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In vitro Antioxidant, PTP-1B Inhibitory Effects and *in vivo* Hypoglycemic Potential of Selected Medicinal Plants

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Abstract: The therapeutic potential of plants varies according to their parts. The present study was aimed to ascertain the antioxidant and antidiabetic potential of crude fractions obtained from different parts of 6 medicinal plants, *Centratherum anthelminticum, Cissus quadrangularis, Terminalia bellerica, Terminalia chebula, Terminalia arjuna* and *Woodfordia fruticosa*. Total phenolic (TPC), total flavonoid (TFC) and total tannin content (TTC) were determined. *In vitro* antioxidant abilities were showed by 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing/antioxidant Power (FRAP) assays. Furthermore, anti-diabetic potential was determined using *in vitro* protein tyrosine phosphatase-1B (PTP-1B) inhibition assay and blood glucose lowering effects were evaluated on streptozotocin (STZ)-induced diabetic rats. The result of our study showed that *T. chebula* fruit exhibited highest amount of TPC (910.43±37.45 mg GAE g^{-1}) and TTC (65.6±6.83 mg Catechin g^{-1}), respectively. Whereas *C. anthelminticum* seeds contained highest amount of TFC (98.2±27.6 mg Quercetin g^{-1}). The free radical scavenging capacity of *T. chebula* fruits was the highest among the six plants as determined by DPPH (3.6±0.13 μg mL^{-1}) and FRAP (109.6±2.5 μg mL^{-1}) assays. *C. anthelminticum* seeds (9.16±0.62 μM mL^{-1}) demonstrated highest oxygen radical absorbance capacity in ORAC test. In addition, *C. anthelminticum* seeds (38±5.8 μM) showed highest PTP-1B inhibitory effects and maximum blood glucose lowering effects in STZ-induced diabetic rats. Altogether, our findings suggest that *T. chebula* fruit is potent in ameliorating oxidative damage whereas, *C. anthelminticum* seeds possess highest antidiabetic and antioxidant properties.

Key words: Antioxidant, antidiabetic, PTP-1B, DPPH, ORAC, FRAP

INTRODUCTION

Medicinal plant parts are commonly rich in phenolic compounds (e.g., flavonoids, phenolic acids), which have multiple biological effects on oxidative stress, diabetes, inflammation and cancers (Cai *et al*., 2003; Zheng and Wang, 2001). Phenolics and flavonoids in green tea or grape seeds are good examples which have raised public interest as natural antioxidant and have been recognized by many investigations (Rice-Evans *et al*., 1996; Mukai *et al*., 2005). It is well understood that poly-phenolics shows antioxidative abilities by their redox potential to chelate metals thereby quenching of singlet oxygen (Tachakittirungrod *et al*., 2007). Report on certain flavonoids has also shown positive response in inducing insulin secretion on pancreatic β-cells by targeting insulin signaling pathways (Kim *et al*., 2007).

Diabetes mellitus is a disorder characterized by hyperglycemia resulting from increased hepatic glucose production, diminished insulin production and function. Increasing evidences have suggested that oxidative stress generated by Reactive Oxygen Species (ROS) through endogenous or exogenous processes may play a major role in the pathogenesis of Diabetes Mellitus (DM) by attacking lipids, proteins, nucleic acids and also by altering energy metabolism in the mitochondria of the living cells (Valko *et al*., 2006; Poli *et al*., 2004). Oxidative stress also appears to be the pathogenic factor in diabetic complications, leading to pancreatic β-cell necrosis (Ceriello and Motz, 2004). To determine oxidative stress, various biomarkers have been developed at cellular levels and some have been projected for thorough evaluation of oxidative damage in diabetic complications (Hwang and Kim, 2007; Jones, 2006; Sachdev and Davies, 2008). Many studies have shown strong correlation of medicinal plants containing phenolic and poly-phenolic compounds in the prevention of diabetes caused by oxidative stress (Konczak and Zhang, 2004; Williamson and Manach, 2005).

