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ALOE VERA PHYTOCHEMICALS INHIBITS DIPEPTIDYL PEPTIDASE IV (DPP-IV), AN ANTI-DIABETIC TARGET

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ABSTRACT

Deterioration of β-cells in the pancreas is a crucial factor in the progression of Type 2 diabetes. Inhibition of enzyme DiPeptidyl Peptidase (DPP) -IV is considered as an anti-diabetic drug target which enhances the active Glucagon Like Peptide (GLP) -1 in the blood stream resulting in restoration of pancreatic β-cell mass and function. A number of plant and plant products are being used for the treatment of diabetes. *Aloe vera* is known to have anti-diabetic potential and the work carried out at our Centre by Noor et.al, 2008 had shown that oral feeding of *Aloe vera* extract to diabetic rats exhibited anti-diabetic potentials by restoring pancreatic islet mass and functions. In this study, we characterized the phytochemical constituents of *Aloe vera* extract and demonstrated that the *Aloe vera* extract inhibits DPP-IV with an IC_{50} value of 2.716 mg. We further discussed the implications of using such natural products in treating diabetic patients.

KEY WORDS: ***Aloe vera*, phytochemical analysis, Diabetes, DPP-IV inhibition.**

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INTRODUCTION

Type 2 diabetes mellitus is a life style metabolic disorder characterized by hypoinsulinemia and hyperglycaemia. The pancreas plays a major role in maintaining the glucose homeostasis. The deterioration of β-cells in the pancreas is a crucial factor in the progression of the disease. Currently, there is no cure for diabetes, and all the pharmacological agents are aimed at maintaining the glucose homeostasis (Fasting Plasma Glucose between 90-120 mg/dl). Therefore, the restoration of β-cell mass and its function is of vital importance for an effective treatment. Understanding the mechanism underlying pancreatic rejuvenation has increased considerably upon discovery of incretins such as Glucagon-like peptide – 1 (GLP-1) and Glucose-dependent insulinotropic polypeptide (GIP), which helps in maintaining the glucose homeostasis (i.e. incretin effect) with increased β-cell mass and function. GLP-1 is a glucose-dependent insulinotropic gut hormone secreted by the intestinal L-cells that stimulates insulin secretion. But, it is rapidly degraded by the ubiquitous proteolytic enzyme DiPeptidyl Peptidase-4 (DPP-IV) (E.C No.:3.4.14.5). Inhibition of DPP-IV, the enzyme that makes GLP-1 biologically inactive, enhances the incretin effect and is now considered as one of the target for the treatment of type 2 diabetes. For over 5000 years, Ayurvedic medicine has been practiced in India for the treatment of many diseases, including diabetes, and is primarily based on the use of plants and one such plant used in Ayurveda is *Aloe vera*. *Aloe vera* is a succulent herb that belongs to the Liliaceae family. It forms the basis for many medicianial, pharmaceutical creams and cosmetic products. [1] It has also grabbed the attention of scientist for making use of it in other health care applications. Numerous studies have been carried out to test for the anti-diabetic activity of *Aloe vera*. Okyar *et al*., in the year 2001 showed that *Aloe vera* leaf pulp showed hypoglycaemic activity in Insulin dependent diabetes mellitus (IDDM) and Non insulin dependent diabetes mellitus (NIDDM) rats.[2] A significant reduction in blood glucose levels was observed after oral feeding of Streptozotocin (STZ)-induced diabetic rats with *Aloe vera* ethanolic extract. Previous animal experiments on anti diabetic activity of *Aloe vera* at 300mg/kg bw restored the plasma glucose levels with a concomitant increase in insulin levels.[3] The standardized *Aloe vera* extract was shown to have increased pancreatic islet cell size, number and volume, which indicated that the pancreatic islet cells were regenerated in streptozotocin induced diabetic rats. In continuation of earlier work, this work focuses on identifying the targets that contributes for the pancreatic β-cell rejuvenation. One of the pathways by which β-cells are rejuvenated is through increased levels of active GLP-1.[4] This increased active GLP-1 levels, stimulates β-cell proliferation and neogenesis (development from precursor cells), thereby contributing to the increased β-cell mass and function. Therefore, it was hypothesized that, the *Aloe vera* may stimulate pancreatic rejuvenation either by stimulating GLP-1 secretion or by DPP-IV inhibition, or by both mechanisms. This work aims at identifying the presence of DPP-IV inhibitors in *Aloe vera* ethanolic extract.GLP-1(Glucagon Like Peptide-1) an insulinotropic gut hormone released in response to nutrient ingestion from L-cells. It enhances glucose induced insulin secretion. In addition, it decelerates gastric emptying and suppresses glucagon secretion. After secretion, the GLP-1 molecule is rapidly degraded by the plasma enzyme DPP-IV. The half-life of GLP-1 has been calculated as ~2 min for intact GLP-1 (7–36 amide) .[5] The DPP-IV activity is increased in patients with type 2 diabetes .[6] Therefore, it becomes a requisite to identify DPP-IV inhibitors that act as potential antidiabetic agent. To understand the therapeutic activity of *Aloe vera* in the treatment of non-insulin dependent diabetes mellitus (NIDDM), the ethanolic extract of *Aloe vera* extract was tested for its ability to inhibit DPP-IV.

