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Effects of 15 Local Medicinal Plant Species on Protein Tyrosine Phosphatase 1B and Dipeptidyl Peptidase 1V Activity as Herbal Antidiabetic Type 2 Remedies

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Abstract

This study aimed at investigating plant species that are used locally in Zimbabwe to manage diabetes type 2 and their respective modes of action using *in vitro* studies. The plants for this study were identified by ethnobotanical studies and their respective antidiabetic potentials were evaluated using protein tyrosine phosphatase 1B (PTP 1B) and dipeptidyl peptidase IV (DPP IV) inhibition assays. Out of the 15 plant species used to manage diabetes in traditional practices, 9 inhibited PTP 1B with half maximal inhibitory concentrations (IC_{50}) ranging from 0.03 ± 0.00 to 61.31 ± 0.70 µg/mL. The mechanism of inhibition deduced from Lineweaver-Burk plot was non-competitive inhibition for *Bridelia micrantha*, *Brachylaena discolor*, *Artemisia afra*, *Sutherlandia frutescens* and *Euclea undulate*, while *Ziziphus mucronta* extract inhibited PTP 1B enzyme in a competitive pattern. The inhibition mechanism was demonstrated by decreasing or constant K_m and V_{max} values. K_m values ranged from 1.00 to 3.33 mM. Also 9 plant species out of the 15 studied showed DPP IV inhibition activity. Four plant species, *Sutherlandia frutescens, Momordica balsamina, Bulbine latifola,* and *Spirostachys africanus* exhibited inhibitory activity that was comparable to the approved DPP IV tight binding drug, sitagliptin with percentage inhibition ≥ 73.55 ± 0.32%. The present results provide a scientific base for the current uses of the plant species in local management of diabetes type 2. Thus these plants are not only useful in polyherbal formulations for management of diabetes type 2, but also serve as sources of lead compounds for further studies toward developing drugs for treating diabetes type 2.

Keywords: Diabetes type 2; traditional medicinal plants; enzyme inhibition; polyherbal remedies.

Introduction

Diabetes type 2 is an adult onset metabolic disorder that arises following deficiency of insulin or cells insensitivity to insulin (Shah et al. 2016). The worldwide trends of increasing cases of diabetes type 2 have created threats to human health globally (WHO 2018). The disease causes several secondary complications, particularly, atherosclerosis, renal dysfunction and failure and diabetic retinopathy (Wang et al. 2015). Currently, diabetes type 2 is treated by eight types of oral hypoglycaemic drug types; α-glucosidase inhibitors, glucagon-like peptide-1 receptor agonist, amylin analogs, insulin secretagogues, thiazolidinediones, biguanides, dipeptidyl peptidase-4 inhibitors and sodium-glucose cotransporter-2 inhibitors (Bösenberg and Van Zyl, 2008, Riyanti et al. 2016). Nevertheless, the complications associated

with their administration to patients, markedly; gastrointestinal complaints, weight gain, peripheral oedema, headache and hypertension, these drugs are effective hypoglycaemic agents and have been widely used to treat diabetes type 2. Those limitations are because mechanistically they are not targeting insulin mediated cell signaling mechanism (Moller 2001). Thus, drugs that target the negative regulation of insulin signaling are urgently needed. Recently, polyherbal formulations have gained attention because they are affordable and have demonstrated less side effects.

Protein tyrosine phosphatase constitutes enzymes that catalyze tyrosine dephosphorylation-dependent cellular functions, including the PTP 1B which catalyzes the negative regulation of insulin signaling mechanism (Quang et al. 2015). Several studies have shown that the cytosolic non-receptor protein tyrosine phosphatase 1B (PTP 1B) obstruct insulin signal transduction (Zhang and Lee 2003, Koren and Fantus 2007). Thus PTP 1B has proved to be a crucial drug target for management of diabetes type 2. Many inhibitors of PTP 1B such as vanadium compounds, sulphonic acids, phosphonic acids, carboxylic acids and imides have been synthesized. These are non-hydrolysable p-tyrosine surrogates that bind on either the catalytic site or the allosteric pocket or both (Combs 2010, Jiang et al. 2012). However, these compounds lack in vivo potency due to both poor oral bioavailability and permeability to membranes. The compounds have also failed to discriminate PTP 1B among the several protein tyrosine phosphatases (Cragg et al. 2009). Thus, the searches for novel compounds that selectively inhibit protein tyrosine phosphatase 1B are still on going.

