

In Vitro Antioxidant Potential and Type II Diabetes Related Enzyme Inhibition Properties of Traditionally Processed Legume-based Food and Medicinal Recipes in Indian Himalayas

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ABSTRACT

In vitro antioxidant potential of methanolic extracts of six legume-based traditional plant recipes used in Indian Himalayas were evaluated by trolox equivalent antioxidant capacity (TEAC), scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide radical, hydrogen peroxide, ferric reducing antioxidant power (FRAP) and inhibition of β -carotene degradation activity (IBDA). Type II diabetes-related enzyme inhibition capacity of recipes was assayed on α -amylase and α -glucosidase activity under *in vitro* assay. The methanolic extracts of six recipes showed substantially high total phenolic and flavonoid content along with TEAC, radical scavenging activities, FRAP and IBDA in significantly different magnitudes. Apart from high magnitude of antioxidant potential, the three recipes namely 'methi paste', 'arhar dal' and 'gheh simi' used during diabetes by local people exhibited moderate to high level of enzyme inhibition capacity. Present results suggest that methanolic extracts of six indigenous recipes are rich in polyphenols and antioxidant activity. The three medicinal preparations have high potential to inhibit type II diabetes-related enzyme activity, and may be integrated into dietary management of type II diabetes.

INTRODUCTION

Various experimental and pharmacological studies suggest that consumption of foods rich in antioxidant polyphenols is significantly associated with reduced risk of various non-communicable human diseases, including diabetes (Arts *et al.*, 2005; Kumari *et al.*, 2011). Legumes such as beans, peas and lentils are highly rich in bioactive compounds, and different polyphenols have high antioxidant potential, hindering the formation of free radicals, chelating catalytic metals and scavenging of reactive oxygen species in biological systems (Graham and Vance, 2003; Hooda and Pal, 2012; Talukdar, 2012a). In recent years, management of type II diabetes through dietary practices has become more prevalent. An ideal anti-diabetic compound should possess both hypoglycemic and antioxidant properties, without any adverse side effects. Growing evidences indicate that plant polyphenols can inhibit carbohydrate hydrolyzing enzymes such as α -amylase and α -glucosidase, which is extremely important in lowering postprandial hyperglycemia (Arts *et al.*, 2005). Recently, stress markers of this disease have been

identified in certain Indian Himalayan population (Bhutia *et al.*, 2011). Accumulating reports suggest that besides abnormal rise in blood glucose concentrations, reduction in plasma antioxidant level is another risk of type II diabetes (Chhetri *et al.*, 2005). Therefore, search for "hypoglycemic foods," showing α -amylase and α -glucosidase inhibition activities are gradually gaining momentum. Although synthetic drugs such as acarbose, metformin are in use as enzyme inhibitors for clinical treatment of type II diabetes, these drugs are not without side effects (Arts *et al.*, 2005). Hence, at present, there is an increasing demand among food scientists to find alternative natural sources of α -amylase and α -glucosidase inhibitors with potential antioxidant activity-without any side effects-to manage the diet-linked challenges of type II diabetes. Many people throughout the world use plants as safe and alternative medicine for their everyday health care needs (Talukdar, 2011c; Kumari *et al.*, 2011; Talukdar and Talukdar, 2012a). However, scientific evaluation of their functionality is necessary to develop them as effective phytochemicals. Legumes constitute an important part in hill-based traditional and indigenous formulations of plant natural products for food and medicinal purposes in different parts

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of the Himalayas (Chhetri *et al.*, 2005; Kumari *et al.*, 2011; Talukdar and Talukdar, 2012a,b). The study revealed that various legume recipes/preparations are being used regularly in this region in treating chronic diseases such as jaundice, gastrointestinal diseases and diabetes (Kumari *et al.*, 2011; Talukdar and Talukdar, 2012a). However, reports regarding antioxidant potential and diabetes-linked enzyme inhibition activity of these recipes are not available. The objective of the present study was, therefore, framed to analyze the total free phenolic content, total flavonoids, antioxidant activity (TEAC, free radical scavenging capacity, reducing power and inhibition of β -carotene oxidation) and type II diabetes-related enzyme inhibition properties in the methanolic extract of six traditional recipes of food and medicinal items used extensively in Indian Himalayas. Obtained results may be helpful to formulate polyphenol rich legume-based therapeutic foods with pharmacological importance.