MATERIALS AND METHODS

All chemicals, including solvents were of analytical grade. Ethanol, methanol, Acetic acid was obtained from SRL (Mumbai, India). HPLC grade acetonitrile was obtained from Fluka (Seelze, Germany). Sitagliptin was purchased from Sellekchem (Houston, USA). Soxhlet apparatus, mantle, rotary evaporator was used for the preparation of the extract. Leaves of *Aloe vera* barbadensis miller were collected from Hosur, Tamilnadu (exact location of collection.)

(i) Preparation of extract

Aloe vera crude extract was prepared according to Noor *et al*., 2008 with slight modifications. The leaves were washed with tap water and weighed. The lower leaf base, the tapering point at the leafy top and the short spines located along the leaf margins are removed by sharp blades. The blade was then introduced into the mucilage layer below the green rind avoiding the vascular bundles, and the top rind was removed. The epidermis of the leaves were peeled off, and the parenchymatous tissue was collected. The colourless, solid mucilaginous gel was cut into pieces. The gel was lyophilized and ground. The lyophilized gel powder was then packed into soxhlet apparatus and extracted with 90 % ethanol at 90° C for 4 hrs. The ethanol containing the extract is filtered and concentrated using rotary evaporator and stored at 4° C.

(ii) Phytochemical analysis

Phytochemicals are the substances that are naturally produced in plants for its growth and development and further to protect themselves from bacteria, virus and fungi. Many of these phytochemicals possess antioxidant properties. The antioxidant activity of the extract is due to the presence of various flavonoids, polyphenols, carbohydrates, etc. Phytochemicals also promote human health by strengthening the immune system .[7] Because of these properties and to ensure the consistency and to avoid the batch to batch variations, the extract was subjected to phytochemical analysis.

(iii) Preparation of samples

100mg of dried crude *Aloe vera* extract was weighed and dissolved in 1 ml water. Kept for shaking overnight,

centrifuged and the supernatant was used for all the assays. All the assays were performed in triplicates.

(iv) Determination of total antioxidant capacity

The antioxidant capacity was estimated by phosphomolybdenum method. The phosphomolybdenum method was performed according to the procedure of Prieto, Pineda and Aguilar (1999).[8] The principle is based on the reduction of Mo (VI) - MO (V) by the sample and subsequent formation of green phosphate/ Mo (V) complex at acidic pH. The sample is mixed with the antioxidant reagent (0.6 M H_2SO_4:28mM Sodium phosphate: 4mM Ammonium Molybdate) and methanol. The reaction tube was then mixed well and incubated for 1.5 Hrs at 95°C. Absorbance was measured at 695 nm. The antioxidant activity was expressed as ascorbic acid equivalents.

(v) Determination of polyphenol content

The total phenolic content was determined using Folin-Ciocalteu's reagent and Gallic acid as standard, according to the modified methods of Singleton and Rossi (1965).[9] Samples (50 µL, triplicates) were introduced into test tubes; 20 µL of Folin-Ciocalteu's reagent and 50 µL of sodium carbonate (5%) were added. The tubes were mixed and incubated for 1 hour in darkness. Absorbance was measured at 725nm. The total phenolic content was determined and expressed as gallic acid equivalents (GAE)/mg of extract.

(vi) Determination of flavonoids

The total flavonoid content was determined using aluminium chloride (AlCl3) colorimetric method .[10] 0.5 ml of the samples and 20-150µg/ml of standard solutions (0.5 ml) were separately mixed with 1.5ml of ethanol (95%), 0.1 ml of 10 % aluminium chloride, 0.1 ml of potassium acetate and made up to 3 ml with water. Incubated at room temperature for 30 min and absorbance of the reaction mixture was measured at 415 nm. Blank was set along with the working standard. The amount of 10 % aluminium chloride was substituted by the same amount of distilled water in the blank. Results are expressed as quercitine equivalents (mg/g of dry extract).

(vii) Determination of carbohydrates

Reducing sugars in *Aloe vera* extract were determined by anthrone test described by Balasubramanian and S. Sadasivam (1996).[11] In brief, 20 mg of the extract was boiled in 1 ml of 2.5N HCl for 3hrs. Neutralized with Na_2CO_3, centrifuged and the supernatant was used for the assay. 5 µL of the sample was mixed with 985 µL water and 4 ml of anthrone reagent was added and incubated at 100°C for 10 min. Absorbance was measured at 630 nm and plotted against the dextrose standard. Reducing sugar content was determined as dextrose equivalents and expressed in mg/gm of extract.

(viii) Determination of protein content

The protein concentration in the *Aloe vera* extract was measured using the method proposed by Bradford. The principle of the assay is based on the ability of the proteins to bind with Coomassie brilliant blue dye. The procedure involves the mixing of the test sample with the Bradford reagent and incubated for 5 min at room temperature. Absorbance was then measured at 595nm. The absorbance value is plotted against the standard graph using Bovine Serum Albumin (BSA) as a reference standard and the results are expressed in mg/gm of the extract.