DPP IV is a serine protease which degrades proline or alanine containing peptides (Havale and Pal 2009). The enzyme degrades incretins, specifically glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide hormones which are released into the intestine in response to nutrient intake and stimulate insulin secretion (Brubaker and Drucker 2004, Calero et al. 2014). Therefore, incretins peptides which maintain insulin homeostasis are short lived due to their rapid degradations by DPP IV which result into diabetes type 2. Thus, the inhibition of DPP IV enzymes increases the shelf-lives of glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide which maintains the secretion of insulin with overall effects on deterrence of increasing level of glucose in the blood.

Plants are well known for varieties of compounds with diverse bioactivities, thus offer more opportunities for searching novel drugs and lead compounds (Jiang et al. 2012). Based on this, researchers are now concerned with screening plants for potential PTP 1B and DPP IV inhibitors that can reduce insulin resistance and normalize plasma glucose without inducing hypoglycaemia. In line with this thrust, this study was designed to investigate local plants purported to have antidiabetic activities in traditional practices and screen for PTP 1B and dipeptidyl peptidase IV (DPP IV) inhibition with an overall aim of providing impetus in the use of the plants to formulate polyherbal drugs to fight diabetes type 2.

Materials and Methods

Materials

All solvents and buffers were of analytical grade. Except for buffers that were sourced from local chemical suppliers, the rest of the chemicals and reagents (solvents, enzymes and standards) were purchased from Sigma Aldrich, Germany. A UV-Vis spectrophotometer, GENESYS 10S UV-Vis v4.003 2L9Q129001 ThermoFisher Scientific, USA) was used to monitor the progress of reactions.

Ethnobotanical surveys and collection of plant materials

Plant materials were collected from Mashonaland Central and East Forests with

the help of traditional practitioners certified by Zimbabwe National Traditional Healers Association (ZINATHA) and local elderly people. Ten traditional practitioners from each village were orally interviewed. The study area consisted of a total of 8 villages. Plants yielding a quote frequency > 60% of the respondents were collected. A further literature survey was conducted by searching scientific studies and websites reporting similar uses as those reported by the traditional healers using various search engines such as Google scholar, Google, Pubmed, and SciFinder. The key searching words included medicinal uses, diabetes uses and ethnobotanical use. A total of 15 plant species were identified as anti-diabetes herbs by the traditional healers and were collected. The full plant list and medicinal uses are shown in Table 1. Plant species authentication was done by the help of the Harare National Herbarium and information from literature. Voucher specimens were deposited in the Bindura University of Science Education natural product section for future references.

Ethical approval

All procedures performed in studies involving humans were in accordance with the ethical standards of the Bindura University Ethics Committee and participation was purely voluntary.

Sample extraction

Five fractions of organic extracts from dried powdered materials of each plant were obtained after sequential extraction by organic solvents with increasing polarity (100% n-hexane, 100% ethyl-acetate, 75: 25 % ethyl-acetate/ethanol mixture, 50: 50% ethyl-acetate/ethanol mixture and 100% ethanol). In each extraction, the sample material (10 g) was shaken with solvent (50 mL) for 2 hours before filtered and finally solvent removed to obtain the extracts.

