Effects of 22 traditional anti-diabetic medicinal plants on DPP-IV enzyme activity and glucose homeostasis in high-fat fed obese diabetic rats

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Word count: 3,239 (Main Body)

Figures: 05

Table: 04

Abbreviated title: Anti-diabetic plants and DPP-IV
Abstract

This study investigated the effects of hot water extracts of 22 medicinal plants used traditionally to treat diabetes on Dipeptidyl peptidase-IV (DPP-IV) activity both in vitro and in vivo in high fat fed (HFF) obese-diabetic rats. Fluorometric assay was employed to determine the DPP-IV activity. For in vivo studies, HFF obese-diabetic rats were fasted for 6 hours and blood was sampled at different times before and after the oral administration of the glucose alone (18mmol/kg body weight) or with either of the four most active plant extracts (250mg/5ml/kg, body weight) or established DPP-IV inhibitors (10µmol/5ml/kg). DPP-IV inhibitors: sitagliptin, vildagliptin and diprotin A, decreased enzyme activity by a maximum of 95-99% (P<0.001). Among the 22 natural anti-diabetic plants tested, A. Latifolia exhibited the most significant (P<0.001) inhibitory activity (96 ± 1%) with IC50 and IC25 values of 754µg/ml and 590µg/ml. Maximum inhibitory effects of other extracts: A. marmelos, M. indica, C. colchinchinensis, T. foenum-graecum and A. indica were (44 ±7%; 38 ± 4%; 31±1%; 28±2%; 27±2%, respectively). A maximum of 45% inhibition was observed with >25µM concentrations of selected phytochemicals (rutin). A. latifolia, A. marmelos, T. foenum-graecum and M. indica extracts improved glucose tolerance, insulin release, reduced DPP-IV activity and increased circulating active GLP-1 in HFF obese-diabetic rats (P<0.05-0.001). These results suggest that ingestion of selected natural anti-diabetic plants, in particular A. latifolia, A. marmelos, T. foenum-graecum and M. indica can substantially inhibit DPP-IV and improve glucose homeostasis, thereby providing a useful therapeutic approach for the treatment of T2DM.

Keywords: Diabetes, DPP-IV, Medicinal plants, Glucose, GLP-1, Insulin
Abbreviations

1. DPP-IV: Dipeptidyl peptidase-IV
2. Gly-Pro-AMC: Gly-Pro-7-Amino-4-Methyl-Coumarin
3. AMC: 7-Amino-4-Methyl-Coumarin
4. OGTT: Oral glucose tolerance test
5. GIP: Glucose-dependent insulinotropic polypeptide
6. GLP-1: Glucagon-like peptide-1
7. IC25: 25 percent inhibitory concentration
8. IC50: 50 percent inhibitory concentration
9. T2DM: Type 2 Diabetes mellitus
10. HFF: High fat fed

Introduction

Diabetes Mellitus has become a worldwide concern, manifesting as one of the most major health issues within the world’s population. There are several forms of diabetes, including gestational diabetes, but Type 1 and Type 2 diabetes are by far the most prevalent. Type 2 diabetes (T2DM), most often associated with obesity, is a particularly widespread disease and many patients from all over the globe are afflicted by this condition. T2DM patients are characterized by impaired β-cells function and insulin secretion together with tissue insulin resistance [1]. Since, the prevalence and associated complications of T2DM are so damaging, more effective therapies are being sought to either delay or prevent the progression of T2DM [2]. As so, plants are most reliable source as studies to date found they contains series of potential phyto-groups including alkaloids, glycosides, terpenoids, phenolic, flavonoids and plant-derived peptides, each of them has shown potential antidiabetic activity in different experiment [2]. Besides, the diet of patients with T2DM plays a vital role in helping to maintain blood glucose control involving such factors as energy density, carbohydrate content, dietary fiber and natural products that may directly or indirectly affect the absorption of nutrients or the secretion and action of insulin [3]. Hormones released by the gut in response to nutrient absorption, most notably GIP and GLP-1, also play an important role in modulating postprandial hyperglycemia [4]. These hormones have a very short circulating half-life due to inactivation by the enzyme DPP-IV which cleaves the first two amino acids from the N-terminals producing GIP (3-42) and GLP-1 (9-36) [5]. This is why DPP-IV inhibitors are beneficial in the treatment of T2DM. Anti-diabetic drugs like metformin and nateglinide which
respectively target insulin action and insulin secretion can, at high concentrations also suppress DPP IV enzyme activity and such action may partly explain use of nateglinide as prandial insulin releasing agent that augments GLP-1 levels [6].

Having adequate bio-active insulin in the circulation is the key to control of glucose homeostasis as the hormone is unique in stimulating tissue glucose uptake and limiting hepatic glucose output. DPP-IV interferes with normal insulin action by degrading and therefore diminishing the insulinotropic and other β-cell actions of GLP-1 and GIP [4]. Natural resources are being explored to find new dietary ways to promote healthy blood glucose control including manipulation of the microbiome [7]. Over the years, many studies have revealed the anti-diabetic activity of plants used traditionally for the treatment of diabetes and defined their actions mediated via effects on the gastrointestinal processing of food and both the secretion and action of insulin [8-10]. More recently, dietary components including dairy, tuna, rice, salmon and amaranth have been found to exhibit DPP-IV inhibitory properties in vitro [11, 12].