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Protein tyrosine phosphatase-1B (PTP-1B) inhibition effects evidently demonstrates critical role of Protein Tyrosine Phosphatase (PTPs) in controlling insulin signaling pathway (Evans and Jallal, 1999). Metabolic insulin signal transduction occurs through activation of the Insulin Receptor (IR) and PTP-1B have been implicated in the dephosphorylation of the IR (Tonks and Neel, 2001). Therefore, plant extracts/fractions or natural products that shows PTP-1B inhibitory effects may help type 2 diabetic and obese patients in ameliorating such metabolic complications (Klann *et al*., 2000). Hence, there is a need to explore new natural sources of PTP-1B inhibitors.

Centratherum anthelminticum, *Cissus quadrangularis*, *Terminalia bellerica*, *Terminalia. chebula*, *Terminalia arjuna* and *Woodfordia fruticosa* are used as an alternative medicine for the treatment of oxidative stress and diabetes by local folks in south India and southeast Asia. However, to the best of our knowledge, scientific investigation to compare the antioxidant and antidiabetic effects amongst these plants is not yet available. The objective of this study is to determine antioxidant and antidiabetic potential of the crude fractions of different parts of six plants. Initially, we examined total phenolic, flavonoid and tannin content in correlation with their antioxidant properties. Next, in relation to their antioxidant activity, the fractions were evaluated for *in vitro* PTP-1B inhibition and blood glucose lowering activity *in vivo* to determine the antidiabetic potency.

MATERIALS AND METHODS

Sample extraction: Different parts of six plants; *C. anthelminticum*, *C. quadrangularis*, *T. bellerica*, *T. chebula*, *T. arjuna* and *W. fruticosa* were obtained from Amritum Bio-Botanica Herbs Research Laboratory Pvt. Ltd, Jogli, India. Plants were authenticated by taxonomist from the company. The parts (flower, seeds, leaves, stem or fruits) were coarsely powdered and extracted successively using Soxhlet extractor with hexane, chloroform and finally with methanol. The resulting methanolic fractions were evaporated under reduced pressure at 40°C using a rotary evaporator to derive crude methanolic fractions and stored at -20°C prior to use.

Determination of total phenolic content: The Total Phenolic Content (TPC) was determined by Folin-Ciocalteu method with slight modification (Arya *et al*., 2012a). All the crude fractions were prepared in a concentration of 10 mg mL^{-1} in methanol. Five microliters of these solutions were transferred to 96-well mircoplate (TPP, USA). To this, 80 μL of Folin-Ciocalteu reagent (1:10) were added and mixed thoroughly. After 5 min, 160 μL of sodium bicarbonate solution ($NaHCO_3$, 7.5%) was added and the mixtures were allowed to stand for 30 min with intermittent shaking. Absorbance was measured at 765 nm using microplate reader (Molecular Devices, Sunnyvale, USA). The Total Phenolic Content (TPC) was expressed as Gallic Acid Equivalent (GAE) in mg g^{-1}, obtained from the standard curve of gallic acid.

The gallic acid standard curve was established by plotting concentration (mg mL^{-1}) versus absorbance (nm) ($y = 0.001x+0.045$; $R^2 = 0.9975$) where, y is absorbance and x is concentration in GAE (n = 3).

Determination of total flavonoid content: The Total Flavonoid Content (TFC) was determined by following the method of Arya *et al*. (2012a). In brief, 5 mL of 2% aluminium trichloride was mixed with the same volume of all the crude fractions. Absorbance at 415 nm was taken after 10 min against a blank sample consisting of 5 mL of sample solution and 5 mL of methanol without aluminium trichloride. The total flavonoid content was determined using a standard curve of quercetin at 0-50 μg mL^{-1}. The average of three readings was used and then expressed as Quercetin Equivalents (QE) on a Dry Weight (DW) basis.

Determination of total tannin content (TCC): The TTC in the crude fractions were determined by the method as described by Arya *et al*. (2012a). All the crude fraction samples were marked to 3.0 mL volume and mixed with 3.0 mL of vanillin (4%) in methanol. Thereafter, 1.5 mL con. HCl was added and further incubated in the dark for 10 min. Subsequently, the TTC content of the samples were analyzed with a UV-Vis. spectrophotometer at 500 nm. Results are expressed as mg Catechin (C) equivalents.