MATERIALS AND METHODS

Chemicals

Acarbose, ascorbic acid (AsA), 2,2'-azino-bis(3-ethylbenzthiozoline-6-sulfonic acid (ABTS), β carotene (type I, synthetic), butylated hydroxytoluene (BHT), DPPH, dinitrosalicylic acid, nitroblue tetrazolium salt (NBT), Gallic acid (GAE), Porcine α -amylase, *p*-nitrophenyl- α -D-glucopyranoside, Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) deoxyribose, and yeast α -glucosidase were purchased from Sigma-Aldrich (Bangalore, India). All other chemicals were of analytical grade.

Traditional food and medicinal recipes

Samples of six traditional legume-based food and medicinal recipes namely 'kokti (sprouts of beans and peas as a mixed food preparation), 'panhelo dal' (mixed food preparations of lentil, pea, and mung bean directly cooked at 70-80 °C for 1 h), 'seto maysum dal' (rice bean cooked with peas and urd bean at 90-95 °C for 1 h) 'ghew simi' (tender pods/seeds of lima beans cooked with cabbage, spinach and radish at 70-80 °C for 30-40 min as medicinal item), 'arhar dal' (pigeon pea seeds cooked at 90-95 °C for 30 min to make pulse meal as medicinal) and 'methi paste' (dried raw seed powder, medicinal) were collected (Talukdar and Talukdar, 2012a), frozen at -80 °C, freeze-dried and finely powdered. The collection was carried out in diverse altitudes (900-2400 m, amsl) of Eastern Indian Himalayas during 2009-2011 on traditional legumes grown and used by local ethnic tribes in one of the richest biodiversity sites in The Himalayas (Talukdar and Talukdar, 2012a).

Preparation of methanolic extracts

One gram of dried flour of each recipe was treated with petroleum ether (1:10, w/v) overnight on a magnetic stirrer, centrifuged at $2,500 \times g$ for 20 min; the supernatant was then discarded. The defatted residue was then air-dried and extracted exhaustively with 50 ml of chilled aqueous methanol (methanol:

water, 80:20 v/v) at the ratio of 1:10 (w/v) for 2 h at room temperature. The samples were centrifuged at $3,000 \times g$ for 20 min and the supernatant was removed. Extraction was repeated twice and supernatants were pooled and evaporated using a rotary vacuum evaporator at 40 °C and freeze-dried in a lyophilizer for 1 h. The phenolic concentrate was made to a final volume of 10 ml with distilled water and stored at -80 °C until analysis.

Total phenolic (TP) content

TP content of methanolic extracts were estimated following earlier method with minor modifications (Singleton *et al.*, 1999). Briefly, the extract (500 μ l) was mixed with 500 μ l of freshly prepared Folin-Ciocalteu reagent and 6 ml of distilled water. After 5 min of incubation period, 2 ml of 15% sodium carbonate was added, shaken for 30 sec, and was then brought up to a volume of 10 ml with distilled water. After 2 h of incubation at 37 °C in the dark, the absorbance was measured at 750 nm in a UV-visible spectrophotometer (Perkin-Elmer, Lambda 35). TP content was calculated and expressed as Gallic acid (GAE) equivalent/ g extract on a dry weight basis (dwb).

Total flavonoid (TF) content

TF was estimated spectrophotometrically using the earlier method based on the formation of a flavonoid-aluminium complex with some modifications (Zhishen *et al.*, 1999). An amount of 2 % ethanolic $AlCl_3$ (aluminium chloride) solution (0.5 ml) was added to 0.5 ml of sample. After 45 min incubation at room temperature, the absorbance of the reaction mixture was measured at 420 nm. TF content was calculated and expressed as equivalent to catechin (CAE) in mg/ g of the extracts dwb.

Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay was performed on the reduction of the ABTS radical cation by antioxidants with minor modifications (Re *et al.*, 1999). ABTS stock solution (7 mM in water) was mixed with 2.5 mM potassium persulfate, and was left for 12-24 h in the dark until the reaction was complete and the absorbance at 734 nm was stable. Prior to use, the $ABTS^{+}$ solution was diluted with phosphate buffered saline (PBS) to an absorbance of 0.70 (± 0.04) at 734 nm and equilibrated at 25 °C. Sample extract, AsA (positive control) and Trolox (standard) (20 μ l) were dissolved in PBS, and then added to the $ABTS^{+}$ solution (1.98 ml). The antioxidant capacity of the samples was calculated by determining the decrease in absorbance (taken exactly after 6 min of initial mixing) at different concentrations (0-150 μ g/ml) with solvent blanks.

DPPH radical scavenging activity

The methanolic extract (100 μ l, 1 mg/ml) was added to 3.9 ml of DPPH solution (0.025 g/l) and the reactants were incubated at 25 °C for 30 min. Instead of extract, a positive control of AsA was used. The mixture was shaken and allowed to stand in the dark at room temperature for 35 min. Free radical scavenging activity was calculated from absorbance values at 517 nm

(Wettasinghe and Shahidi, 2000) and expressed as inhibition percentage.

Superoxide radical scavenging activity (SRSA)

The light-induced reduction of nitro-blue tetrazolium (NBT) by superoxide radicals was used for the assay (Zhishen *et al.*, 1999). The reactants comprising riboflavin, methionine and NBT were illuminated at 25 °C for 25 min to generate superoxide radicals by photochemically reduced riboflavin and to reduce the NBT by superoxide radicals to form a blue formazan. The methanolic sample extract (100 µl, 1 mg/ml) was added to the reaction mixture, in which superoxide radicals are scavenged, thereby inhibiting the NBT reduction. Using un-illuminated reaction mixture as a blank, the absorbance was measured at 560 nm, and SRSA % was calculated with AsA as positive control.

Hydrogen peroxide scavenging activity

One milliliter of extract (250 µg/ml) was mixed with 2.4 ml of 0.1 M phosphate buffer (pH 7.4), and then 0.6 ml of a 43 mM solution of H₂O₂ in the same buffer were added (Sowndhararajan *et al.*, 2010). After 40 min, the absorbance of reaction mixture was taken at 230 nm against a blank solution (phosphate buffer without H₂O₂). Percentage scavenging of H₂O₂ was calculated with AsA as control.

Ferric reducing antioxidant power (FRAP)

A working FRAP reagent consisting of 300 mmol/l acetate buffer (pH 3.6), 10 mmol/l 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mmol/l HCL and 20 mmol/l ferric chloride (FeCl₃.6H₂O) was freshly prepared, and 3.0 ml of which was mixed with 100 ml of appropriately diluted samples of methanolic extracts (Benzie and Strain, 1999). After 10 min incubation at 37 °C, the absorbance was recorded at 593 nm. The absorbance changes in the test mixture were compared to those obtained from standard mixture of ferrous sulphate. FRAP value was expressed as µmol of Fe²⁺/g dwb.

β-Carotene-linoleic acid test

For β-carotene-linolate bleaching assay, 10 mg of β carotene was dissolved in 10 ml of chloroform and 3 ml of it was added to 20 µl of linoleic acid and 200 µl Tween ® 40 (Boateng *et al.*, 2008). After removing chloroform under reduced pressure, 100 ml of oxygenated distilled water was added slowly and mixed properly to obtain a stable emulsion. A portion (3 ml) of emulsion were added to mix with 40 µl of sample and incubated for 1 hr at 50 °C. The absorbance was recorded at 0 and after 60 min of incubation at 470 nm against a blank (emulsion without β-carotene). Inhibition% of β-carotene oxidation was calculated with BHT as reference.