In the present work, 22 traditional medicinal plants with proven anti-diabetic activity were selected to assess their effects on DPP-IV enzyme activity in vitro (Table 1 & 2). Furthermore, four of the most effective plants (A. latifolia, A. marmelos, T. foenum-graecum and M. indica), were selected to assess their acute effects on plasma DPP-IV activity, glucose-lowering and insulin-releasing properties in high fat fed obese-diabetic rats.

Materials and Methods

Plant materials and preparation of extract

Twenty-two plants used traditionally to treat diabetes were purchased to assess their ability to inhibit DPP-IV enzyme activity and improve glycaemic control. The plants selected and their traditional and pharmacological actions are given in Tables 1-2. All plant materials were sourced in India where they are the native species. Confirmation of identity for the plants was made by a taxonomist Prof. F. A. Khan, Head of Department of Botany, Benazir Govt. Science & Commerce College, Bhopal, Barkatullah University, Madhya Pradesh, India where the plant specimens have been deposited in the herbarium. The accession numbers (voucher specimen numbers) for 22 traditional medicinal plants are listed in Table 3.

All plant components (Table 1, 2 & 3) were dried and grounded to obtain a fine powder. About 1gm of each dried powder was infused using 40ml of boiled water. Aqueous extracts were
chosen based on traditional use and prior studies of plants selected. The infusion was left for
15 min before being filtered through Whatman no. 1 filter paper. After that, the filtrates were
dried under a vacuum (Savant Speedvac; New York, USA) to produce plant extract that was
used to perform DPP-IV inhibitory experiments. For this purpose, the dried extract was
dissolved in a 100 mM Tris-HCl buffer at an initial concentration of 5 mg/ml.

**Determination of DPP-IV inhibitory activity in vitro**

A fluorometric method was used to determine the DPP-IV inhibitory activity of plant extracts
based on that described previously [6, 13]. For *in vitro* studies, a 100 mM Tris-HCl buffer was
prepared and adjusted to pH 8.0 using 100 mM Tris-base. Reactions were performed in 96-
well black-walled, clear-bottomed microplates (Premier Scientific Ltd, Belfast, UK) using 8
mU/ml of DPP-IV enzyme and 200 µM of fluorescent substrate (Gly-Pro-AMC) with or
without plant extract, known DPP-IV inhibitor or selected phytochemicals. These included
caffeine, catechin, epicatechin, gallic acid, isoquercitrin, quercetin and rutin as well as the small
molecule anti-diabetic drug nateglinide. DPP-IV assay was based on liberation of AMC (7-
amino-4-methyl-coumarin) from DPP-IV substrate, Gly-Pro-AMC. Changes in fluorescence
due to cleavage of the molecule by DPP-IV were measured with an excitation and emission at
370 nm and 440 nm with 2.5 nm slit width using a FlexStation 3 (Molecular Devices,
California, USA). The inhibition of DPP-IV activity was calculated as the percentage of
inhibition by each plant extract at various concentrations. Neither the plant extracts nor plasma
samples showed any loss of activity when stored for many months at 20°C. It was checked in
control experiments that the extracts did not themselves cleave the substrate or interfere with
fluorescence measurements at the concentrations employed.

**Animals**

Forty male Sprague-Dawley rats (Envigo, Huntingdon, UK, approx. 380-400g) were fed a high
fat diet (45% fat, 20% protein, and 35% carbohydrate; 26.15 kJ/g total energy percent; Special
Diet Service, Essex, UK), *ad libitum* for 5 - 6 weeks to induce obesity and glucose intolerance.
An additional 10 age-matched rats were maintained on standard rodent diet (30% protein, 10%
fat, and 60% carbohydrate; 12.99 kJ/g total energy percent; Trouw Nutrition, Cheshire, UK).
High fat fed rats exhibited increased body weight (398.7 ± 1.6 g versus 384.7 ± 1.8 g; P<0.01),
impaired oral glucose tolerance and enhanced glucose-induced insulin responses, indicative of
insulin resistance compared with the lean control rats fed normal diet (Fig 3). These animals
also exhibited significantly elevated HbA1c levels (5.90 ± 0.07 % versus 4.57 ± 0.05 %; P<0.001), measured by the point-of-care A1CNow+ kit (PTS Diagnostic, Indiana), indicative of mild diabetes. The animals were housed individually in an air-conditioned room at 22 ± 2°C with a 12h light/dark cycle. All animal experiments were conducted in accordance with UK Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63EU. All necessary steps were taken to prevent any potential animal suffering. The animal studies were approved by local Ulster Animal Welfare and Ethical Review Body (AWERB) committee (01/10/2016), as well as being covered under a UK Home Office Animal project/personal licence numbers PIL450, PIL1822 and PPL 2804, approved on 06/05/2016. All animals were maintained under specific pathogen-free conditions and experiments were conducted in the Biomedical and Behavioral Research Unit (BBRU) at Ulster University, Coleraine, UK. Blood was collected from the cut tip of the tail of conscious animals without need for anaesthesia. No animals were culled.