PTP1B inhibition assay

PTP 1B inhibition assay was carried out according to a slightly modified literature reported method by Shah et al. (2016). The analyses were carried out in the reaction mixtures composed of extracts in the concentration range of 0-100 µg/mL, p-nitrophenol phosphate (2 mM) in bis-tris buffer (50 mM, pH 7.2) and PTP 1B (10 mM). The mixtures were incubated at 37 °C for 30 minutes followed by termination by adding 20 µL of 10 M NaOH. The amount of p-nitrophenol produced was determined by measuring the increase in absorbance at 405 nm using a UV-Vis spectrophotometer, p-nitrophenol which were formed non-enzymatically were also determined spectrophotometrically at 405 nm without PTP 1B. Similar experiments were repeated with ursolic acid as a reference standard. The assays were carried out in triplicate, and results were used to compute IC_{50} values.

Enzyme kinetic studies

Crude extracts which exhibited inhibitions significantly greater than ursolic acid were selected for kinetic studies in order to determine the type of inhibition. The experimental conditions were as described before in PTP 1B inhibition assy. The assays for the kinetic studies were carried out in different concentrations of extracts, 0-6 µg/mL and p-nitrophenol phosphate, 0-1.5 mM. The rate of increase of absorbance at 405 using a molar extinction coefficient of 18000 $M^{-1}cm^{-1}$ for 0.5 M EDTA (Chen et al. 2010) was used to compute initial rates. Lineweaver-Burk plot analyses were then used to estimate the magnitude of (K_m) and maximum velocity (V_{max}), Table 3.

Dipeptidyl IV (DPPIV) inhibition assay

DPPIV inhibition effectiveness by plants extracts were screened by using a method reported by Bharti et al. (2012) with minor modifications. Using a 96 well plate set up, the chromogenic substrate Gly-pro-p-nitroanilidine (GPPN) is broken down by the

serine protease DPPIV enzyme to paranitroanilidine (pNA), a yellow coloured compound that was monitored at 405 nm. Crude extracts or sitagliptin (standard inhibitor of DPPIV) were diluted to various concentrations 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 µg/mL using tris HCl buffer (50 mM, pH 7.5) to a final volume of 35 µL. Absorbance of the solutions were recorded at 405 nm followed by adding of DPPIV (0.05 U/mL, 15 µL). One unit of enzyme was taken as the amount of an enzyme required to catalyze the production of 1 µM of pNA per minute. The mixture was pre-incubated for 10 minutes at 37 °C to allow maximum contact of enzyme with inhibitor followed by addition of GPPN (0.2 mM, 50 µL) in tris HCl (50 mM, pH 7.5). The resultant mixture was incubated at 37 °C for 30 minutes. The reaction was terminated by adding glacial acetic acid (25%, 25 µL). The absorbance results were compared with results of a control experiment (without inhibitor). The percentage inhibition was computed using the equation;

$$\%I = \frac{A_C - A_s}{A_C}$$

where %I = percentage inhibition, A_C = absorbance of control experiment (without inhibitor) and A_S = absorbance of experiment with standard inhibitor or with extracts.

Statistical analysis

The results are expressed as mean plus or minus standard deviation ($\bar{X}$ ± SD) of three replicate analyses. Differences between the group means were analyzed using the IBM SPSS version 20 software by applying one way ANOVA with the Turkey-Karner post hoc test to identify significance of the differences among groups. A p value < 0.05 was considered to be statistically significant.

Results

PTP1B inhibition assay

A total of 15 plants (Table 1) were reported to be antidiabetic in traditional practices, and among these, only *Spirostachys africanus* and *Keostis nana* showed no PTP 1B inhibitory activity as shown in Table 2. Extracts from *Bridelia micrantha*, *Euclea undulate*, *Euclea crispa*, *Ziziphus mucronta*, *Brachylaena discolor*, *Artemisia afra*, *Sutherlandia frutescens*, and *Bulbine latifola* showed significantly greater ($p < 0.05$) PTP 1B inhibitory activities, with IC_{50} values < 0.2 µg/mL than the standard inhibitor (ursolic acid) that had an IC_{50} value of 3.20 ± 0.20 µg/mL. *Bridelia micrantha*, *Euclea undulate*, *Euclea crispa*, *Ziziphus mucronta*, *Brachylaena discolor*, *Artemisia afra* and *Sutherlandia frutescens* showed the greatest PTP 1B inhibitory activities with IC_{50} values ≤ 0.05 µg/mL. Samples extracted using 100% ethyl acetate and ethyl acetate/ethanol mixture (75:25 v/v) showed the greatest PTP 1B inhibitory activities ($p < 0.05$). With 1:1 ethyl acetate/ethanol extracts, only those of *Artemisia afra* and *Sutherlandia frutescens* showed greater PTP 1B inhibitory activity with IC_{50} values of 0.03 ± 0.00 and 0.08 ± 0.00 µg/mL, respectively.

