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Inhibitory activity of fractions of *Senna nigricans* toward protein tyrosine phosphatase 1B and dipeptidyl peptidase IV

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Protein tyrosine phosphatase (PTP) 1B and dipeptidyl peptidase (DPP) IV are important in down regulation and secretion of insulin, respectively. While PTP 1B regulates insulin binding to its receptor, DPP IV hydrolyses incretin, which is an important regulator of postprandial insulin secretion. The study evaluated the *in vitro* inhibitory effect of different fractions of *Senna nigricans* against PTP IB and DPP IV and the results were compared with standard inhibitors, sumarin and P32/98. The methanolic extract was further fractionated using Soxhlet apparatus and then the most potent fraction eluted through column on silica gel. The result indicated that the methanolic fraction had the highest inhibition percentage of 56.43±3.98 against PTP 1B. The inhibition of PTP 1B by methanolic fraction was significantly (P<0.05) higher than that of the standard inhibitor, sumarin. The PTP 1B inhibition by ethyl acetate fraction (31.34±5.40%) was not significantly (P>0.05) different from that of sumarin, while hexane fraction had the inhibition of 19.03±4.24% which was significantly (P<0.05) decreased as compared with sumarin. The result of DPP IV inhibition indicated that the methanolic, hexane and ethyl acetate fractions were not significantly different, but all the fractions were significantly less active than the standard inhibitor, P32/98 which recorded 63.1±4.67% inhibition of DPP IV. Because of *S. nigricans* as sources of PTP 1B inhibitors most especially and to some extent DPP IV inhibitors, the plant may be a potential source for the discovery of lead compounds as PTP 1B and DPP IV inhibitors to treat type 2 diabetes mellitus.

Key words: Protein tyrosine phosphatase, dipeptidyl peptidase, inhibitors, *Senna nigricans*.

INTRODUCTION

Diabetes mellitus is a metabolic disorder which is due to a defect in insulin secretion, insulin action, or both. A consequence of this is chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism. Insulin deficiency or resistance led to various metabolic alterations in diabetic subjects or animals which increased blood glucose and caused dyslipidaemia (Himma et al., 2014). Type 2 diabetes is the most

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Abbreviations: TLC, thin layer chromatography; **PTP,** protein tyrosine phosphatase; **DTT,** dithiothreitol

common form, accounting for about 85 to 90% of all diabetic cases, and is due to a combination of impaired insulin secretion and insulin resistance (Mlinar et al., 2007). The prevalence of type 2 diabetes mellitus is rapidly increasing worldwide, and it is estimated that more than 430 million people will be affected by 2030 (Shaw et al., 2010). Therefore, a continuous search for new therapeutic options is necessary due to limitations of the current available treatment. The medicinal properties of plant species have made an outstanding impact in the origin and evolution of many traditional herbal therapies. Traditional medicine has gained popularity world over owing to high cost of orthodox medicine (Hudaib et al., 2008). Natural products have been shown to play a significant role in the development of novel drugs for the treatment and prevention of diseases (Gilani and Rahman, 2005).

Senna (Cassia) nigricans is a very important plant of Ayurvedic medicines. The plant is a variable, branching, erect shrub and it is highly drought resistant and suitable for desert. The leaves of the plant have been reported to contain pharmacologically bioactive substances. Studies have shown that this plant possesses anti-inflammatory (Chidume et al., 2001), antioxidant (Kumaran and Karunakaran, 2007), hypoglycaemic (Jalalpure et al., 2004), and anticancer activities (Yadav et al., 2010).