(ix) DPP-IV Enzyme activity assay

The DPP-IV enzyme activity is determined in normal, healthy rat and human plasma. The purpose of the assay is to confirm the presence of DPP-IV activity in rat and human plasma. All the experiments were conducted after obtaining clearance from Instituttional Animal Ethical Committee (VIT IAECV:20 dated 02-03-2012) and University Human Ethical Committee (Ref: VIT/UHEC-5/NO.2; dated 27-08-2012) respectively. The assay is an end-point spectrophotometric determination designed for a 96- well plate reader and is a modification of a previously published method. 0.1 M Tris HCL, pH 8 was used as reaction buffer. A 1 mM stock solution of Gly-Pro-p-nitroanilide was prepared using 0.1 M Tris-HCL buffer, pH 8.0. Standard (p-Nitro aniline) of various concentrations (5-35nM) were made in Tris-HCL buffer and plotted the standard graph. One unit of enzyme will produced 1 µM of p-nitroaniline from Gly-Pro-p-nitroanilide per minute in 0.1 M Tris buffer at pH 8.0 at 37°C. The reaction was initiated by adding 70 µL of substrate to various volumes of plasma (10, 20, 30, 40, 50 µL). The reaction volume was made to 250 µL with reaction buffer and incubated for 30 min at 37°C. After incubation O.D was taken at 405 nm using a microplate reader. The enzyme activity was determined by plotting against the pNA standard graph.

(x) DPP-IV Enzyme inhibition assay

To test for the ability of the *Aloe vera* extract to inhibit DPP-IV enzyme, biochemical assay was performed. The assay was performed in 96- well plate. Healthy human/rat plasma was used as the enzyme source. 0.1 M Tris HCL, pH 8 was used as reaction buffer. A 1 mM Gly-Pro-p-nitroanilide was used as the substrate. All the test samples were dissolved in water. Extract concentrations ranging from 0.6 to 4.5 mg were taken for the assay. The assay was run by mixing 80 µl of 0.1 M Tris buffer, 70 µl of plasma and 30 µl of the test sample. After a 15 minute incubation period at 37°C, 70 µl of substrate solution was added to the appropriate wells to commence the reaction. Following an incubation period of 30 minutes at 37°C, absorbance was determined at 405 nm. The assay was performed in triplicate with appropriate blanks and standard. Percentage of inhibition was calculated as

$$\% \text{ INHIBITION} = \frac{(\text{Absorbance of control} - \text{Absorbance of the test}) \times 100}{\text{Absorbance of control}}$$

Table 1
Enzyme inhibition assay

	BUFFER (TRIS-HCL)	ENZYME (PLASMA)	INHIBITOR (PLANT EXTRACT)	PREINCUBATION AT 37°C FOR 15 MIN.	SUBSTRATE (GLY-PRO-pNA)	INCUBATION AT 37°C FOR 30 MIN. O.D AT 405 NM
CONTROL	110 µL	70 µL	-		70 µL	
TEST SAMPLE	80 µL	70 µL	30 µL		70 L	

(xi) HPLC analysis

The Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) was carried out to substantiate the consistency of the extract prepared and to avoid the batch to batch variation. The reverse phase C18 column consists of 18 carbon chains immobilized to silica matrix. The principle of C18 involves the binding of hydrophobic molecules to the C18 stationary phase. The interaction is disturbed by the low polar mobile phase in a linear gradient mode. The *Aloe vera* extract was dissolved in water at a concentration of 100mg/ml. 25µl was then injected into C-18 RP-HPLC column (Waters, 150 X 3.9mm, i.d., 5µm). The solvents used were (0.1%) acetic acid (Solvent A) and HPLC grade Acetonitrile (Solvent B). The flow rate was set at 1 ml/min. The Column was equilibrated for 30 min before sample injection and absorbance was monitored at 255 and 290nm. Data acquisition and processing were performed using Breeze data (Waters) system. The gradient system involves the following

Table 2
Solvent gradient for RP-HPLC

TIME	SOLVENT A	SOLVENT B
-	88 %	12 %
3	88 %	12 %
8	70 %	30 %
13	45 %	55 %
15	40 %	60 %
17	10 %	90 %
21	10 %	90 %
23	88 %	12 %

RESULTS AND DISCUSSION

1. Phytochemical analysis

The main feature of *Aloe vera* plant is its high water content, ranging from 99.0-99.5%. The remnant solid material of 0.5-1.0% is reported to contain over 75 different potentially active compounds, vitamins, minerals, enzymes, polysaccharides, phenolic compounds and other organic acids [12], while some of them acts as antioxidants. Antioxidants are a diverse group of chemicals that is naturally found in vegetables, fruits and plants. They protect the body from oxidative stress induced by free radicals and reactive oxygen species by suppressing their formation. The phytochemical analysis of the *Aloe vera* ethanolic extract showed the presence of medicinally active constituents like flavonoids, phenolic molecules, carbohydrates and proteins. All these phytoconstituent contributes for the anti-oxidant property of the extract/plant. The total anti-oxidants of the *Aloe vera* crude extract are found to be 31.94 mg/gm of ascorbic acid equivalents. Masis Laboratories (1984) and Terry Corporation reported that, there is considerable variation in the composition of *Aloe vera* depending on the season, location .[13] This explains the discrepancies of different research findings.