Table 1: Summary of the results obtained from ethnobotanical survey and literature search

Plant name	Uses with quote frequency > 60% of interviewee	Number of scientific papers	Number of websites
Bridelia micrantha	Fruit tree, against stomach ache, antidiabetic	7	2
Euclea undulata	Antidiabetic,	3	1
Euclea crispa	Anti-HIV, anti-inflammatory, antidiabetic	5	4
Spirostachys africanus	Against ulcers, antidiabetic, anti-malaria, against cough and headache	4	1
Ziziphus mucronta	Antidiabetic, pain killer, against wound infections	6	3
Terminalia sericea	Wound infections, diabetes,	4	3

Brachylaena discolor	Diabetes, kidney problems, chest pains	7	5
Bulbine latifola	Diabetes, rheumatism, burns, rashes, itchiness	8	4
Lannea edulis	Diabetes, diarrhoea	5	5
Pteronia divaricate	Diabetes, hypertension	6	2
Warburgia salutaris	Malaria remedy, diabetes, cough	7	2
Momordica balsamina	Diabetes, analgesic	6	2
Keostis nana	Diabetes	6	2
Artemisia afra	Diabetes	7	4
Sutherlandia frutescens	Diabetes	4	3

Table 2: PTP 1B inhibitory activity by organic extracts of different plant species and the standard inhibitor, ursolic acid. 1 = 100% n-hexane, 2 = 100% ethyl-acetate, 3 = 75: 25 % ethyl-acetate/ethanol mixture, 4 = 50: 50% ethyl-acetate/ethanol mixture and 5 = 100% ethanol). NI = no inhibition

Plant species	Plant material	Extract	PTP 1B inhibitory activity (IC_{50} values, µg/mL)
Bridelia micrantha	Bark	1	33.70 ± 0.00
		2	0.20 ± 0.02
		3	0.05 ± 0.02
		4	1.20 ± 0.60
		5	31.10 ± 0.50
Euclea undulate	Roots	1	9.20 ± 0.06
		2	0.06 ± 0.02
		3	2.25 ± 0.09
		4	3.00 ± 0.00
		5	31.50 ± 0.50
Euclea crispa	Leaves	1	7.33 ± 0.10
		2	0.13 ± 0.07
		3	0.12 ± 0.05
		4	61.31 ± 0.70
		5	33.18 ± 0.80
Spirostachys africanus	Bark	1	NI
		2	NI
		3	NI
		4	NI
		5	NI
Ziziphus mucronta	Leaves	1	9.06 ± 0.11
		2	5.20 ± 0.12
		3	0.05 ± 0.02
		4	1.21 ± 0.20
		5	5.10 ± 0.10
Terminalia sericea	Bark	1	6.06 ± 0.13
		2	6.20 ± 0.02
		3	7.85 ± 0.02
		4	8.31 ± 0.20
		5	5.12 ± 0.30
Brachylaena discolor	Leaves	1	5.02 ± 0.00
		2	0.20 ± 0.02
		3	0.05 ± 0.00
		4	1.01 ± 0.00
		5	6.20 ± 0.10

Table 2 (Ctd)