**Determination of the acute effects of plant extracts on DPP-IV activity in vivo**

High fat-fed rats were used to study DPP-IV inhibitory activity of four medicinal plants (*A. latifolia, A. marmelos T. foenum-graecum and M. indica*) *in vivo*. Sitagliptin and vidagliptin were used in comparison as positive controls. Animals were fasted for approximately 6 hours prior to experimentation. Blood samples were collected from the cut tip of the tail from conscious rats before and after oral administration of glucose with or without plant extract (250mg/5ml/kg) or an established DPP-IV inhibitor (10µmol/5ml/kg) at 0, 30, 60, 120, 180, 240, 360 and 480 min. Samples were collected in chilled fluoride/heparin-coated micro centrifuge tubes followed by centrifugation at 12000 rpm for 5 min. Plasma was stored at -20°C for measurement of insulin, DPP-IV activity and active GLP-1 (7-36). An Ascencia Contour glucose meter (Bayer, Newbury, UK) was used to measure blood glucose and insulin was determined by dextran-charcoal radioimmunoassay [14]. Active GLP-1 (7-36) was determined in the plasma samples collected at 60 min using a specific GLP-1 (Active) ELISA Kit (EGLP-35K, Merck Millipore, Dorset, UK).

**Statistical Analysis**

Statistical analysis tests were performed by using Graph Pad-Prism 5. The results are represented as mean±SEM. Data was analysed using by unpaired Student’s t test
(nonparametric, with two-tailed P values) and one-way ANOVA with repeated measures was used and adjusted using Bonferroni correction. P value of < 0.05 was considered significant.

Results

*In vitro* DPP-IV Inhibitory effects of plant extracts

The extracts from 22 different plants were evaluated *in vitro* to assess their effects on DPP-IV activity. Established DPP-IV inhibitors namely sitagliptin, vildagliptin and diprotin A were used as positive controls (Table 4, Fig 1 (A-C)). These inhibitors decreased DPP-IV enzyme activity by up to 99 ± 2%, 99 ± 3% and 95 ± 3%, with IC$_{50}$ values of 2.04x10$^{-2}$, 1.70x10$^{-2}$ and 2.39x10$^{-3}$ µg/ml (P<0.05 - 0.001, respectively, (Table 4, Fig 1 (A-C)). In the presence of *A. latifolia* (bark), enzymatic AMC liberation from Gly-Pro-AMC was inhibited by 20 ± 1% to 96 ± 1% (P<0.05-0.001, Table 4, Fig 1D) at concentrations of 200-5000µg/ml when compared to control. Moreover, *A. marmelos* (leaves), *M. indica* (seeds) and *T. foenum-graecum* (seeds) significantly inhibited DPP-IV enzyme activity at concentrations ranging from 200-5000µg/ml (P<0.05-0.001, respectively, Table 4, Fig 1 (E-G). The highest inhibitory effects of plant extracts were observed at 5000 µg/ml (44 ± 7%, 38 ± 4%, 31 ± 1% and 28 ± 2%, P<0.001, respectively, Table 4) as compared to control. The other plant extracts were found to inhibit DPP-IV activity in between 9 ± 1% to 27 ± 2% (P<0.05-0.001, Table 4) when tested at a concentration of 5000 µg/ml. The phytochemicals responsible for the inhibitory action are unknown but several possible candidates known to be present in the plant collection were tested. These included caffeine, catechin, epicatechin, gallic acid, isoquercitrin, quercetin and rutin. As shown in Fig 2A-G, each inhibited DPP-IV with the majority exhibiting lower effective concentrations of 125-200µM. Isoquercitrin and quercetin inhibited at 25-50µM whereas rutin was particularly effective with maximal inhibition of 45% and IC$_{25}$ value of 306µM. The effect was similar to the established insulintropic drug nateglinide (Fig 2H).

Acute effects of plants extract on glucose tolerance and plasma insulin in high fat-fed rats

Four plants (*A. latifolia*, *A. marmelos*, *T. foenum-graecum* and *M. indica*), the most potent in inhibiting *in vitro* DPP IV enzyme activity, were selected for evaluation of effects on DPP IV activity and oral glucose tolerance in high fat fed rats. Hot water extracts (250mg/5ml/kg) substantially improved the glycemic excursion from 30 to 240 min and increased plasma insulin from 30 to 120 min as compared to oral administration of glucose alone (P<0.05-0.001;
Fig 3A & C). Established DPP-IV inhibitors (sitagliptin & vildagliptin) also improved glucose
tolerance and insulin release following oral administration (P<0.05-0.001; Fig 3 B & D).