Table 3
Phytochemical analysis of standardized aloe vera extract

TOTAL ANTI OXIDANTS	31.94 ± 0.0091 (ASCORBIC ACID EQU)
POLYPHENOLS	8.117 ± 0.0078 (GALLIC ACID EQU)
FLOVAONOIDS	3.84 ± 0.009 (QUERCETIN EQU)
CARBOHYDRATES	20.598 ± 0.0064 (DEXTROSE EQU)
PROTEIN CONTENT	0.86 ± 0.01 (BSA EQU)

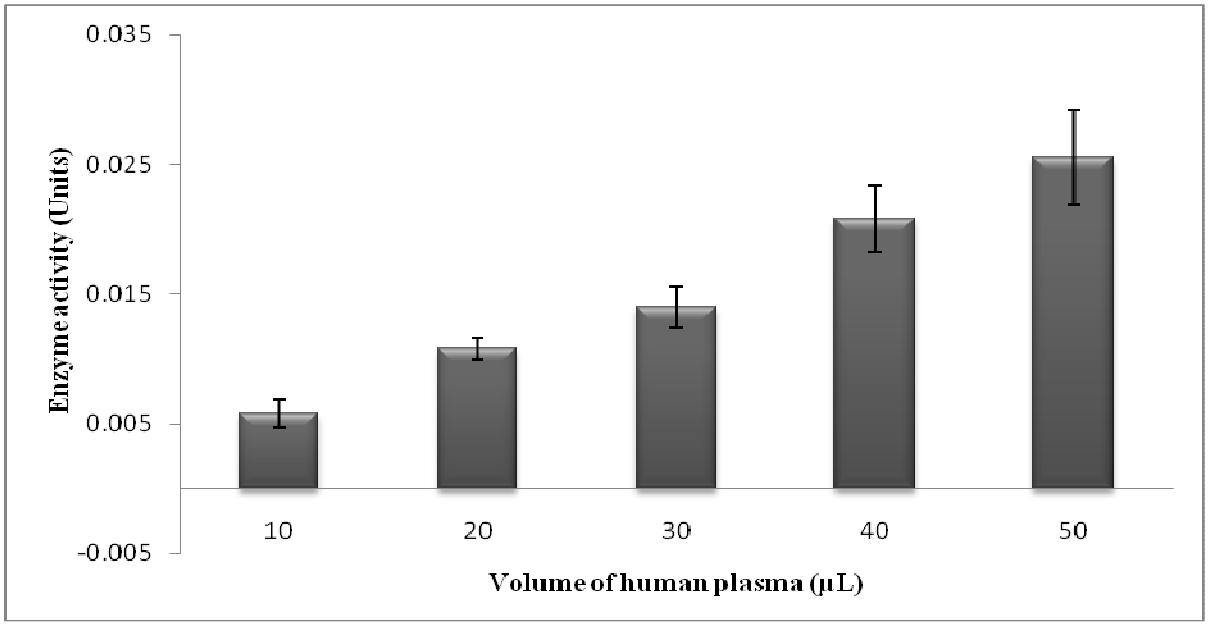
[* All the values are average ± SD for 3 independent analysis]

Polyphenols are a group of chemical substances consisting of more than one phenol group per molecule. Phenolic compounds are the principle antioxidant constituents of natural products that act as potent radical terminators. [14, 15] Polyphenols are the most abundant group of plant compounds, known to provide much of the flavour, color, and taste of the fruits and vegetables. The health benefits of polyphenols have been associated with their antioxidant, antibacterial, anti-inflammatory and anti-allergenic property. The antioxidant property of phenolics is due to their redox properties, which allows them to act as reducing agents, hydrogen donors, metal chelators and single oxygen quenchers .[16] The total phenolic content of the *Aloe vera* ethanolic extract is found to be 8.117 ± 0.0078 mg/gm of gallic acid equivalent. The results are given in table 3. On the other hand, the previously reported phenolic content of *Aloe vera* gel powder is 5.16 mg/gm .[17] This shows that, the ethanol (90%) used for extraction has enriched the phenolic content present in the gel. Flavonoids are water soluble plant pigments that belong to a part of a polyphenol group of plant compounds. Flavonoids are one of the most diverse and widespread group of natural compounds that are the most important natural phenolics.[18] These compounds possess a broad spectrum of chemicals and biological activities like radical scavenging properties. Flavonoids can be classified into several subclasses according to their chemical structure. These groups include flavonols, dihydroflavonols, flavones, isoflavones, flavanones, anthrocyanins and anthrocyanidins. The ethanol extract of *Aloe vera* is found to contain 3.84 mg/gm in terms of quercetin. Moniruzzaman *et al*., 2012 reported that, *Aloe vera* gel ethanol extract (80%) is found to contain 5.43 mg/kg of catechein equivalents. This shows that, increasing the percentage of ethanol increases the amount of flavonoid extracted .[19] The extract is rich in carbohydrates which might help to boost the immune system .[20] Some of the previously reported carbohydrate includes acemannan [21], mannan, cellulose galactan, xylan, mannose etc .[22] Lectin, a glycoprotein present in this plant has anti cancer and wound healing property .[23, 24] This extract also contains a very little amount of proteins that might help in the normal functioning/activity of the plant. In conclusion, the extract has high antioxidant activity because of the presence of flavonoids, phenols, etc. Hence, each molecule present in the extract might contribute to the anti-diabetic property synergistically. There are several studies that show the anti diabetic effect of many medicinal plants .[25] Traditionally used herbs in treating diabetes, had led to the isolation of potential molecule with hypoglycaemic activity. These herbs have been used for generations, and so it can be expected that the active principle isolated would have low toxicity. Interestingly, *Galega officinalis* L (goats rue) was used in treating diabetes in Europe during 10th century. This led to the isolation of its active ingredient “galegine” which exhibited the antihyperglycemic activity. After a slight modification in the structure, it was named metformin and introduced into the market. This is how “galegine” served as a template for metformin, which is a successful hypoglycaemic drug for type 2 diabetics .[26] Likewise, few plants are said to have the same effect as like chemically synthesized insulin secretagogues (i.e. sulfonylureas and meglitenides). They are Banaba (*Lagerstroemia speciosa*) [27], Fenugreek (*Trigonella foenum-graecum*).[28], Gymnema (*Gymnema sylvestre*) [29], etc. and polypeptide-P from the seeds of *Momordica charantia* exhibited insulin like activity when injected subcutaneously into humans .[30] Oral administration of Gowar plant and seed at a dose of 40mg/kg bw showed hypoglycaemic activity similar to tolbutamide.