Protein tyrosine phosphatase (PTP) 1B has been implicated in the negative regulation of insulin signaling by dephosphorylating the insulin receptor as well as insulin receptor substrate and selective inhibitions of this enzyme have emerged as a new drug target for the treatment of type 2 diabetes mellitus (Teng et al, 2011). Dipeptidyl peptidase (DPP) IV is a serine protease which causes breakdown of incretin hormones; glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are released into the intestine in response to nutrient ingestion and stimulate insulin secretion induced by glucose (Mentlein et al., 1993). Both peptides, GLP-1 and GLP are rapidly degraded by DPP IV into an inactive form (Vilsboll et al., 2003). Thus, inhibition of DPP IV activity has the potential to be a novel therapeutic strategy to treat type 2 diabetes mellitus (Ahren, 2005). Current antidiabetic drugs in use for long-term therapy are found to be associated with various toxic effects owing to which the developmental process in antidiabetic drug research has shifted its emphasis to natural plant sources having minimal side effects (Nayak et al., 2009; Veerapur et al., 2010). Therefore, the study is aimed at isolating PTP 1B and DPP IV inhibitors from *S. nigricans*

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used are of analytical grade. PTP IB and DPP IV drug discovery assay kits were purchased from Enzo® Life Sciences.

Plant

In the current study, *S. nigricans* was collected from Sokoto and the plant was identified by a taxonomist from Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

Fractionation of the extract

The plant sample was shade dried and ground to powder using laboratory pestle and mortar. Ten grams of the powdered sample was extracted in 100 ml of methanol for 72 h at room temperature. At the end of the 72nd hour, the extract was filtered using Whatman No 1 filter paper. The filtrate was concentrated using rotary evaporator. The obtained residue was left to dry in a drying cabinet and stored in an air tight labeled container at 4°C until required. Further extraction of the plant material was carried out in a soxhlet apparatus successively 5 h each with hexane, ethyl acetate and methanol. The percentage yield of crude methanol was 29.8%, while 12.3, 9.6 and 8.5% were recorded for hexane, ethyl acetate and methanol, respectively after soxhlet extraction.

Column chromatographic separation of the *S. nigricans*

The methanol soxhlet fraction of the *S. nigricans* was further fractionated using column chromatography on silica gel. The methanol fraction was fractionated with gradient column chromatography with silica gel 60 (Merck, Germany) as the stationary phase and hexane:ethyl acetate and ethyl acetate:methanol mixture as the mobile phase starting with the least polar and gradually increasing the polarity. The fractions were initially collected from column in 10 ml volumes, rerun on TLC and the fractions with same RF values were merged for bioassay of PTP 1B and DPP IV inhibitory activities.

DPP IV inhibition assay

The fractions were screened for DPP IV inhibition at 100 µg/ml in a total volume of 100 µl using DPP IV drug discovery assay kits, a product of Enzo Life Sciences. The inhibitor (P32/98) was diluted in the assay 1 in 10 µl of assay buffer (50 mM Tris, pH 7.5). The substrate, H-Gly-Pro-pNA was diluted in 1 in 50 µl of assay buffer. The plant samples were reconstituted in buffer (50 mM Tris, pH 7.5) to give 1 µg/µl. The DPP IV (BML-SE434-9090) was reconstituted 1 in 50 µl of the assay buffer. The assay mixture was prepared in a 96 well plate to contain 10 µg per 100 µl assay mixture of the sample, 15 µl of DPP IV (17.3 µU/µl) and 50 µl of the substrate and the volume made up to 100 µl with the assay buffer. The inhibitor well contained 10 µl of the inhibitor (P32/98) in place of the extract. The control tube had neither inhibitor nor the extract and the blank was prepared using the substrate and the buffer only. The plate was read continuously at 405 nm, in a microplate reader at 1 min intervals for 10 min.

PTP 1B inhibition assay

The kit components were thawed and held on an ice bath except BIOMOL RED™ that was stored at room temperature. The substrate, insulin receptor β residues 1142-1153, pY-1146 (IR5) was reconstituted to 1.5 mM by assay buffer and dH_2O. The asssay buffer, 100 mM MES, pH 6.0 containing 300 mM NaCl, 2 mM EDTA, 2 mM DTT and 0.1% NP-40 was diluted with equal volume of dH_2O and kept on ice untill required. The enzyme, PTP 1B (human recombinant) was prepared in x1 cold assay buffer. Stock