α-Amylase inhibition assay

To 100 µl of 0.02 M Na-phosphate buffer (pH 6.9) and 100 µl of porcine α-amylase solution (4.5 Units/ml/min) sample extract (100 µl, 1 mg/ml) was added and pre-incubated at

25 °C for 10 min (Worthington, 1993). After addition of 1% starch solution, the reaction mixture was incubated for 30 min. The reaction was stopped with 1.0 ml of dinitrosalicylic acid. The test tubes were then incubated in a boiling water bath for 5 min, cooled to 25 °C, diluted and the absorbance was taken at 540 nm with the control (buffer, no extract). Percent of α-amylase enzyme inhibition was calculated.

α-Glucosidase Inhibition Assay

Methanolic extract (100 µl, 1 mg/ml) was mixed with 100 µl of 0.1 M phosphate buffer (pH 6.9) and 100 µl of yeast α-glucosidase solution (1 Unit/ml/min) and pre-incubated at 25 °C for 5 min (Worthington, 1993). Then, 100 µl of p-nitrophenyl-α-D-glucopyranoside was added and the reaction mixture was incubated at 25 °C for 10 min. After the incubation, the absorbance were taken at 405 nm and allegorized to a control (100 µl of buffer instead of the extract). The results were expressed on a percent basis. Synthetic anti-diabetic drug acarbose (1mg/ml) was used as positive control for both α-amylase and α-glucosidase activity inhibition bioassay.

Statistical analysis

All data were expressed as means ± standard errors of five replicates. Percent scavenging activity was estimated by: % scavenging = [(A₀ - A_s)/A₀] × 100, where A₀ is absorption of control, A_s is absorption of tested extract solution. One-way ANOVA with Duncan's Multiple Range Test was performed using the SPSS v.10 statistical software (SPS Inc., USA) for multiple comparison and separation of means. Correlation analysis was carried out using 'Microsoft data analysis tool pack v. 2007' (Roselle, IL, USA) with significance levels at P < 0.05.

Results and Discussion

Six legume-based food and medicinal preparations were selected in the present study based on their extensive use by local people (Talukdar and Talukdar, 2012a). Among these, 'methi paste', 'ghew simi' and 'arhar dal' have been used as traditional medicine during diabetes, while others three are used as food items. The 'kokti' which is traditionally taken during auspicious festivals and the 'panhelo dal' which is taken as regular nutritious meal has tremendous food value owing to significantly high value of their phenolic and flavonoid contents (Fig. 1). The values are considerably higher compared to the earlier report in raw leguminous seeds (Gharachorloo *et al.*, 2012; Talukdar, 2012a) and supported the beneficial effect of sprouting and direct cooking to enhance polyphenols in comparison to raw seeds (Vadivel *et al.*, 2011). Besides more of preparations, the variation in TP and TF contents among six items might be due to genotypes and agronomic practice in determining the seed polyphenol content of beans and peas (Talukdar, 2009a, b; Xu and Chang, 2010). TP and TF levels in the 'methi paste' and 'arhar dal' were higher than 'seto maysum dal' and 'ghew simi' but lower than 'kokti' and 'panhelo dal' (Fig. 1). Among the medicinal items, 'arhar dal' and 'methi paste' were purely composed of legumes while 'ghew simi'

was prepared with beans mixed with leafy vegetables (spinach, cabbage and radish). High TF content in 'ghew simi' strongly favored the idea that mixing of green leafy vegetables with legume-based preparation significantly enhances antioxidant capacity of cooked item (Gupta and Prakash, 2009). Present results suggest that the six recipes, including three medicinal items are rich in polyphenols.