Bulbine latifola	Leaves	1	8.15 ± 0.01
		2	0.12 ± 0.02
		3	0.10 ± 0.01
		4	1.01 ± 0.00
		5	10.00 ± 0.01
Lannea edulis	Roots	1	10.13 ± 0.11
		2	13.22 ± 0.12
		3	9.15 ± 0.08
		4	11.21 ± 0.21
		5	9.27 ± 0.20
Pteronia divaricate	Bark	1	10.26 ± 0.30
		2	8.55 ± 0.12
		3	9.09 ± 0.08
		4	12.21 ± 0.24
		5	12.20 ± 0.22
Warburgia salutaris	Bark	1	7.62 ± 0.30
		2	13.23 ± 0.12
		3	6.15 ± 0.10
		4	11.21 ± 0.10
		5	9.10 ± 0.09
Momordica balsamina	Fruits	1	9.12 ± 0.10
		2	8.30 ± 0.22
		3	10.32 ± 0.15
		4	5.21 ± 0.10
		5	9.23 ± 0.07
Keostis nana	Roots	1	NI
		2	NI
		3	NI
		4	NI
		5	NI
Artemisia afra	Leaves	1	3.08 ± 0.00
		2	0.03 ± 0.00
		3	0.05 ± 0.00
		4	0.03 ± 0.00
		5	4.50 ± 0.10
Sutherlandia frutescens	Bark	1	5.02 ± 0.02
		2	5.20 ± 0.02
		3	0.05 ± 0.00
		4	0.08 ± 0.00
		5	3.20 ± 0.10
	Leaves	1	NI
		2	10.40 ± 0.02
		3	8.51 ± 0.06
		4	7.11 ± 0.02
		5	9.33 ± 0.11
Ursolic acid	-	5	3.20 ± 0.20

Enzyme kinetic studies

In order to determine the kinetics of PTP 1B inhibitions by the extracts, each extract was assayed in different constant concentrations while the concentrations of pNPP were varied (Figure 1). The kinetic constants (K_m) and maximum velocity (V_{max}) were estimated from the intercept of the vertical and

horizontal axis of the double reciprocal plots, Figure 1 (i) and (iii-vi) which demonstrated the reduction in V_{max} values without changes in $K_{m.}$ (Table 3). These results are translated to non-competitive inhibition patterns. Figure 1 (ii) and Table 3 demonstrate change of K_m while V_{max} remained constant as the concentrations of the extract were increased. This showed that *Ziziphus mucronta* extract inhibited PTP 1B enzyme inhibition in a competitive pattern.

Dipeptidyl IV (DPPIV) inhibition assay

Nine of the plant species reported to be remedies for diabetes in ethnopharmacological study showed dipeptidyl IV (DPPIV) inhibition activities (Table 4). *Sutherlandia frutescens*, *Momordica balsamina*, *Bulbine latifola*, and *Spirostachys africanus* showed inhibitory activities that were comparable to that of the standard inhibitor **(**sitagliptin), ≥ 73.55 ± 0.32%. Sitagliptin is a fast, tight binding and reversible DPP IV inhibitor. Of all the plants evaluated using DPPIV inhibition assays, *Sutherlandia frutescens* showed the greatest inhibitory activity.