DPPH radical scavenging activity: The scavenging activity of all the methanolic fractions on DPPH (1,1-diphenyl-2-picrylhydrazyl) was determined by following the method as described by Arya *et al*. (2012b). This method is based on the reduction of purple DPPH to a yellow colored diphenylpicryl hydrazine. Changes in color were measured at 518 nm. All the fractions were tested at concentrations ranging 10-600 μg mL^{-1} in ethanol. One milliliter of 0.3 mM DPPH ethanol solution was added to 2.5 mL of sample solution in different concentrations to produce the test solutions, while 1 mL of ethanol was added to 2.5 mL of sample to produce the blank solutions. The negative control consisted of 1 mL of DPPH solution plus 2.5 mL of ethanol. The solutions were allowed to react at room temperature for 30 min in the dark. The

absorbance values were measured at 518 nm and converted into percentage antioxidant activity using the following equation:

$$\text{Inhibition (\%)} = [(AB\text{-}AA)/AB]\times 100$$

where, AB: Absorption of blank sample; AA: Absorption of tested samples.

The half maximal inhibitory concentration (IC_{50}) and the kinetics of DPPH scavenging activity were determined. ascorbic acid and Butylated Hydroxy Toluene (BHT) were used as positive controls in this assay.

ORAC antioxidant activity assay: The Oxygen Radical Absorbance Capacity (ORAC) assay was carried out based on the procedure described with slight modifications (Cao *et al*., 1997). Briefly, 175 μL of the sample/blank were dissolved with Phosphate Buffer Solution (PBS) at concentrations of 160 μg mL^{-1} and at 7.4 pH. Serial dilutions of the standard Trolox was prepared from 75 mM. To 96-well black microplates, 25 μL each of samples (fractions), standard (Trolox), blank (solvent/PBS), or positive control (quercetin) were added. Subsequently, 150 μL of fluorescent sodium salt solution was added and the plate was incubated for 45 min at 37°C. 2, 20-azobis (2-amidinopropane) dihydrochloride (AAPH) solution (25 μL) was added to make up a total volume of 200 μL/well. Fluorescence was recorded at 37°C until it reached 0 (excitation at 485 nm, emission at 535 nm) using a fluorescence spectrophotometer (Perkin-Elmer LS 55) equipped with an automatic thermostatic autocell-holder. Data were collected every 2 min for 2 h and were analyzed by calculating the differences of areas under the fluorescein decay curve; Area under Curve (AUC) between the blank and the sample. Values are expressed as Trolox equivalents.

FRAP assay: The FRAP (ferric reducing/antioxidant power) assay was modified from the method used by Benzie and Strain (Benzie and Strain, 1996). The stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ solution. A fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ and 2.5 mL $FeCl_3.6H_2O$. The temperature of the solution was raised to 37°C before use. Fractions (10 μL) were allowed to react with 190 μL of FRAP solution for 30 min in the dark. Colorimetric readings of the product, i.e., the ferrous-TPTZ complex, were taken at 593 nm for 10 min and a steady state was reached within 5 min for the different test substance concentrations. The IC_{50} value was calculated from the regression curve as the concentration of antioxidant (1 M) giving an absorbance reading equivalent to that obtained with a 1 mM Fe (II) solution. The standard curve was linear between 200 and 1000 μM $FeSO_4$. Results are expressed as μM Fe (II)/g dry mass and compared with those of ascorbic acid and BHT.

