the management of type 2 diabetes. The two main hormones involved in this endocrine signaling from the gut are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [21]. These incretin hormones play a significant role in insulin biosynthesis, secretion, and  $\beta$ -cell survival and provide therapeutic potential for the management of T2DM [22]. However, due to rapid DPP-IV-mediated degradation of incretin hormones, their function is inactivated [23]. Thus, DPP-IV inhibitors offer a promising strategy for treating T2DM by extending the circulating half-life of incretin hormones.

Natural products offer great potential for bioactive components with numerous health benefits. These positive effects are associated with a mixture of phytochemicals or a single compound. Several plants and phytocomponents (s) have been reported to have DPP-IV inhibitory potential [24]. *In silico* studies demonstrated that flavonoids isolated from *Lens culinaris* Medikus (Fabaceae) seeds inhibited DPP-IV in a dose-dependent manner [25,26]. Furthermore, the bark of *Berberis aristata* and its secondary metabolites are known to play an important role in DPP-IV inhibition, suggesting their effectiveness in glucose homeostasis [27]. Also, berberine isolated from *Cardiospermum halicacabum* demonstrated its DPP-IV inhibitory capacity with an  $IC_{50}$  of  $16.328 \pm 1.344 \mu\text{M}$ , and *in silico* studies demonstrated that berberine was found to bind to the active site of DPP-IV [28].

Our data offer *in vitro* evidence that the glucose-lowering ability of *A. vera* is, in part, due to its DPP-IV inhibitory potential. In the present study, two molecules were isolated through activity-guided fractionation using three chromatographic steps with m/z values of 226.94 and 543.15 Da exhibiting DPP-IV inhibitory potential. The molecules were eluted in the flow-through phase during RP-HPLC, indicating their hydrophilic nature. The IR spectrum of the two molecules demonstrated the presence of various functional groups, including carbonyl and amine groups at 1710-1720  $\text{cm}^{-1}$  and 3200-3600  $\text{cm}^{-1}$ , respectively. There is a sharp peak corresponding to the C-C bond that appears around 1600-1700  $\text{cm}^{-1}$ , and a peak for the C-H bond stretch appears around 3000  $\text{cm}^{-1}$ , which might indicate the presence of an alkene. There is a C-N stretch at 1020-1250  $\text{cm}^{-1}$ , which could indicate the presence of amines [29]. However, there are structural differences between fractions A and B in the present study corresponding to the two molecules with m/z of 226.94 and 543.15, respectively. There are varying intensities in the 1600-1700  $\text{cm}^{-1}$  and 900-1000  $\text{cm}^{-1}$  regions on the IR spectrum. These findings are consistent with earlier research that identified a water-soluble molecule (dipyrrole derivative) from *A. vera* extract with a mass of 225.175 Da and demonstrated the presence of carbonyl and amine groups. A dipyrrole derivative (3, 6-dioxo-3, 3a, 6, 6a-tetrahydropyrrolo [3,4-c] pyrrole-1,4-dicarboxamide) inhibited DPP-IV with an  $\text{IC}_{50}$  of  $8.59 \pm 2.61$  mM in a non-competitive manner [19]. Thus, the beneficial effects of *A. vera*, as reported in numerous studies, in restoring pancreatic  $\beta$ -cell function and mass [14], may be due to its ability to inhibit DPP-IV and increase the half-life of GLP-1.

Furthermore, the potential effects of incretin-based therapeutics have received widespread attention because of the expression of GLP-1 receptor in various tissues, including hepatocytes, and administering GLP-1 agonists reduced triglyceride stores and improved liver function [30]. DPP-IV, on the contrary, is reported to be expressed ubiquitously, and DPP-IV inhibitors are known to have beneficial effects independent of incretin activity [31]. Further, natural products with antioxidant potential play an important role in minimizing the effects of diabetes by scavenging free radicals generated by oxidative stress or by dual functions that target the causes of metabolic syndromes/diseases and minimize free radical generation [24]. Since numerous naturally occurring DPP-IV inhibitors have been reported to possess antioxidant potential, these molecules may exhibit dual activity, acting as DPP-IV inhibitors and exhibiting antioxidant properties [32,33]. Various researchers have reported the antioxidant capacity of *A. vera* using ORAC, FRAP, DPPH, and ABTS assays [34], and that its antidiabetic effects are partially mediated by its strong antioxidant activity [35]. Earlier research from our lab demonstrated that ethanolic *A. vera* extract is rich in polyphenols, with a total antioxidant content of  $17.81 \pm 0.087$  mg equivalent/g [36]. Thus, the therapeutic effects of *A. vera* and its molecules may be due to their dual roles as DPP-IV inhibitors and antioxidants. To conclude this hypothesis, though, more research is required to characterize these molecules using NMR for elucidating the structure and understand their mode of action. DPP-IV inhibitors, along with their antioxidant nature, may influence the immune system and its function; therefore, a longer duration is required for their safety and effectiveness evaluations [37].

#### 4. Conclusion

Our findings divulge support for the anti-hyperglycemic potential of *A. vera* through inhibition of DPP-IV. More research is needed to characterise these molecules using NMR to determine their structures, and enzyme kinetics studies should be carried out to determine their

IC50 values and modes of inhibition. Also, their potential in different animal models, along with additional research on efficacy, safety, and the bioavailability of these isolated DPP-IV inhibitors, needs to be elucidated. *A. vera* appears to be a versatile medicinal plant that is widely utilized in pharmacology, cosmetics, and the food industry. The current increase in demand for functional food products such as *A. vera* is a response to the growing need for solutions to manage type 2 diabetes.

### **Author Contributions**

Conceptualization, K.V. and N.D.; methodology, K.V. and N.D.; software, N.D.; validation, K.V. and N.D.; formal analysis, N.D.; investigation, N.D.; resources, K.V.; data curation, N.D.; writing—original draft preparation, K.V. and N.D.; writing—review and editing, K.V. and N.D.; visualization, K.V. and N.D.; supervision, K.V.; project administration, K.V.; funding acquisition, N.D. All authors have read and agreed to the published version of the manuscript.

### **Institutional Review Board Statement**

Not applicable.

### **Informed Consent Statement**

Not applicable.

### **Data Availability Statement**

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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### **Conflicts of Interest**

The authors declare no conflict of interest.

### **Abbreviations**

The following abbreviations are used in this manuscript:

<b>Abbreviation</b>	<b>Definition</b>
A. vera	Aloe vera
DPP-IV	Dipeptidyl Peptidase-IV

Abbreviation	Definition
FT-IR	Fourier Transform Infrared Spectroscopy
GLP-1 Levels	Glucagon-Like Peptide-1
RP-HPLC	Reverse Phase High Performance Liquid Chromatography

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