**Acute effects of plant extracts on circulating DPP-IV activity and active GLP-1 (7-36) in high fat-fed rats**

Sitagliptin and vildagliptin significantly reduced DPP-IV activity in high fat fed rats (P<0.001, Fig 4 (A-D)). Hot water extracts (250mg/5ml/kg) of *A. latifolia, A. marmelos, T. foenum-graecum* and *M. indica* also significantly decreased *in vivo* DPP-IV enzyme activity compared to glucose alone (P<0.05-0.01, Fig 4 (A-D)). The effects of *A. latifolia* and *T. foenum-graecum* were particularly prominent with a sustained inhibition of DPP-IV activity from 30 min onwards, (P<0.05-0.01, Fig 4A & C). Lesser but still significant effects were observed with *A. marmelos* and *M. indica* extracts (P<0.05, Fig 4B & D). As shown in Figure 5, active GLP-1 (7-36) concentrations in plasma were significantly increased by 32-45% (P<0.05-0.01) at 60 min after administration of each plant extract. An 81-89% increase was observed with sitagliptin and vidagliptin (P<0.001; Fig 5).

**Discussion**

DPP-IV inhibitors are used in the treatment of T2DM based on their ability to extend postprandial levels of circulating plasma GLP-1 and GIP, thereby improving insulin secretion and helping to maintain good blood glucose control. Since their introduction to the clinic [15], this drug class has proven to be effective and highly popular. Weight reduction and glycaemic control are inferior to the related family of GLP-1 mimetics but DPP-IV inhibitors have the advantage of being orally active, thereby avoiding the need for daily injections and increasing patient compliance. In certain developing countries, the limited availability and cost of these and other modern medicines, such as metformin, sulphonylureas, thiazolidinediones, SGLT2 inhibitors and insulin formulations, have resulted in increasing attention being paid to traditional plant medicines with reputed anti-diabetic activity for treatment of T2DM [3, 8, 10, 16-18].

A considerable number of plants have been used traditionally for the treatment for diabetes and its complications but only a limited number have been subjected to scientific scrutiny and fewer still scrutinized for the mechanisms responsible for their anti-diabetic effects [9, 16, 19]. In the present study, we have examined 22 medicinal plants with proven glucose-lowering ability (Table 2) to assess whether part of their mode of action relates to an ability to inhibit DPP-IV.
Hot water extracts of every plant studied exhibited some degree of DPP-IV inhibition in vitro ranging from 9-96%, but the most substantial effects were observed (in descending order) with A. latifolia (bark), A. marmelos (leaves), M. indica (seeds), T. foenum-graecum (seeds), C. colchicinensis (bark), and A indica (seeds). These plants inhibited DPP-IV by 27-96% with IC25 values of 446-4720 µg/ml. Although considerably less effective than pure preparations of sitagliptin and vildagliptin, these observations suggest that a component of the anti-diabetic actions of these plants may be due to inhibition of DPP-IV. This adds to the results of previous studies which have highlighted gastrointestinal effects of anti-diabetic plants and their ability to enhance insulin secretion and/or action [20-23].

Based on these in vitro results, the four most active plants were selected from the 22 initially screened for in vivo evaluation of effects on oral glucose tolerance, insulin secretion and plasma DPP-IV activity using high fat fed rats. This included A. latifolia, A. marmelos, T. foenum-graecum and M. indica. The first two were particularly effective in inhibiting DPP-IV in vitro with up to 44-96% inhibition and IC50 values of 754-790 µg/ml. This compares with almost total inhibition of DPP-IV by sitagliptin and vildagliptin with IC25 and IC50 values of 2.04x10^-3 – 2.43x10^-3 µg/ml and 2.04x10^-2 – 1.70x10^-2 µg/ml, respectively. As expected, administration of either sitagliptin or vildagliptin orally to high fat fed obese-diabetic rats, together with glucose, induced a remarkable improvement in glucose tolerance and glucose-stimulated insulin secretion. This was associated with a 70-72% decrease in plasma DPP-IV activity known to result in strong augmentation of the stimulatory insulin-releasing effects of the incretin hormones GLP-1 and GIP. Consistent with this, circulating concentrations of active GLP-1 (7-36) were increased by 81-89% at 60 min following administration of these DPP-IV inhibitors. Extracts of A. latifolia, A. marmelos, T. foenum-graecum and M. indica also significantly inhibited DPP-IV enzyme activity and increased active GLP-1 (7-36) but by lesser extents of 12-18% and 32-45% respectively. Interestingly, the glucose lowering actions of the four plant extracts were very similar to the DPP-IV inhibitors despite a smaller plasma insulin response. This indicates that other factors such as a delayed glucose absorption make a major contribution to the acute anti-hyperglycaemic activity of these plants in vivo [9, 23]. Further long-term studies are required to determine how their effects compare with other plant-derived substances that exhibit anti-diabetic properties, such as metformin.