PTP-1B inhibition assay: The present study investigated all the crude fractions for their inhibitory effects on PTP-1B activity in an *in vitro* assay. ursolic acid and 3- hexadecanoyl-5-hydroxymethyl tetronic acid (RK-682) were used as positive controls in the assay (Hoang *et al*., 2009). To each well of a 96-well plate (final volume: 200 μL), 2 mM p-nitrophenyl phosphate (p-NPP) as a substrate and PTP-1B human recombinant enzyme (BIOMOL International LP USA) (0.1 μg) were added in a 50 mM citrate buffer of pH 6.0, containing, 0.1 M NaCl, 1 mM EDTA and 1 mM dithiothreitol (DTT) with or without fraction samples and incubated for 30 min at 37°C. Thereafter, 10 M NaOH was added and the reaction was terminated. The amount of p-nitro phenol produced was estimated by measuring the absorbance at 405 nm. The non enzymatic hydrolysis of 2 mM p-NPP was measured at the same absorbance in the absence of PTP-1B enzyme.

Animals: Male Sprague Dawley (SD) rats weighing 180-200 g were procured from the Animal Care Unit, UMMC (University Malaya Medical Centre) Kuala Lumpur, Malaysia and were maintained under pathogen-free conditions in the animal housing unit in a temperature-controlled (23±2°C) and light-controlled (12 h light/dark cycle) room and 35-60% humidity. The animals were acclimatized for 10 days prior to start the experiments and were provided rodent chow and water *ad libitum*. Animal experiments were performed in accordance with the guidelines for animal experimentation issued by the Animal Care and Use Committee, University of Malaya (Ethics Number: FAR/10/11/2008/AA(R)). Animals were divided into 15 groups (n = 6); first group with normal control (non-diabetic), second group with diabetic control and other 13 groups of diabetic animals; one group treated with glibenclamide (50 mg kg^{-1} b.wt.) and remaining 12 groups with plant fractions (200 mg kg^{-1} b.w.). All the treated group animals were orally fed with their respective doses of the plant samples and glibenclamide for 4 days. Everyday, blood glucose was measured in all the groups by tail snipping in non-fasting conditions with free access to food and water by using a standardized glucometer (Accu-Chek; Roche, Mannheim, Germany).

Induction of diabetes mellitus in rats: Diabetes was induced in overnight-fasted normal male rats through

intraperitoneal (i.p) administration of 45 mg kg^{-1} of STZ (Sigma-Aldrich, Germany) in 0.1 M citrate buffer (pH 4.5) in a volume of 1 mL kg^{-1} (b.wt.). Hyperglycemia was confirmed by elevation in blood glucose levels, determined at 96 h after the STZ administration. Rats with a fasting blood glucose range of 9-12 mmol L^{-1} were considered diabetic and subsequently used for the study.

Statistical analysis: The results were expressed as mean±standard deviation (SD). Significant differences between the means of the experimental groups were identified with analysis of variance (ANOVA), followed by the Tukey-Kramer multiple comparisons test (GraphPad version 5.0; GraphPad Software Inc., San Diego, CA, USA).

RESULTS AND DISCUSSION

The crude methanolic fractions of the selected plants were initially tested for total phenolic, total flavonoid and total tannin content, followed by investigating fractions for antioxidant and anti-diabetic effects using *in vitro* and *in vivo* study models.

Total phenolic, flavonoid and tannin content: Poly-phenolic compounds in plants are known to be the most active antioxidant constituents and possess wide range of biological activities (Heim *et al*., 2002). Table 1 demonstrates the results for total phenolic, flavonoid and tannin contents. The crude methanolic fraction of *T. chebula* fruits showed the highest phenolic (910.43±37.45 mg GAE g^{-1}) and tannin (65.6±6.83 mg Catechin/g) contents, respectively. The total flavonoid content was highest in *C. anthelminticum* seeds (98.2±27.6 mg Quercetin/g) amongst all tested crude fractions. The relationship between total phenolic content and antioxidant activity has been controversial. Previous report showed a strong correlation between total phenolic content and antioxidant activity of crude extracts from certain vegetables, fruits and grain products (Velioglu *et al*., 1998). Whereas other study on polyphenolic compounds from a few plant extracts did not show such correlation (Kahkonen *et al*., 1999).

***In vitro* antioxidant activities:** To examine whether there is a correlation between total phenolic and antioxidant activity, the antioxidative abilities of selected crude fractions were determined with DPPH, ORAC and FRAP assays. These assays were measured in triplicate at different concentrations to determine the IC_{50} values.