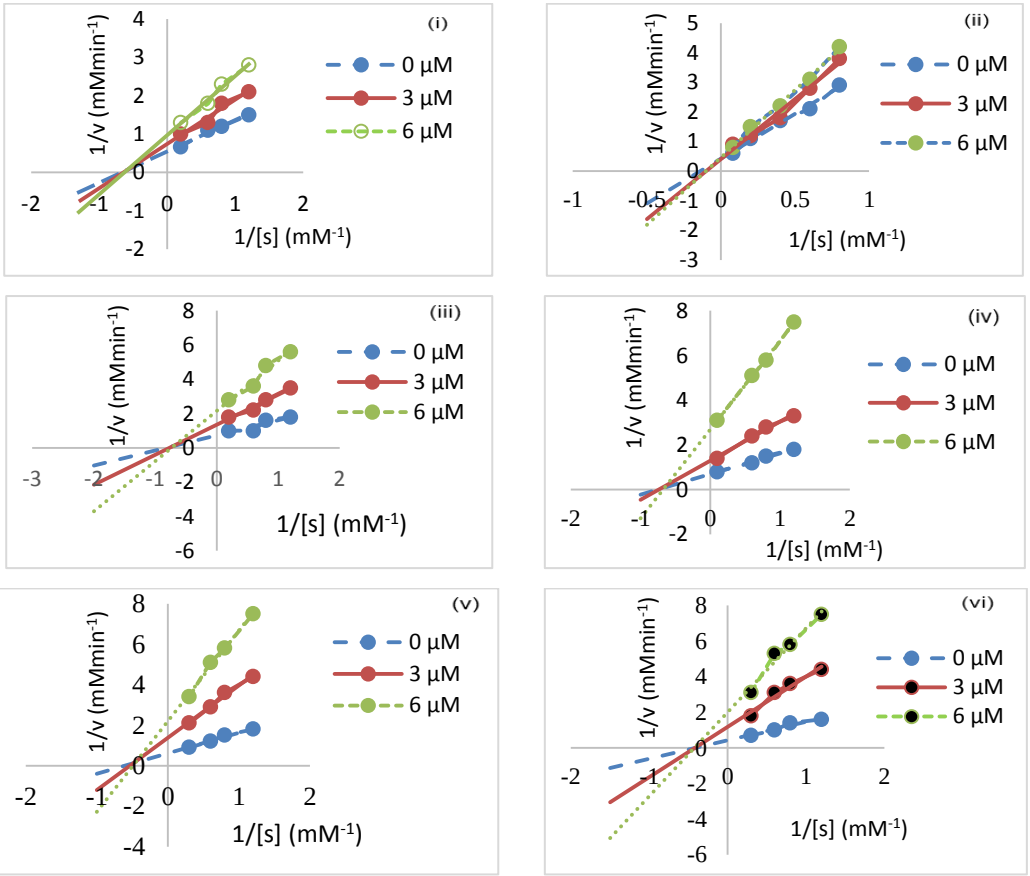

Figure 1: Lineweaver-Burk plots for the inhibition of PTP 1B hydrolysis of p-NPP by ethyl acetate/ethanol (75:25) extracts of (i) *Bridelia micrantha*, (ii) *Ziziphus mucronta*, (iii) *Brachylaena discolor*, (iv) *Artemisia afra*, (v) *Sutherlandia frutescens* and (vi) ethyl acetate (100%) extract of *Euclea undulate*.

Table 3: K_m and V_{max} values for the extracts with significant PTP IB inhibition percentages

	Extract	**K_m (mM)**	**V_{max}(mMmin^{-1})**
Without inhibitor	Ethyl acetate/ethanol (75:25)	2.00	2.00
With *Bridelia micrantha* extract	Ethyl acetate/ethanol (75:25)	2.00	1.00
Without inhibitor	Ethyl acetate/ethanol (75:25)	3.33	3.30
With *Ziziphus mucronta* extract	Ethyl acetate/ethanol (75:25)	2.50	3.30
Without inhibitor	Ethyl acetate/ethanol (75:25)	1.00	2.50
With *Brachylaena discolor* extract	Ethyl acetate/ethanol (75:25)	1.00	0.43
Without inhibitor	Ethyl acetate/ethanol (75:25)	1.11	1.43
With *Artemisia afra* extract	Ethyl acetate/ethanol (75:25)	1.11	0.40
Without inhibitor	Ethyl acetate/ethanol (75:25)	1.67	3.33
With *Sutherlandia frutescens* extract	Ethyl acetate/ethanol (75:25)	1.67	0.50
Without inhibitor	Ethyl acetate (100%)	2	4
With *Euclea undulate* extract	Ethyl acetate (100%)	2	0.50