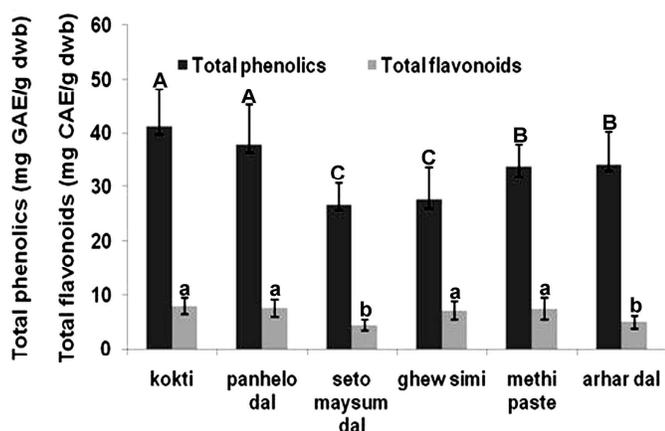


Fig. 1: Total phenolics and flavonoids in methanolic extract of six traditionally prepared food (kokti, panhelo dal, seto maysum dal) and medicinal items (ghew simi, methi paste, arhar dal). Values are means and \pm standard error of five independent determinations ($n = 5$). Bars with different letters denote significant differences ($P < 0.05$) among the items by Duncan's Multiple Range Test.

TEAC (mM TE/g) indicates the relative ability of hydrogen or electron-donating antioxidants to scavenge the ABTS radical cation compared with that of Trolox. In the present study, all the six preparations exhibited radical scavenging activity in the range between 4.23 ('Kokti') and 2.05 ('seto maysum dal'). Results in fig 2 also indicated that the scavenging ability of 'kokti' and 'panhelo dal' was higher than that of AsA, while that of 'methi paste', 'ghew simi' and 'arhar dal' was comparable to AsA. Occurrence of high TEAC in these traditional recipes might be due to high antioxidant capacity of their ingredient beans and peas and/or preparation procedures (Djuric and Powell, 2001; Lobo *et al.*, 2010). Similar evidence of radical scavenging capacity determined by TEAC assay was also reported in different prepared food items, herbal and medicinal formulations (Djuric and Powell, 2001; Lobo *et al.*, 2010).

The free radical scavenging capacity by methanolic extracts of six items was presented in fig 2. The results indicated that DPPH free radical scavenging capacity was the highest in 'kokti' (95.43%), closely followed by 'panhelo dal' (92.28%) 'arhar dal' (89.67%) and 'ghew simi' (90.11%). The values were higher than positive control AsA (70.33%, not in figure) and previous reports on *Phaseolus vulgaris* (Boateng *et al.*, 2008) *Cassia hirsuta* (64.40%) (Vadivel *et al.*, 2011), mung bean (24.9%) (Kim *et al.*, 2012) and *Vigna aconitifolia* (Siddhuraju, 2006). 'Methi paste' and 'seto maysum dal' showed 65.34% and 57.67% activity, respectively (Fig. 2).

The SRSA values among the six recipes varied between 93.34% (highest in 'ghew simi') and 55.23% (lowest in 'seto

maysum dal'). The highest value was immediately followed by 'kokti' 'arhar dal' and 'panhelo dal', exhibiting 90.78%, 87.65% and 86.54% activity, respectively (Fig. 2). The values were higher than that of AsA (50.65%, not in figure) as well as earlier reports on moth bean (Siddhuraju, 2006) and cowpea (Siddhuraju and Becker, 2007).