DPPH radical scavenging activity: DPPH is a free radical compound and has been extensively utilized to evaluate the free radical scavenging aptitude of samples and is usually used as a substrate to examine antioxidant activity. Upon treatment with samples which are hydrogen atom donors, the DPPH radical is converted into a stable DPPH radical, indicated by a color change from purple to yellow (Thaipong *et al*., 2006).

Table 2 illustrates the DPPH radical scavenging capacity of all the tested fractions. The result showed that *T. chebula* fruits (3.6 μg mL^{-1}) and *C. anthelminticum* seeds (5.6 μg mL^{-1}) possessed highest scavenging capacity among all the 12 crude fractions tested, as these two plants recorded the lowest IC_{50} value of all the fractions. In contrast, *T. arjuna* (21.8 μg mL^{-1}) demonstrated the highest IC_{50} with lowest scavenging capacity. Whereas, standard drug ascorbic acid and BHT showed the lowest IC_{50} (IC_{50} = 1.6 and 1.7 μg mL^{-1}, respectively). The DPPH results correlates with the

Table 1: Total phenolic, flavonoid and tannin contents in crude fractions of different plants

Plants	TPC	TFC	TTC
C. anthelminticum leaves	312.8±023.4	18.23±00.9	12.30±1.9
C. anthelminticum seeds	665.3±188.8[R1]	98.20±27.6[R1]	33.30±2.7
C. quadrangularis leaves	306.4±087.5	29.17±01.8	09.00±0.32
C. quadrangularis stems	431.8±105.3	42.31±02.7	22.20±2.6
W. fruticosa leaves	211.6±057.4	18.23±02.6	13.20±2.5
W. fruticosa flowers	389.6±076.3	38.17±03.4	23.10±1.84
T. arjuna leaves	147.23±02.34[R2]	08.19±01.41[R2]	06.31±0.17[R2]
T. arjuna barks	203.12±014.4	14.49±02.56	53.43±3.48
T. bellerica leaves	259.28±06.42[R2]	16.15±02.42[R2]	04.68±1.31[R2]
T. bellerica fruits	451.65±102.4	31.42±04.9	59.35±5.8
T. chebula leaves	266.16±07.81[R2]	29.23±03.81[R2]	08.36±0.37[R2]
T. chebula fruits	910.43±37.45	48.41±05.61	65.60±6.83

TPC: Total phenolic content (mg GAE/g), TFC: Total flavonoid content (mg quercetin/g), TTC: Total tannin content (mg catechin/g), Values expressed are Mean±SD of triplicate measurements, [R1, R2]Superscript denoting published result of Arya *et al*. (2012a, b), respectively

Table 2: Effects of different crude fractions on DPPH, ORAC and FRAP assays

Samples	[a]DPPH	[a]ORAC	[a]FRAP
C. anthelminticum leaves	09.8±0.31	16.20±0.61	1790±2.3
C. anthelminticum seeds	05.6±0.33	09.16±0.62	118.0±1.9
C. quadrangularis leaves	18.3±0.41	26.70±1.4	221.0±6.7
C. quadrangularis stems	12.8±0.26	17.90±2.13	187.5±8.3
W. fruticosa leaves	15.3±0.29	39.10±1.39	212.0±4.7
W. fruticosa flowers	09.6±0.11	17.30±1.13	163.2±3.3
T. arjuna leaves	21.8±0.37[R]	42.31±2.7[R]	232.0±8.3[R]
T. arjuna barks	12.2±0.19	22.60±0.21	192.0±3.8
T. bellerica leaves	16.4±0.55[R]	29.10±1.8[R]	265.0±6.7[R]
T. bellerica fruits	09.9±0.35	21.20±0.42	152.4±5.8
T. chebula leaves	11.6±0.43[R]	18.23±0.9[R]	243.0±5.8[R]
T. chebula fruits	03.6±0.13	12.12±0.29	109.6±2.5
Ascorbic acid	01.6±0.14	Not applicable	03.70±0.8
BHT	01.7±0.07	Not applicable	03.20±0.5
Quercetin	Not applicable	12.16±1.3	Not applicable