Table 4: Percentage dipeptidyl IV (DPPIV) inhibition activities of different plant species and the standard inhibitor, sitagliptin. 1 = 100% n-hexane, 2 = 100% ethyl-acetate, 3 = 75: 25 % ethyl-acetate/ethanol mixture, 4 = 50: 50% ethyl-acetate/ethanol mixture and 5 = 100% ethanol). NI = no inhibition

Plant species	Plant material	Extract	DPPIV inhibitory activity (%)
Bridelia micrantha	Bark	1	NI
		2	NI
		3	NI
		4	NI
		5	NI
Euclea undulate	Roots	1	NI
		2	22.12 ± 0.31
		3	33.02 ± 1.22
		4	21.00 ± 0.21
		5	52.27 ± 0.09
Euclea crispa	Leaves	1	55.13 ± 1.24
		2	35.17 ± 0.55
		3	33.11 ± 1.52
		4	50.00 ± 0.21
		5	52.04 ± 2.01
Spirostachys africanus	Bark	1	75.52 ± 2.00
		2	77.44 ± 1.61
		3	79.81 ± 2.02
		4	70.00 ± 0.92
		5	78.14 ± 1.01
Ziziphus mucronta	Leaves	1	65.87 ± 1.33
		2	72.04 ± 0.92
		3	33.18 ± 1.00
		4	59.00 ± 0.10
		5	52.84 ± 2.00
Terminalia sericea	Bark	1	NI
		2	NI
		3	NI
		4	NI
		5	NI

Table 4 (Ctd)

Brachylaena discolor	**Leaves**	**1**	**NI**
		2	NI
		3	NI
		4	NI
		5	NI
Bulbine latifola	Leaves	1	81.10 ± 1.14
		2	76.00 ± 0.55
		3	83.21 ± 2.02
		4	80.00 ± 2.21
		5	52.04 ± 3.10
Lannea edulis	Roots	1	NI
		2	NI
		3	NI
		4	NI
		5	NI
Pteronia divaricate	Bark	1	NI
		2	NI
		3	NI
		4	NI
		5	NI
Warburgia salutaris	Bark	1	5.12 ± 1.02
		2	5.18 ± 2.05
		3	3.18 ± 0.90
		4	5.00 ± 0.51
		5	2.01 ± 0.01
Momordica balsamina	Fruits	1	75.86 ± 1.83
		2	78.32 ± 1.51
		3	73.41 ± 1.22
		4	70.00 ± 2.23
		5	82.04 ± 2.40
Keostis nana	Roots	1	NI
		2	NI
		3	NI
		4	NI
		5	NI
Artemisia afra	Leaves	1	35.10 ± 1.33
		2	22.07 ± 1.63
		3	13.18 ± 1.22
		4	30.10 ± 1.33
		5	22.06 ± 1.01
Sutherlandia frutescens	Bark	1	75.18 ± 1.00
		2	85.13 ± 1.55
		3	73.13 ± 1.22
		4	76.55 ± 2.11
		5	72.44 ± 1.50
	Leaves	1	85.16 ± 1.89
		2	75.12 ± 1.65
		3	73.19 ± 1.92
		4	77.30 ± 2.20
		5	72.14 ± 1.93
Sitagliptin	-	Water	73.55 ± 0.32

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Discussion

Despite the ethnobotanical survey description of folklore medicine anti-diabetic uses of the 15 plants reported in the present studies (Ribeiro et al. 2010, Deutschländer et al. 2009), scientific information to justify their traditional uses has remained limited. PTP 1B has long been used as a validated drug target for the search of anti-diabetic drugs. The in vitro studies showed that a number of the plants showed PTP 1B inhibitory activities that were significantly greater than that by a pentacyclic triterpenoid acid (ursolic acid) that is widely used as standard natural inhibitor (Zhang et al. 2006). The IC_{50} values of the bioactive plants ranged from 0.03 ± 0.00 to 61.31 ± 0.70 µg/mL, while that for ursolic acid is 3.20 ± 0.20 µg/mL.