These results suggest that many plants used traditionally to treat diabetes have orally available constituents that inhibit DPP-IV, thereby contributing to their spectrum of actions which in the
case of some might be significant [24, 25]. Indeed, additional *in vitro* and *in vivo* studies on individual plant extracts have reported that other plant species inhibit DPP-IV activity [26, 27]. These observations suggest that these plant extracts exert part of their insulinotropic effects via inhibition of DPP-IV with resultant preservation of active forms of GLP-1 (7-36) and GIP (1-42) released from intestinal enteroendocrine cells by feeding. Overall, our results together with these previous studies indicate that net effects on insulin secreting cells reflect combination of direct actions of glucose and other nutrients compounded by potentiating effects of incretin hormones that are favored by the concurrent inhibition of DPP-IV. Further extensive studies will be required to measure active and total forms of GLP-1 and GIP following administration of the plants studied but the few reports in the literature suggest that some plants with reputed anti-diabetic activity increase circulating GLP-1 [28]. The extent to which this may reflect enhanced secretion as opposed to decreased degradation by DPP-IV is unknown.

Although the present study describes DPP-IV lowering activity of many medicinal plants with anti-diabetic actions, few precise details are known about the nature of the phytochemicals that are absorbed and subsequently inhibit DPP-IV. All 22 plants examined inhibited DPP-IV activity *in vitro* to some extent suggesting that such chemicals are commonly encountered in the plant kingdom. Studies to date suggest that these include alkaloids, glycosides, terpenoids, phenolic, flavonoids as well as protein hydrolysates and peptides [27, 2]. These may act at the molecular level by binding to the active site of the enzyme, thereby inhibiting interactions with natural substrates. Small peptides may also serve as competitive substrates as is the case with diprotin-A. Indeed, we demonstrated DPP-IV inhibitory action for caffeine, catechin, epicatechin, gallic acid, isoquercitrin, quercetin and rutin which may realistically contribute to the observed effects as they have been reported to be present in plants at levels of up to 2-30% by weight, not accounting for losses during our extraction procedure [29]. Rutin is the most abundant of these phytochemicals and was also shown to be the most effective inhibitor of DPP-IV, with an action broadly similar to that observed with the established anti-diabetic drug, nateglinide. The active plant constituents might, like this meglitinide, serve as effective prandial blood glucose regulators stemming partly from their ability to preserve active forms of GLP-1 and GIP released by feeding [6].

In the light of the DPP-IV inhibitory actions of the 22 plants tested plus the selected phytochemicals, it is notable that flavonoids and their metabolites have been reported to exhibit anti-diabetic activities. An inverse relationship has been suggested also between flavonoid intake and T2DM risk [30]. Phytochemicals other than those tested, such as anthocyanin,
aspalathin, chrysin, eriodictyol, hispidulin, kaempferol, leptoside, mangiferin, naringenin, naringin, procyanidin, rhamnoside, terpenoids, vitexin and *Lens culinaris* extracts have been shown also to exhibit DPP-IV inhibitory activity [27, 31]. In addition, a number of other plants (such as *A. catechu*, *M. indica* and *A. marmelos*) have been reported to contain potential phenolic compounds that exert antioxidant effects and DPP-IV inhibitory activity [32].

**Conclusions**

In conclusion, these findings indicate that a substantial proportion of plants used traditionally for the treatment of diabetes exhibit DPP-IV inhibitory activity which may contribute to their multiple glucose lowering actions. Such medicinal plants could provide an accessible therapy for diabetes particularly in populations without easy access to the recognized drugs. More work is required for isolation, identification and characterization of agents responsible for inhibition of DPP-IV but there is a good chance that multiple phytochemicals are involved in mediating such effects [33].

**Acknowledgments**

The authors would like to thank Ulster University Strategic Research Funding and the award of a Vice Chancellor’s Research Studentship to PA.

**Conflict of interest**

The authors declare that there is no duality of interest associated with this manuscript.

**Funding**

There is no outsource of funding for this project.

**Author contributions**

P.R.F. and Y.H.A.A.W. were responsible for the conception and design of research and contributed equally to the supervision of the study; M.P.H.F. was responsible for reading and revising manuscript; P.A. performed the experiments, analysed the data, interpreted the results, prepared the figures and drafted the manuscript with P.R.F.; P.R.F. and P.A. edited the revised manuscript; All authors approved the final version of the manuscript.

**Institutional Animal Care**
All animal experiments were conducted in accordance to UK Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63EU. All necessary steps were taken to prevent any potential animal suffering. The animal studies were approved by local Ulster Animal Welfare and Ethical Review Body (AWERB) committee (01/10/2016), as well as being covered under a UK Home Office Animal project/personal licence numbers PIL450, PIL1822 and PPL 2804, approved on 06/05/2016. All animals were maintained under specific pathogen-free conditions, and all experiments were conducted in the Biomedical and Behavioral Research Unit (BBRU) at Ulster University, Coleraine, UK. Blood samples were collected from the cut tip of the tail of conscious animals and they are not scarified.