DPPH: 1,1-diphenyl-2-picrylhydrazyl (μg mL^{-1}), ORAC: Oxygen radical absorbance capacity (μM mL^{-1}), μM: Concentration equivalent to Trolox (20 μg mL^{-1}), FRAP: Ferric reducing/antioxidant power (μg mL^{-1}), Standard deviation (SD) values of a minimum of 3 replicates, IC_{50} (Maximum inhibitory concentration up to 50%) demonstrating low values indicate high antioxidant activity, [R]superscript denoting published result of Arya (2012b)

relatively high total phenolic and flavonoids contents of *T. chebula* fruits and *C. anthelminticum* seeds, implying that polyphenolic compounds may be major contributor to the scavenging activity of the fractions.

ORAC assay: ORAC assays measure the ability of compound/extracts to inhibit peroxyl-radical-induced oxidation initiated by thermal decomposition of azo-compounds such as [2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH)] (Glazer, 1990). The result of ORAC assay is depicted in Table 2. Among all the tested fractions, *C. anthelminticum* seeds exhibited lowest IC_{50} values (9.16 μM TE mL^{-1}) followed by *T. chebula* (12.12 μM TE mL^{-1}) fruits and *C. anthelminticum* leaves (16.2 μM TE mL^{-1}), whereas positive control (Quercetin) had the lowest IC_{50} value (12.16 μM TE mL^{-1}); IC_{50} values are expressed in Trolox (μM mL^{-1}) equivalent (TE) concentrations.

Based on our result, *C. anthelminticum* seeds possess highest oxygen radical absorption capacity, which correlates with their high total flavonoid content. The ORAC assays provide a measure of the scavenging capacity of antioxidants against peroxyl radical which is one of the most common Reactive Oxygen Species (ROS) (Ou *et al*., 2001). ROS are hazardous to cellular constructions (i.e., DNA, proteins, lipid) as they act as vigorous oxidizing mediators or free radicals. Natural antioxidants with high oxygen radical absorption capacity are useful in eliminating all of the free radicals, oxygen ions and peroxides that may damage tissues and organs.

FRAP assay: The principle of the FRAP assay involves the reduction of ferric ions to ferrous ions due to the presence of reducing substances in the crude fractions. The ability of electron-donating antioxidant fractions to reduce ferric ions is capable of reducing the ferric-TPTZ (Fe (III)-TPTZ) complex to a blue ferrous-TPTZ (Fe (II)-TPTZ) complex that exhibits strong absorbance at 593 nm (Benzie and Strain, 1996).

Result of FRAP assay is showed in Table 2. *T. chebula* demonstrated highest ferric reducing antioxidant ability, with IC_{50} value 109.6 μg mL^{-1} followed by *C. anthelminticum* seeds (118 μg mL^{-1}) and *T. bellerica* (152.4 μg mL^{-1}). Lower IC_{50} value means higher ferric reducing antioxidant ability of the fraction. The standard BHT and ascorbic acid (3.2 and 3.7 μg mL^{-1}) had greater ferric ion reducing capability than others. The reducing capacity of these fractions may have been due to a large number of poly-phenolic compounds with electron-donating hydroxyl groups.

PTP-1B inhibition assay: The results for PTP-1B inhibition assay are presented in Table 3. Result showed that *C. anthelminticum* seeds exhibited strong inhibition of PTP-1B enzyme activity with IC_{50} values (38 μM) followed by *W. fruticosa* flowers (53 μM), *C. anthelminticum* leaves (64 μM) and *T. chebula* fruits (66 μM), while other fractions demonstrated least inhibition of PTP-1B with IC_{50} more than or near to 100 μM. RK-682 and ursolic acid (IC_{50} values of 4.1 and 3.5 μM, respectively) were used as positive controls as phosphatase inhibitors.