Crude extracts obtained using ethyl-acetate (100%), ethyl-acetate/ethanol (75: 25 % v/v), ethyl-acetate/ethanol (50: 50% v/v) showed the greatest inhibitory activities than hexane (100%) and ethanol (100%). This shows that the bioactive compounds are moderately polar. These results are interesting since the current thrust in PTP 1B inhibitors search is to find orally available lipophilic compounds that do not pose a challenge in passing through the highly lipophilic cell membranes (Johnson et al. 2002). All the known clinical PTP 1B inhibitors contain functional groups that mimic a phosphate, therefore are negatively charged. Such charged compounds have reduced abilities to permeate cell membranes and are not favourable for intestinal transcellular absorption (Palm et al. 1997). *Bridelia micrantha*, *Ziziphus mucronta*, *Brachylaena discolor*, *Artemisia afra* and *Sutherlandia frutescens* crude extracts IC_{50} values less than 3.20 ± 0.20 µg/mL, exhibited inhibitory activities that were approximately 100 fold greater than that for ursolic acid. The IC_{50} values were ≤ to 0.05 µg/mL.

Lineweaver-Burk plots were used to establish the mechanisms of inhibition for *Bridelia micrantha*, *Ziziphus mucronta*, *Brachylaena discolor*, *Artemisia afra* and *Sutherlandia frutescens* crude extracts. The results in Figure 1 and Table 3 show that the crude extracts exhibit a non-competitive PTP 1B inhibitory pattern. This implies that the bioactive compounds target the allosteric sites of the enzyme. Previous studies postulated the potency of noncompetitive PTP 1B inhibitors that target the enzyme allosteric site and prospects of developing efficacious anti-diabetic drugs from such inhibitors (Liu et al. 2008). Therefore, the present study has also identified the plants as sources of lead compounds for developing drugs to treat diabetes type 2.

Nine out of the 15 plants reported in the present study showed dipeptidyl IV (DPP IV) inhibition activities. DPP IV is involved in the degradation of glucagon like peptide-1, a potent insulinotropic peptide (Gallwitz 2008). Besides supporting the current uses of plants locally, these results also illustrate the significance of inhibiting DPP-IV as an effective approach to treat type 2 diabetes mellitus by promoting insulin secretion. *Bridelia micrantha*, *Terminalia sericea*, *Brachylaena discolor*, *Lannea edulis and Pteronia divaricate* showed PTP 1B inhibitory activities. However, the plants could not inhibit DPP IV. Out of the nine plants which showed PTP IB inhibitory activities, only 4 plants, that is, *Sutherlandia frutescens*, *Momordica balsamina*, *Bulbine latifola*, and *Spirostachys africanus* portrayed activities that were significantly comparable to that of the commercial drug sitagliptin. Since the current anti DPP IV active drugs; vildagliptin, sitagliptin and sexagliptin have a number of considerable side effects like

tremors, dizziness, nausea, weakness, weight gain and leg edema due to excessive fluid retention to some patients, *Sutherlandia frutescens, Momordica balsamina, Bulbine latifola,* and *Spirostachys africanus* can function as alternative drugs or as sources of lead compounds for the development of new diabetes mellitus type 2 drugs. The herbal drugs may be useful in treatment or management of impaired glucose tolerance, insulin resistance and hyperinsulinemia. Plants which did not show either PTP IB or DPP IV inhibition might be acting by different modes such as α glucosidase or amylase inhibition.

Conclusion

The present study shows the importance of folklore medicine in management of diabetes type 2. Out of the 15 plants encountered in the present study, 9 are active against PTP IB. Also 9 plants inhibited DPP IV. Only 4 plants had both PTP IB and DPP IV inhibitory activities. The PTP IB inhibition activity depends on the solvent used in extraction. Ethyl acetate extracts showed the greatest activity which implied the anti-diabetic active components of the extracts are moderate polar.

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Conflict of interests

The authors declare no conflict of interest.

Authors' contributions

PD and TC collected samples, performed the experimental work and statistical analysis. PD managed literature, wrote the first and final draft. All the authors read and approved the final draft.

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