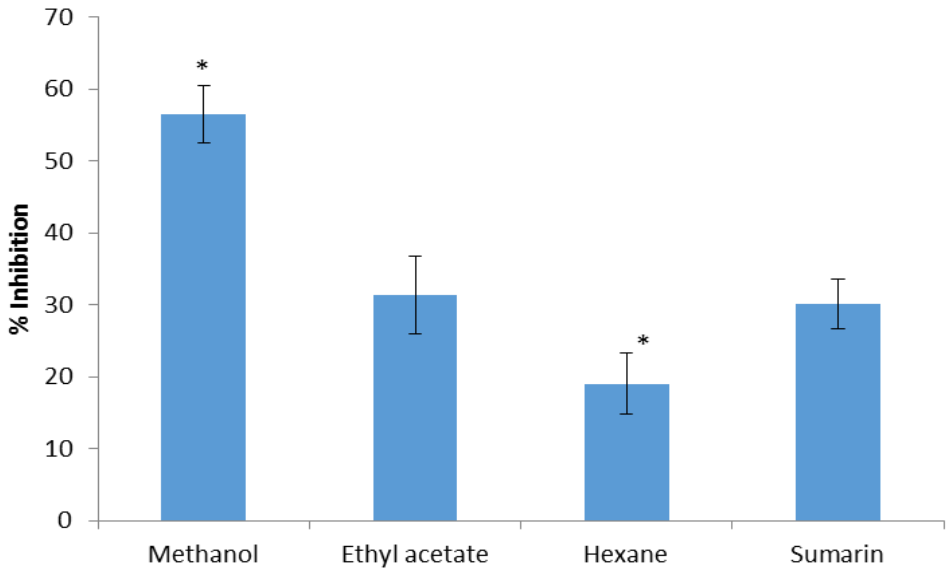

Figure 1. PTP 1B Inhibition of Soxhlet Fractions of *S. nigricans*. Data are expressed as Mean ± SD, n = 3 replicate, *P< 0.05 when compared with sumarin.

of 10 mM of the inhibitor, suramin was prepared in assay buffer. The assay mixture was prepared in a 96 well plate to contain 10 µg per 100 µl assay mixture of the sample. Other components of the the assay mixture were the buffer, the enzyme and the substrate. The substrate was added last. This was done strictly according to the manufacturers specifications.

Data analysis

The data are expressed as mean ± standard deviation (SD) of 3 replicate. The values were expressed as percentage inhibition. SPSS (version 17.0) was used for data analysis and P value of <0.05 was considered significant.

RESULTS

The percentage PTP 1B and DPP IV inhibitions of Soxhlet fractions of *S. nigricans* are presented in Figures 1 and 2, respectively. The results show that methanolic fraction had the highest percent inhibition of 56.43±3.98% for PTP 1B (Figure 1) as against the standard inhibitor, sumarin (30.12±3.46%). The percent PTP 1B inhibition of ethyl acetate fraction (31.34±5.40%) was not significantly (P>0.05) different from that of sumarin, while hexane fraction had the inhibition of 19.03±4.24% which was significantly (P<0.05) lowered as compared to sumarin (Figure 1).

DPP IV inhibition (Figure 2) indicated that the methanolic fraction (32.6± 4.33%), hexane fraction (28.2±5.36%) and ethyl acetate fraction (27.3±6.06%) were not significantly different, but all the fractions were significantly less active than the standard inhibitor, P32/98 which recorded 63.1± 4.67% inhibition of DPP IV.

The column chromatographic fractions of the methanolic fraction (Soxhlet) were merged based on the results of the thin layer chromatography into 19 fractions. The results of the PTP 1B and DPP IV inhibition studies are presented in Table 1. The results indicated further that fraction 12, which was eluted from the column using 1:3 ethyl acetate in hexane, had the highest PTP 1 B inhibition of 56.43% as compared to 30.12% for the standard inhibitor, suramin. The effect of the fractions on DPP IV indicated that the fractions that were active were significantly less active than the standard inhibitor.

Extracts with NI showed no inhibition, and actually activated the activities of the respective enzymes.