2. DPP-IV Enzyme activity assay

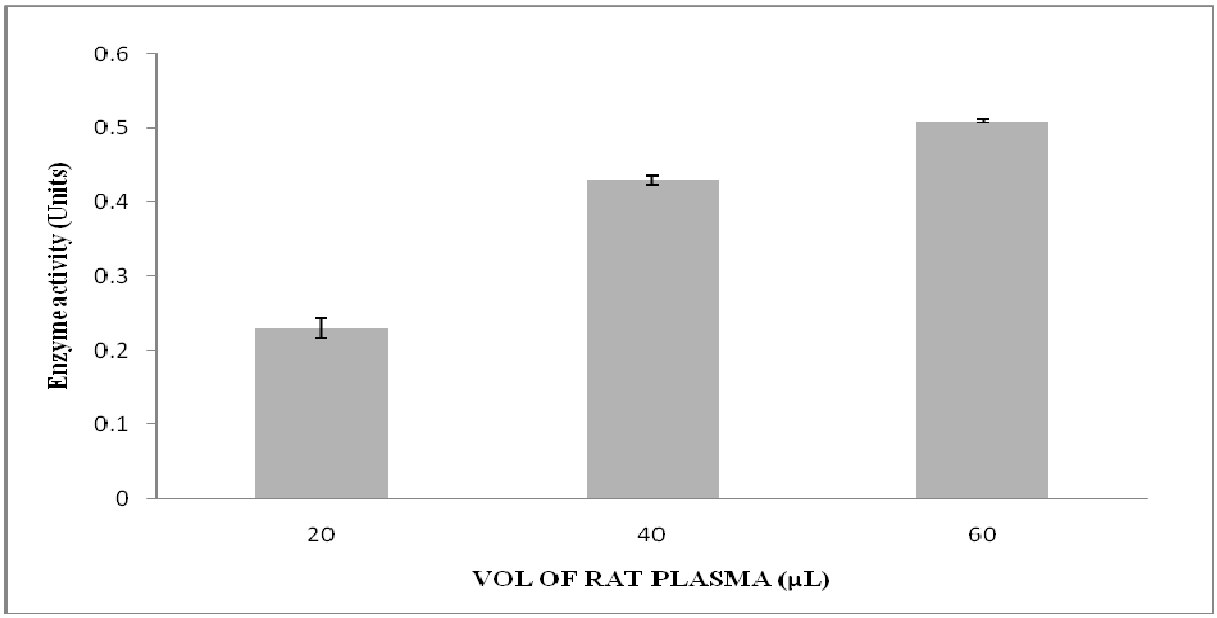
DPP-IV is a ubiquitous enzyme that is present in both soluble and membrane bound form distributed almost in all tissues in humans and animals .[31] It makes the GLP-1 biologically inactive. Graph 1 shows the dose dependent increase in DPP-IV activity in healthy human and rat plasma. This illustrates the presence of DPP-IV enzyme in normal human/rat plasma. The DPP-IV activity present in the plasma of healthy human and rat is 0.5228 and 0.300933 U/mL respectively. But, its activity is an increased during diabetic condition. Inhibiting the enzyme DPP-IV, thereby increasing the half life of GLP-1 in the circulation is a key target in treating type 2 diabetes.

Graph 1
Human Plasma DPP-IV activity



Mean Error bars in the graph represents the mean ± standard error from the triplicate assays.