**Data Availability Statement**

All data are included in the manuscripts and the identified participant information (PA) is included in the Author contributions section for the data collections.
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Table 1: Traditional use of selected medicinal plants treatment for diabetes

| Plants                                      | Traditional Uses                                                                 | References |
|---------------------------------------------|---------------------------------------------------------------------------------|------------|
| *Acacia catechu* (L.f.) Willd.              | Diabetes, Obesity, asthma, bronchitis, anaemia, diarrhoea                       | [34, 35]   |
| *Bunium persicum* (Boiss.) B.Fedtsch.       | Obesity, gastrointestinal and urinary disorders, diarrhoea, asthma               | [36]       |
| *Eugenia jambolana* Lam.                    | Diabetes, cancer, enteric disorders, renal problems                             | [37, 38]   |
| *Linum usitatissimum* L.                    | Gastrointestinal disorders, asthma, bronchitis, pulmonary tuberculosis, gingival disorders, atherosclerosis | [39, 40]   |
| *Santalum album* L.                         | Inflammation, anti-septic, fever, carminative, diuretic, hypotensive, memory booster | [41]       |
| *Selaginella bryopteris* (L.)               | Jaundice, chronic tracheitis, lung cancer, venereal diseases, colitis, diuretic problems | [42, 43]   |
| *Sesamum indicum* (White)                   | Dietary fibre, joint inflammation, toothache, scrapes, cuts                    | [44, 45]   |
| *Tamarindus indica*                         | Inflammation, rheumatism, diarrhoea, dysentery, respiration conditions, malaria, gonorrhoea | [46]       |
| *Terminalia arjuna* (Roxb. ex DC.)          | Diabetes, cirrhosis, anaemia, cardiovascular disorders, viral diseases         | [47, 48]   |
| *Azadirachta indica* (Roxb. ex DC.)         | Diabetes, urinary and gastrointestinal problems, skin diseases, blood pressure and cholesterol | [49]       |
| *Anogeissus latifolia* (Roxb. ex DC.)       | Diabetes, haemorrhages, diarrhoea, dysentery, skin diseases, leprosy, hepatopathy | [50]       |
| *Albizia lebbeck* (L.) Benth.               | Respiratory disease, skin diseases, inflammation, diarrhoea, edema             | [51, 52]   |
| *Cudrania cochinchinensis* (Lour.)          | Gonorrhoea, rheumatism, jaundice, hepatitis, boils, scabies, bruising.         | [53]       |
| *Cassia fistula* L.                         | Diabetes, jaundice, piles, rheumatism ulcers, skin eruptions, eczema, heart diseases, asthma, liver disorder | [54, 55]   |
| *Dalbergia sissoo* DC.                      | Bronchitis, inflammations, gonorrhoea, digestive disorders, colorectal cancer, bacterial infections | [56]       |
| *Swertia chirayita* (Roxb.)                | Diabetes, hypertension, liver disorders, malaria, hepatitis, inflammation, digestive diseases, epilepsy | [57, 58]   |
| *Withania coagulans* (Stocks)              | Chronic degenerative diseases, diabetes                                         | [59]       |
| *Glycyrrhiza glabra* L.                     | Dyspepsia, belching, gas stomach ache, intestinal & liver colics, ulcerated wounds & gastritis | [60]       |
| *Momordica charantia* L.                    | Diabetes, hypertension, obesity, cancer, hyperlipidaemia, digestive disorders, microbial infections | [61, 62]   |
| *Mangifera indica* L.                      | Diabetes, hypertension, anaemia, haemorrhage, asthma, gastric disorders         | [63, 64]   |
| *Aegle marmelos* (L.) Corrêa               | Diabetes, inflammations, asthma, ophthalmia, diarrhoea, dysentery, cardiac ailments | [65]       |
| *Trigonella foenum-graecum* L.              | Diabetes, hypercholesterolemia, edema lung congestion sinus, indigestion, baldness | [66, 67]   |
### Table: 2 Antidiabetic actions of selected traditional plants treatment for diabetes

| Plants                          | ↑Hyperglycemia | ↑Insulin secretion | ↑Glucose uptake and metabolism | References |
|---------------------------------|----------------|--------------------|---------------------------------|------------|
| Acacia catechu                  | ↓              | ↑                  | ND                              | [68]       |
| Bunium persicum                 | ↓              | ND                 | ↑                               | [69]       |
| Eugenia jambolana               | ↓              | ↑                  | ↑                               | [70]       |
| Linum usitatissimum             | ↓              | ↑                  | ↑                               | [71, 72]  |
| Santalum album                  | ↓              | ↑                  | ↑                               | [73]       |
| Selaginella bryopteris          | ↓              | ↑                  | ↑                               | [74]       |
| Sesamum indicum (White)         | ↓              | ND                 | ↑                               | [75]       |
| Tamarindus indica               | ↓              | ↑                  | ND                              | [76]       |
| Terminalia arjuna               | ↓              | ↑                  | ↑                               | [77, 78]  |
| Azadirachta indica              | ↓              | ↑                  | ↑                               | [79, 80]  |
| Anogeissus latifolia            | ↓              | ↑                  | ND                              | [81]       |
| Albizia lebbeck                 | ↓              | ↑                  | ↑                               | [82, 83]  |
| C. cochinchinensis              | ↓              | ↑                  | ↑                               | [84]       |
| Cassia fistula                  | ↓              | ↑                  | ↑                               | [85, 86]  |
| Dalbergia sissso                | ↓              | ND                 | ND                              | [87, 88]  |
| Swertia chirrayita              | ↓              | ↑                  | ↑                               | [89]       |
| Withania coagulans              | ↓              | ND                 | ND                              | [90, 91]  |
| Licorice glyceriza              | ↓              | ↓                  | ND                              | [16, 92, 93]|
| Momordica chirantia             | ↓              | ↑                  | ND                              | [94, 95]  |
| Mangifera indica                | ↓              | ↑                  | ↑                               | [96, 97]  |
| Aegle marmelos                  | ↓              | ↑                  | ↑                               | [21]       |
| T. foenum graecum               | ↓              | ↑                  | ND                              | [22, 98]  |