Table 3: Inhibitory effects of different crude fractions on PTP-1B inhibition assay

Samples (n = 3)	PTP-1B
C. anthelminticum leaves	64±5.8
C. anthelminticum seeds	38±5.8
C. quadrangularis leaves	>100
C. quadrangularis stems	88±5.8
W. fruticosa leaves	>100
W. fruticosa flowers	53±5.8
T. arjuna leaves	>100
T. arjuna barks	100
T. bellerica leaves	>100
T. bellerica fruits	93±5.8
T. chebula leaves	>100
T. chebula fruits	66±5.8
RK-682	4.1±0.6
Ursolic Acid	3.5±0.5

PTP-2B: Protein tyrosine phosphatase inhibitory effects (μM), IC_{50} (Maximum inhibitory concentration up to 50%) demonstrating low value indicates high PTP-1B inhibition effects

Protein Tyrosine Phosphatase (PTPs) are enzymes that catalyze the dephosphorylation of phosphotyrosine (Cheng *et al*., 2006). More than a hundred PTPs exist in humans which may act either as negative or positive modulators in various signal transduction pathways, including insulin and leptin signaling (Saltiel and Pessin, 2002; Han *et al*., 2005). The inhibition of targeted PTP-1B generates potentials for developing plant based medicines by combating dephosphorylation of PTPs, which localized to the cytoplasmic face of the endoplasmic reticulum and expressed ubiquitously, including the classical insulin-targeted tissues such as liver, muscle and fat (Cebula *et al*., 1997; Chen *et al*., 2002).

Antidiabetic activity of crude fractions in diabetic rats: Plant based supplements or certain nutritional functional foods, taken concurrently with oral anti-diabetic agents or insulin therapy may be helpful in controlling postprandial hyperglycemia and could be one of the beneficial therapies in the management of type 2 diabetes mellitus. Postprandial hyperglycemia displays crucial role in the generation of type 2 diabetic complications such as micro- or macro-vascular disorders and participate in the development of Cardio Vascular Diseases (CVD) (Li *et al*., 2005). Initial investigation of postprandial hyperglycemia and its control may offer

Table 4: Effect of different crude fractions on the blood glucose level of diabetic rats

	Non-fasting blood glucose level (mmol L^{-1})				
	Pretreatment period (day)				
Group	0	1	2	3	4
Normal control	4.30±0.44	5.10±0.46	4.70±0.89	05.5±1.09	05.9±0.53
Diabetic control	9.60±0.68[a]	10.1±1.54[a]	9.80±0.64[a]	11.3±1.14[a]	10.9±1.32[a]
Glibenclamide	10.5±0.89	6.80±0.31[b]	7.20±0.53[b]	05.9±0.72[b]	05.6±0.63[b]
C. anthelminticum leaves	11.6±0.76	10.7±1.01	9.20±0.55[b]	08.9±0.73[b]	08.2±0.61[b]
C. anthelminticum seeds	11.9±0.54	7.90±0.48[b]	7.10±0.23[b]	06.3±0.92[b]	05.9±0.65[b]
C. quadrangularis leaves	10.3±1.01	10.9±0.32	9.90±0.76	09.2±0.39	09.6±0.85
C. quadrangularis stems	10.9±0.71	9.60±0.66	8.80±0.36[b]	08.9±0.98[b]	09.2±0.78
W. fruticosa leaves	11.8±0.57	10.7±0.66	9.90±0.47	10.3±0.71	09.4±0.76[b]
W. fruticosa flowers	11.2±0.39	10.8±0.71	9.60±0.79	09.3±0.21[b]	08.7±0.81[b]
T. arjuna leaves	10.7± 0.56	10.1±0.87	11.5±0.79	10.2±0.91	09.4±0.67
T. arjuna barks	12.0±0.56	11.7±0.97	10.5±1.12	09.9±0.27[b]	10.2±0.42
T. bellerica leaves	9.90±0.49	10.7±0.88	10.2±0.42	09.8±0.68	10.3±0.98
T. bellerica fruits	10.5±0.23	9.70±0.64	9.30±0.80	09.6±0.65	08.9±0.89
T. chebula leaves	11.8±1.11	10.9±0.75	10.10±0.61	09.4±0.37	09.5±0.76
T. chebula fruits	10.9±0.66	9.50±0.76	9.10±0.91	08.5± 0.81[b]	08.2±0.59[b]

[a]Represents statistical significance compared to normal control ($p<0.05$), [b]Represents statistical significance compared to diabetic control ($p<0.05$), Each value represent Means±SD n = 6

the potential for early intervention and prevention of diabetic complications (Ratner, 2001).