Rat Plasma DPP-IV activity

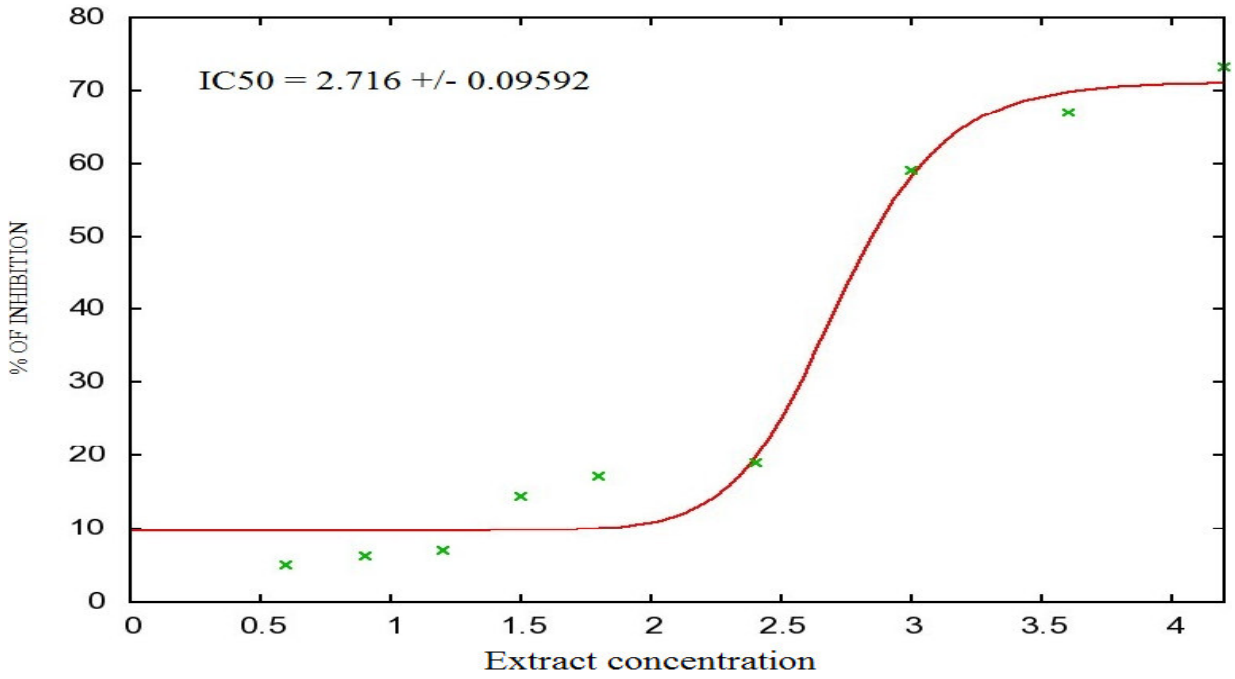


Mean Error bars in the graph represents the mean ± standard error from the triplicate assays.

3. DPP-IV Enzyme inhibition assay

There are few plant molecules like berberine, naringenin that shows inhibition to DPP-IV .[32, 33] Similarly, an attempt of exploring the mechanism of antidiabetic activity of *Aloe vera*, DPP-IV inhibitory assay was carried out. In our study, the *Aloe vera* ethanolic crude extract is able to inhibit the enzyme DPP-IV of both human and rat plasma. Graph 2 shows the DPP-IV inhibition of human plasma in a dose dependent manner. The results are expressed as percentage of inhibition. The IC50 i.e the concentration of the extract required to bring 50% of the enzyme inhibition is 2.716 ± 0.09592 mg. This illustrates the presence of some natural available DPP-IV inhibitor in the *Aloe vera* ethanolic extract. This could be a single molecule or a group of molecules that might act synergistically to bring the action.