Effects of plant: ↑, increase; ↓, decrease (beneficial effect on hyperglycemia); ND, effect not determined

1Effects on hyperglycemia were demonstrated in mice or rats given streptozotocin or alloxan or high fat diet to induce diabetes. 2Effects on insulin secretion were demonstrated in vitro using pancreatic β-cells or in vivo using blood plasma of rats or mice. Beneficial actions in vitro were dose-dependent and did not affect cellular viability at low concentrations. 3Effects on glucose uptake and metabolism were demonstrated in vitro using isolated mouse abdominal muscle.
Table 3: List of confirmation of identity of 22 traditional medicinal plants with their herbarium numbers

| Plants                        | Collected parts of plants | Voucher specimen numbers |
|-------------------------------|---------------------------|--------------------------|
| Acacia catechu (L.f.) Willd.  | Bark                      | 1721                     |
| Bunium persicum (Boiss.) B.Fedtsch. | Seed                   | 1844                     |
| Eugenia jambolana Lam.        | Seed                      | 1681                     |
| Linum usitatissimum L.        | Seed                      | 1531                     |
| Santalum album L.             | Bark                      | 1168                     |
| Selaginella bryopteris (L.)   | Leaf                      | 1135                     |
| Sesamum indicum (White)       | Seed                      | 1219                     |
| Tamarindus indica             | Seed                      | 866                      |
| Terminalia arjuna (Roxb. ex DC.) | Bark                   | 535                      |
| Azadirachta indica            | Seed                      | 1610                     |
| Anogeissus latifolia (Roxb. ex DC.) | Bark                  | 1734                     |
| Albizia lebbeck (L.) Benth.   | Bark                      | 1761                     |
| Cudrania cochinchinensis (Lour.) | Bark                   | 1241                     |
| Cassia fistula L.             | Stalk                     | 1321                     |
| Dalbergia sissoo DC.          | Bark                      | 335                      |
| Swertia chirayita (Roxb.)     | Bark                      | 581                      |
| Withania coagulans (Stocks)   | Fruit                     | 1196                     |
| Glycyrrhiza glabra L.         | Root                      | 2212                     |
| Momordica charantia L.        | Seed                      | 2378                     |
| Mangifera indica L.           | Seed                      | 2391                     |
| Aegle marmelos (L.) Corrêa    | Leaf                      | 1733                     |
| Trigonella foenum-graecum L.  | Seed                      | 681                      |
Table 4: DPP-IV inhibitory activity of established inhibitors and hot water extract of various traditional plants.

| Plants/ Inhibitors | Lower effective concentration (µg/ml) | Maximum Inhibitory effect (%) | IC<sub>25</sub> (µg/ml) | Estimated IC<sub>50</sub> (µg/ml) |
|--------------------|-------------------------------------|-----------------------------|---------------------|---------------------|
| Sitagliptin (Inhibitor) | 4.07x10⁻⁴ | 99 ± 2 *** | 2.04x10⁻³ | 2.04x10⁻² |
| Vidalagiptin (Inhibitor) | 6.07x10⁻⁴ | 99 ± 3 *** | 2.43x10⁻³ | 1.70x10⁻² |
| Diprotin A (Inhibitor) | 1.02x10⁻³ | 95 ± 3 *** | 1.02x10⁻³ | 2.39x10⁻³ |
| Anogeissus latifolia | 200 | 96 ± 1 *** | 590 | 754 |
| Aegle marmelos (L.) Corrêa | 200 | 44 ± 7 ** | 446 | 790 |
| Mangifera indica L. | 200 | 38 ± 4 *** | 2000 | ----- |
| Cudrania cochinchinensis (Lour.) | 1000 | 31 ± 1 *** | 4,050 | ----- |
| Trigonella foenum-graecum L. | 200 | 28 ± 2 *** | 4,700 | ----- |
| Azadirachta indica | 1000 | 27 ± 2 *** | 4,720 | ----- |
| Tamarindus indica | 200 | 23 ± 2 *** | ----- | ----- |
| Terminalia arjuna (Roxb. ex DC.) | 200 | 22 ± 1 *** | ----- | ----- |
| Acacia catechu (L.f.) Willd. | 5000 | 22 ± 7 * | ----- | ----- |
| Withania coagulans (Stocks) | 1000 | 17 ± 3 ** | ----- | ----- |
| Sesamum indicum (White) | 40 | 19 ± 5 * | ----- | ----- |
| Albizia lebbeck (L.) Benth. | 5000 | 19 ± 4 ** | ----- | ----- |
| Cassia fistula L. | 5000 | 19 ± 6 * | ----- | ----- |
| Santalum album L. | 40 | 17 ± 5 * | ----- | ----- |
| Eugenia jambolana Lam. | 40 | 14 ± 5 * | ----- | ----- |
| Selaginella bryopteris (L.) | 1000 | 13 ± 4 * | ----- | ----- |
| Momordica charantia L. | 1000 | 13 ± 2 ** | ----- | ----- |
| Swertia chirayita (Roxb.) | 5000 | 12 ± 1 ** | ----- | ----- |
| Glycyrrhiza glabra L. | 200 | 12 ± 2 * | ----- | ----- |
| Bunium persicum (Boiss.) | 200 | 11 ± 3 * | ----- | ----- |
| Dalbergia sissoo DC. | 5000 | 10 ± 2 * | ----- | ----- |
| Linum usitatissimum L. | 40 | 9 ± 1 * | ----- | ----- |