DISCUSSION

Type 2 diabetes mellitus which accounts for about 85 to 95% of diabetes cases is associated with considerable morbidity and mortality. In this study, the effects of fractions of *S. nigricans* on the activities of PTP I and DPP IV were investigated. The effect of methanolic fraction against PTP 1B was significantly higher than that of the standard inhibitor, sumarin. The PTP 1B and DPP IV inhibition assays were used in this study to elucidate the inhibitory effect of the fractions of *S. nigricans.* The methanolic extracts of plants such as *Psidium guajava* (Oh et al., 2005), *Salvia miltiorrhiza* (Han et al., 2005) and *Centratherum anthelminticum* (Arya et al., 2013) have been reported to exhibit significant effect against PTP 1B activities. Consistently, our findings indicated methanolic fraction displayed the highest percentage inhibition of 56.43%. This observation suggests that

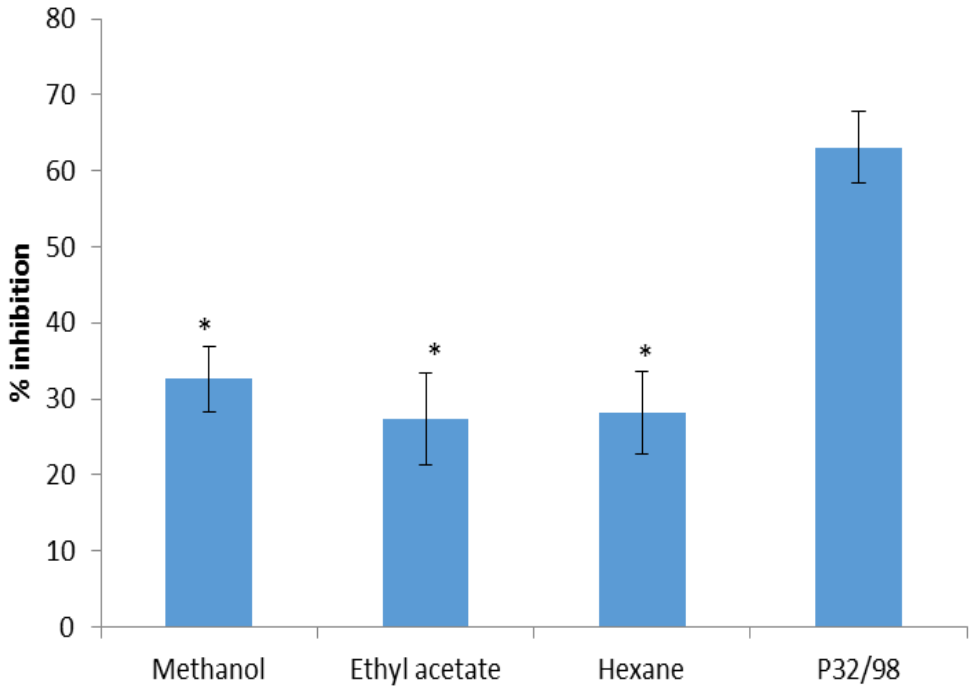

Figure 2. DPP IV Inhibition of Soxhlet fractions of *S. nigricans*. Data are expressed as Mean ± SD, n = 3 replicate, *P<0.05 when compared with P32/98.

Table 1. Inhibition of PTP 1B and DPP IV of the column chromatographic fractions of the methanolic fraction.

Fraction**	Percentage Inhibition	
	PTP 1 B	DPP IV
1	20.48	9.12
2	10.95	NI
3	NI	NI
4	NI	NI
5	37.38	NI
6	28.38	12.11
7	8.64	NI
8	28.78	NI
9	NI	NI
10	31.34	NI
11	44.48	7.23
12	56.43	12.12
13	13.51	NI
14	17.58	NI
15	NI	NI
16	17.93	NI
17	NI	6.23
18	NI	NI
19	NI	NI
20	23.48	NI
Inhibitor*	30.12	63.1