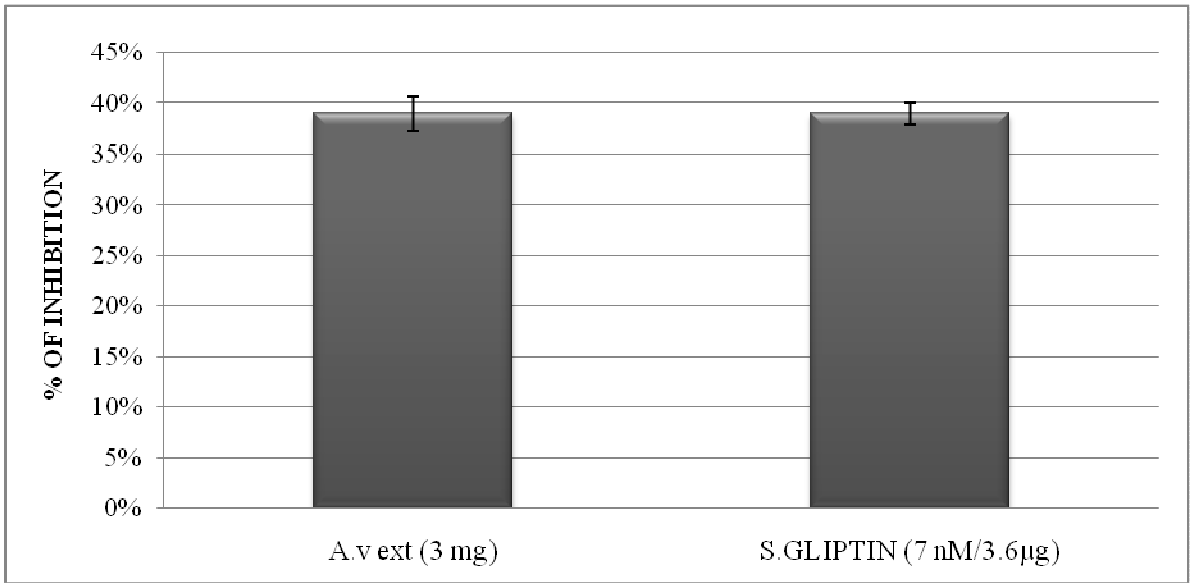
IC 50 Determination



Graph 2

The DPP-IV inhibitory activity in human plasma. Aloe vera crude ethanolic extract showed a concentration dependent increase in DPP-IV inhibition with an IC50 of 2.716 ±0.09592 mg in human plasma.

DPP-IV INHIBTION IN RAT PLASMA



Graph 3

The bar diagram represents the DPP-IV inhibitory activity of Aloe vera crude ethanolic in healthy rat plasma. Sitgliptin (DPP-IV inhibitor) was used as positive control.

4. HPLC analysis

The HPLC analysis using C18 reverse phase column showed the presence of various molecules at 265 nm. This shows the presence of molecules with varying hydrophobicity. These compounds might individually or synergistically in bringing the DPP-IV inhibition.

C18 RP-HPLC Profile

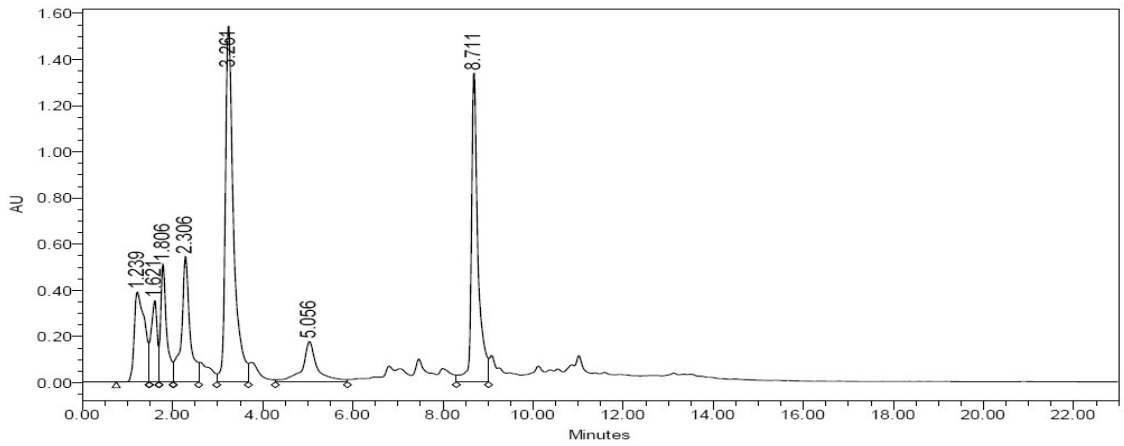


Figure 1

The RP-HPLC profile at 265 nm showed the presence of presence of various molecules (peaks), which might act principally or synergistically for its potent biological activity.

CONCLUSION AND FUTURE PROSPECTIVES

So in conclusion, the *Aloe vera* ethanolic extract has various molecules such as polyphenols, flavonoids, carbohydrates and proteins. These molecules contribute to its antioxidant activity, which is an added beneficial effect to be used for human healthcare. The presence of these molecules shows that *Aloe vera* is a good source of antioxidants. Although this concept has been confirmed in previous studies [34, 35, 36], our study is the first to show that the *Aloe vera* extract is able to inhibit the enzyme DPP-IV confirmed by the biochemical enzyme assay. The crude extract is also able to show DPP-IV inhibition in streptozotocin induced diabetic rats (Unpublished data). Now, the future work is to identify/isolate the molecule that is involved in DPP-IV inhibition and to characterized the molecule. Overall, in exploring the pathway by which *Aloe vera* extract stimulates pancreatic rejuvenation, we conclude here that one of the pathways by which *Aloe vera* acts is by inhibiting the enzyme DPP-IV.

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REFERENCES

1. Sharma A, Gautam S. An overview on medicinal properties of Aloe vera: antibacterial & antifungal aspects. Int J Pharm Bio Sci 2013; 4 (3) 694-705.
2. Okyar AC, Akev A, N Baktir N, Sutlupinar T. Effect of *Aloe vera* leaves on blood glucose level in type I and type II diabetic rat models. Phytother Res 2001;15(2):157-61.
3. Noor A, Gunasekaran S, Soosai MA, Vijayalakshmi MA. Antidiabetic activity of *Aloe vera* and histology of organs in Streptozotocin induced diabetic rats. Curr Sci 2008;94(8):1070-76.
4. Garber AJ. Incretin effects on beta-cell function. Replication and mass. Diabetes care 2011;34(2):S258-S263.
5. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gasteroenterology 2007;132(6):2131-57.
6. Ryskjaer J, Deacon CF, Carr RD, Krarup T, Madsbad S, Holst J, *et al*., Plasma dipeptidyl peptidase-IV activity in patients with type 2 diabetes mellitus correlates positively with HbA1c levels, but is not acutely affected by food intake. Eur J Endocrinol 2006;155(3):485-93.
7. Garcia-Lafuente A, Guillamon E, Villares A, Roatagno MA, Martinez JA. Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. Inflamm Res 2009;58(9):537-52.
8. Prieto P, Pineda M, Aquilar M. Spectrometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999;269(2):337-41.
9. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic acid reagents. Am J Enol Viticult 1965;16(3):144-58.
10. Chang C M, Wen YH, Chem J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Analysis 2002;10(3):178-82.
11. Balasubramanian T, Sadasivam S. Changes in carbohydrate and nitrogenous components and amylase activities during germination of grain amaranth. Plant foods HumNutr 1989;39(4):327-30.
12. Atherton P. *Aloe vera* revisited. Br J Phytother 1998;4:76-83.