DPP-IV inhibitory activity of hot water extracts of various plants when incubated with Gly-Pro-AMC (200 µM) plus DPP4 (8 mU/mL⁻¹) for 30 minutes at 37°C. Sitagliptin, Vidalagiptin and Diprotin A were used as established inhibitors. Values are Mean±SEM with n= 4, *P<0.05, **P<0.01 and ***P<0.001, compared to control group Gly-Pro-AMC (200 µM) + DPP4 (8 mU/mL⁻¹) alone. The calculated IC<sub>50</sub> (µg/ml) was an estimate.
Figure legends:

Figure 1: DPP-IV inhibitory activity of (A) Sitagliptin, (B) Vidaliglptin, (C) Diprotin-A and hot water extract of four most potent plants (D) A. latifolia, (E) A. marmelos, (F) T. foenum graecum and (G) M. indica expressed as the bar chart (A, B, C, D, E, F & G). Values are Mean±SEM with n = 4, *P< 0.05, **P< 0.01 and ***P< 0.001, compared to control. Sitagliptin: 1-10000nM; Vidaliglptin: 1.6-5000nM and Diprotin A: 2.5-40nM.

Figure 2: DPP-IV inhibitory activity of phytochemicals: (A) caffeine, (B) catechin, (C) epicatechin, (D) gallic acid, (E) isoquercitrin, (F) quercetin, (G) rutin and (H) the antidiabetic small molecule drug, nateglinide. Values are Mean±SEM with n = 4, *P< 0.05, **P< 0.01 and ***P< 0.001, compared to control.

Figure 3: Acute effects of hot water extract of four most potent plants (A & C) A. latifolia, A. marmelos, T. foenum graecum, M. indica and (B & D) DPP-IV inhibitors: sitagliptin and vidagliptin on (A & B) glucose tolerance and (C & D) plasma insulin in high fat fed rats expressed as line graphs and area under the curve. Blood glucose and plasma insulin were measured prior to and after oral administration of glucose alone (18mmol/kg body weight, control) or in combination with plant extract (250mg/5ml/kg body weight,), Sitagliptin or Vidaliglptin (both at 10µmol/5ml/kg body weight). Values are Mean±SEM with n = 6, *P<0.05, **P< 0.01 and ***P< 0.001, compared to lean rats and ∆P< 0.05, ∆∆P< 0.01 and ∆∆∆P< 0.001 compared to high fat fed controls.

Figure 4: Acute effects of hot water extract of four most potent plants (A) A. latifolia, (B) A. marmelos, (C) T. foenum graecum and (D) M. indica on DPP-IV activity in high fat fed rats expressed as line graphs and area under the curve. Plasma DPP-IV activity was measured prior to and after oral administration of glucose alone (18mmol/kg body weight, control) or in combination with plant extract (250mg/5ml/kg body weight), Sitagliptin or Vidaliglptin (each at 10µmol/5ml/kg body weight). DPP-IV activity was determined by Gly-Pro-AMC (200 µM) cleavage. Values are Mean±SEM with n = 6, *P< 0.05, **P< 0.01 and ***P< 0.001, compared to lean rats and ∆P< 0.05, ∆∆P< 0.01 and ∆∆∆P< 0.001 compared to high fat fed controls.
Figure 5: Acute effects of hot water extract of four most potent plants: *A. latifolia*, *A. marmelos*, *T. foenum graecum* and *M. indica* on plasma active GLP-1 (7-36) in high fat fed rats. Plasma active GLP-1 (7-36) concentrations was measured at 60 min after oral administration of glucose alone (18mmol/kg body weight, control) or in combination with plant extract (250mg/5ml/kg body weight), Sitagliptin or Vidagliptin (both at 10µmol/5ml/kg, body weight). Values are Mean±SEM with n = 6, **P< 0.01 compared to lean rats and ΔP< 0.05, ΔΔP< 0.01 and ΔΔΔP< 0.001 compared to high fat fed controls.