*Suramin for PTP IB and P32/98, MW=260.4 for DPP IV, **Fractions were initial collected from column in 10 mL volumes, rerun on TLC and the fractions with same RF values were merged for bioassay.

methanol may be the best solvent for extraction of PTP 1B inhibitors from *S. nigricans*. The DPP IV inhibitory activity of the three fractions were not significantly different, but were significantly lower than that of the control, P32/98. Many other studies have reported inhibition of DPP IV enzymatic activity by plant extract; the aqueous leaves extract of *Cistus incanus* L. (Lendeckel et al., 2002), methanolic leaves extract of *Mangifera indica* (Yogisha and Raveesha, 2010), hexane extract of *Annona squamosa* (Davis et al., 2012), ethanolic extract of *Urena lobata* (Yudi et al., 2015) and leaf extract of *Ocimum sactum* and fruit extract of *Momordica charantia* (Singh et al., 2014). Therefore, since prevalence of diabetes mellitus has escalated, a multi-model therapeutic strategy is urgently required for the treatment of the disorder and to large extent; *S. nigricans* and other plants that have been reported to be potential source(s) of inhibitors of PTP 1B and DPP IV could be isolated to treat type 2 diabetes mellitus. Fractionation using soxhlet apparatus followed by column chromatography and then thin layer chromatography indicated the fractionation of the PTP 1 B inhibitor(s) into fraction 12 of the column fractions. Unfortunately, it appears that the DPP IV inhibitory activity of *S. nigricans* has been lost upon the fractionation. This may be attributed to the fact that most of the bioactive components of plants act in synergy and this could be responsible for loss in the effect of *S. nigricans* against DPP IV inhibition.

Based on the findings in this study, it was hypothesized that crude extract which contains varieties of bioactive components may be more beneficial to multifactorial diseases like diabetes than any single bioactive compound. Although, further studies such as *in vivo* inhibitory and kinetic studies of PTP IB and DPP IV using crude fractions and pure isolate from *S. nigricans* are required to validate this hypothesis.

Conflict of Interests

The authors have not declared any conflict of interests.

Conclusion

The results of the current study demonstrated the potential of *S. nigricans* as a source of lead compound(s) in the development of inhibitors(s) of PTP 1B and DPP IV in the management of type 2 diabetes mellitus.

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REFERENCES

Ahren B (2005). Inhibition of dipeptidyl peptidase 4 (DPP-4) a novel approach to treat type 2 diabetes mellitus. Curr. Enzym. Inhib. 1(1):65-73.

Arya A, Looi CY, Wong WF, Noordin MI (2013). *In vitro* antioxidant, PTP IB inhibitory effects and in vivo hypoglycemic potential of selected medicinal plants. Int. J. Pharmacol. 9(1):50-57.

Chidume FC, Gamaniel K, Amos S, Akah P, Obodozie O, Wambebe C (2001). Pharmacological activity of the methanolic extract of Cassia nigricans leaves. Ind. J. Pharmacol. 33:350-356.

Davis JA, Sharma S, Mittra S, Sujatha S, Kanaujia A, Shukla G, Katiyar C, Lakshmi BS, Bansal VS, Bhatnagar PK (2012). Antihyperglycemic effect of *Annona squamosa* hexane extract in type 2 diabetes animal model: PTP1B inhibition, a possible mechanism of action? Ind. J Pharmacol. 44:326-332.

Gilani AH, Atta-ur-Rahman (2005). Trends in Ethnopharmacology. J. Ethnopharmacol. 100:43-49.

Han YM, Oh H, Na M, Kim BS, Oh WK, Kim BY, Jeong DG, Ryu SE, Sok DE, Ahn JS (2005). PTP1B inhibitory effect of Abietane Diterpenes isolated from *Salvia miltiorrhiza*. Biol. Pharm. Bull. 28:1795-1797.

Hudaib M, Mohammad M, Bustanji Y, Tayyem R, Yousef M, Abuirjeie M, Talal A (2008). Ethno pharmacological survey of medicinal plants in Jordan, Mujib Nature Reserve and surrounding area. J. Ethnopharmacol. 120:63-71.