13. Grindlay D, Reynolds T. The *Aloe vera* phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. J Ethanopharmacol 1986;16(2):117-51.
14. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 1999;47(10):3954-62.
15. Shahidi F, Wanasundara PKJPD. Phenolic antioxidants. Crit Rev Food Sci Nutrit 1992;32(1):67-103.
16. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends in plant science 1997;2(4):152-59.
17. Aslani A, Ghannadi A, Raddanipour R. Design, formulation and evaluation of *Aloe vera* chewing gum. Adv Biomed Res 2015;4:175-81.
18. Agarwal PK. Carbon-13 NMR of Flavonoids. *Elsevier* 1989(6);564-71.
19. Moniruzzaman M, Rokeya B, Ahmed S, Bhowmik A, Khalil MI, Gan SH. In vitro antioxidant effects of Aloe barbadensis miller extracts and the potential role of these extracts as antidiabetic and antilipidemic agents on steptozotocin-induced type 2 diabetic model rats. Molecules 2012;17(11):12851-67.
20. Petrovsky N, Cooper PD. Carbohydrate-based immune adjuvants. Expert Rev Vaccines 2011;10(4):523-37.
21. Femenia A, Sanchez ES, Simal S, Rossello C. Compositional features of polysaccharides from Aloe vera (*Aloe barbadensis* Miller) plant tissues. Carbohydrate Polymers 1998;39(2):109-17.
22. Hamman J H. Composition and applications of *Aloe vera* leaf gel. Molecules 2008;13(8):1599-1616.
23. Reynolds T, Dweck AC. *Aloe vera* leaf gel: A review update. Ethanopharmacol 1999;68(1):3-37.
24. Chitra R, Sajithal GB, Chandrakasan C. Influence of *Aloe vera* on collegan characteristic in healing dermal wound in rats. Mol Cell Biochem 1998;181(1):71-76.
25. Nahas R, Moher M. Complementary and alternative medicine for the treatment of type 2 diabetes. Can Fam Physician 2009;55(6):591-96.
26. Fabricant D S, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect 2001;109(1):69-75.
27. Liu R, Zhang J, Liu W, Kimura Y, Zheng Y. Anti-Obesity effects of protopanaxdiol types of Ginsenosides isolated from the leaves of American ginseng (Panaz quinquefolius L.) in mice fed with a high fat diet. Fitoterapia 2010;81(8):1079-87.
28. Mohammad RH, Mohammad I, Mohammad RS, Mohammad RN, Kenneth NW. The effect of fenugreek 4-hydroxyisoleucine on liver function biomarkers and glucose in diabetic and fructose-fed rats. Phytother Res 2009;23(1):61-64.
29. Mohammad B, Abderrahim Z, Hassane M, Abdelhafid T, Abdelkhaleq L. Medicinal plants with potential antidiabetic activity – A review of ten years of herbal medicine research. Int J Diabetes & Metabolism 2006;14:1-25.
30. Farog T, Sapna SL, Meenakshi M, Kumar U. A Review: Medicinal plants and its impact on diabetes. World J Pharm Res 2012;1(4):1019-46.
31. Mentlein, R. Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. Regul Pept 1999;85(1):9-24.
32. Ai Masri IM, Mohammed MK, Tahaa MO. Inhibition of dipeptidyl peptidase IV is one of the mechanisms explaining the hypoglycaemic effect of berberine. J Enzyme Inhib Med Chem 2009;24(5):1061-66.
33. Parmar HS, Jain P, Chaunhan DS, Bhinchar MK, Munjal V, Yusuf M, Choube K, Tawani A, Tiwari V, Manivannan E, Kumar A. DPP-IV inhibitory potential of naringin: an in silico, invitro and in vivo study. Diabetes Res Clin Pract 2012;97(1):105-11.
34. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 2009;14(6):2167-80.
35. Botes L, Westhuizen FHV, Loots DT. Photochemical contents and antioxidant capacities of two *Aloe greatheadii* var *Davyana* extracts. Molecules 2008;13(9):2169-80.
36. Hu Y, Xu J, Hu Q. Evaluation of antioxidant potential of *Aloe vera* (*Aloe barbadensis Miller*) extracts. J. Agric. Food Chem 2003;51(26):7788-91.