In order to determine a scientific basis for the utilization of different fractions in the treatment of diabetes, we investigated anti-diabetic effects of the crude fractions on diabetic rats. Amongst the tested crude fractions at 200 mg kg^{-1} dose, *C. anthelminticum* seeds demonstrated significant reduction in blood glucose levels (7.9±0.48, 7.1±0.23, 6.3±0.92 and 5.9±0.65 mmol L^{-1}) at 1, 2, 3 and 4th day of oral administration, followed by *C. quadrangularis* stems (9.6±0.66, 8.8±0.36, 8.9±0.98 and 9.2±0.78 mmol L^{-1}), *W. fruticosa* flowers (10.8±0.71, 9.6±0.79, 9.3±0.21 and 8.7±0.81 mmol L^{-1}), *T. chebula* fruits (9.5±0.76, 9.1±0.91, 8.5±0.81 and 8.2±0.59 mmol L^{-1}). Positive control glibenclamide showed maximum reduction (6.8±0.31, 7.2±0.53, 5.9±0.72 and 5.6±0.63 mmol L^{-1}) at 50 mg kg^{-1} dose whereas, other fractions showed non-significant reduction in blood glucose levels Table 4. It is interesting to note that *T. chebula* fruits only showed significant result on day 3 and day 4, whereas *C. anthelminticum* seeds displayed consistent therapeutic effect from day 1 to day 4. We previously reported certain poly-phenolic principles, namely quercetin glycoside, 3,4-0-dicaffeoylquinic acid, caffeic acid, naringenin-7-0-glucoside and kaempferol as major compounds in the seeds of *C. anthelminticum* (Arya *et al*., 2012a). Of note, quercetin glycoside has been shown to exert antidiabetic activity by stimulating the insulin-independent AMP-activated protein kinase (AMPK) pathway (Eid *et al*., 2010). The fruits of *T. chebula* also contained various bioactive phytoconstituents such as chebulanin, chebulagic acid and chebulinic acid, which showed potent α-glucosidase inhibitory activity (Gao *et al*., 2008). Thus, we speculate that the mixture of these compounds in the plant extracts may be responsible for the healing effect observed in diabetic rat model at different doses.

PTP-1B is a central switch in controlling insulin signalling and adipogenesis (Muthusamy *et al*., 2010). Our study showed *C. anthelminticum* seed exhibited highest effect in decreasing elevated blood glucose levels in diabetic rats probably due to its maximum PTP-1B inhibitory effects. Moreover, *C. anthelminticum* seeds are rich in flavonoid and phenolic contents, which involved in the healing process of chronic inflammatory diseases, including diabetes (Arya *et al*., 2012d). On the other hand, the hypoglycemic effect of *T. chebula* fruits may be due to its highest antioxidant property as recent study suggests that most diabetic complications could be mediated through oxidative stress (Arya *et al*., 2012c). Production of ROS and its related oxidative stress was reported as the root cause for the development of insulin resistance, β-cell dysfunction, impaired glucose tolerance and type 2 diabetes mellitus (Wright *et al*., 2006).

CONCLUSION

The present study indicates that crude methanolic fraction from the seeds of *C. anthelminticum* possesses potent antioxidant and antidiabetic effects, which correlates with higher phenolic and flavonoidal contents. On the other hand, fruits of *T. chebula* showed highest antioxidant effects among all the tested fractions. Therefore, our findings suggest the use of *C. anthelminticum* seeds and *T. chebula* fruit as potential nutraceuticals in reducing oxidative stress

related diabetic complications. Future study on the *C. anthelminticum* seed and *T. chebula* fruits crude fraction will pave the way to yield new lead molecules on the management of oxidative stress generated diabetes and its associated complications.

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