Himma BR, Mohana LS, Himaja N, Nirosha K, Pooja M (2014). Formulation, characterization and antidiabetic evaluation of *Talinum portulacifolium* (Forssk) loaded solid lipid nanoparticles in streptozotocin and high fat diet induced diabetic rats. J. Glob. Trends Pharm. Sci. 5(4):2108-2114.

Jalalpure SS, Patil MB, Pai A, Shah BN, Salahuddin MD (2004). Antidiabetic activity of Cassia auriculata seeds in alloxan induced diabetic rats. Niger. J. Nat. Prod. Med. 8:22-23.

Kumaran A, Karunakaran RJ (2007). Antioxidant activity of *Cassia auriculata* flowers. Fitoterapia 78(1):46-47.

Lendeckel U, Arndt M, Wolke C, Reinhold D, Kahne T, Ansorge S (2002). Inhibition of human leukocyte function, alanyl aminopeptidase (APN, CD13) and dipeptidylpeptidase IV (DP IV, CD26) enzymatic activities by aqueous extracts of *Cistus incanus* L. ssp. Incanus. J. Ethnopharmacol. 79:221-227.

Mentlein R, Gallwitz B, Schmidt W (1993). Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36) amide, peptide histidine methionine and is responsible for their degradation in human serum. Eur. J. Biochem. 214:829-835.

Mlinar B, Marc J, Janez A, Pfeifer M (2007). Molecular mechanisms of insulin resistance and associated diseases. Clin. Chim. Acta 375:20-35.

Nayak Y, Veerapur VP, Nagappa AN, Unnikrishnan MK (2009). Perspectives in efficacy, safety and clinical evaluation of bioactive natural products. Compendium of bioactive natural products, 2, USA:M/S. Stadium Press llc. pp. 1-30.

Oh WK, Lee CH, Lee MS, Bae EY, Sohn CB, Oh H, Kim BY, Ahn JS (2005). Antidiabetic effects of extracts from *Psidium guajava*. J. Ethnopharmacol. 96(3):411-415.

Shaw JE, Sicree RA, Zimmet PZ (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. Diabet. Res. Clin. Pract. 87:4-14.

Singh AK, Jatwa R, Joshi J (2014). Cytoprotective and dipeptidase IV (DPP-IV/Cd6) inhibitory roles of *Ocimum sactum* and *Momordica charantia* extract. Asian J. Pharm. Clin. Res. 7:1.

Teng BS, Wang CD, Yang HJ, Wu JS, Zhang D, Zheng M, Fan ZH, Pan D, Zhou P (2011). Protein Tyrosine Phosphatase 1B activity inhibitor from the fruiting bodies of *Ganoderma lucidum* (Fr.) Karst and its hypoglycemic potency on streptozotocin -induced type 2 diabetic mice. J. Agric. Food Chem. 59:6492-6500.

Veerapur VP, Prabhakar KR (2010). Antidiabetic effect of *Dodonaea viscosa* (L). Lacq aerial parts in high fructose-fed insulin resistant rats: a mechanism based study. Ind. J. Exp. Biol. 48:800-810.

Vilsboll T, Agerso H, Krarup T, Holst JJ (2003). Similar elimination rates of glucagon-like peptide -1 in obese type 2 diabetic patients and healthy subjects. J. Clin. Endocrinol. Metab. 88(1):220-224.

Yadav JP, Arya V, Yadav S, Panghal M, Kumar S, Dhankhar S (2010). *Cassia occidentalis* L.: A review on its ethnobotany, phytochemical and pharmacological profile. Fitoterapia 81:223-230.
Yogisha S, Raveesha KA (2010). Dipeptidyl Peptidase IV inhibitory activity of *Mangifera indica*. J. Nat. Prod. 3:76-79.
Yudi P, Djoko WS, Sutiman BS, Mochamad AW (2015). Antidiabetic potential of *Urena lobata* leaf extract through inhibition of dipeptidyl peptidase IV activity. Asian Pac. J. Trop. Biomed. 5(8):645-649.