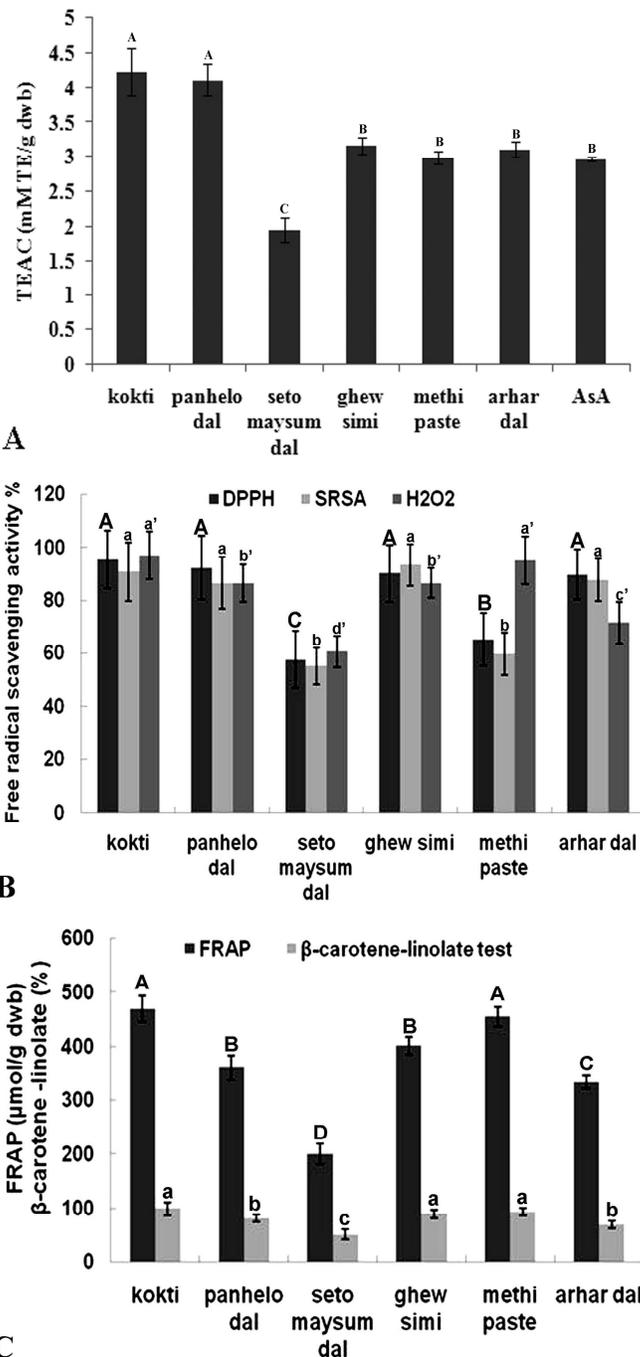


Fig. 2: TEAC values (A), DPPH scavenging, superoxide radical scavenging activity (SRSA), H₂O₂-scavenging (B), and FRAP (μ mol/g dwb) and β -carotene oxidation inhibition (C) in methanolic extract of six traditionally prepared food (kokti, panhelo dal, seto maysum dal) and medicinal items (ghew simi, methi paste, arhar dal). Values are means \pm standard error of five independent determinations ($n = 5$). Bars with different letters denote significant differences ($P < 0.05$) among the items by Duncan's Multiple Range Test.

For H₂O₂-scavenging capacity, 'methi paste' was the best with 97.13 % scavenging activity (Fig. 2) closely followed by 'kokti' (95.11%), 'ghew simi' (86.56%), and 'panhelo dal' (86.44%). Low to moderate scavenging power was observed in 'arhar dal' (71.56%) and 'seto maysum dal' (60.65%), comparable to AsA (43.22%, not in figure). Since phenolic compounds present in the extract are good electron donors, they may accelerate the conversion of H₂O₂–H₂O. As a diffusible free radical, dual roles of H₂O₂ in stress signaling and induction of oxidative stress have been revealed in human (Arts *et al.*, 2005) and in plants experiencing stresses (Talukdar, 2011a, c, 2012b,c; Zia-Ul-Haq *et al.*, 2012). Increasing oxidative damage due to excess free radicals appears to be a feature of most human diseases (Arts *et al.*, 2005), the risk of which is increasing due to wide spread metal contamination of human foods (Talukdar, 2012d, 2013a, b). Therefore, dietary antioxidants from fresh legumes may be supplemented against free radical damage of cellular DNA, lipids and proteins.

The antioxidant effect exponentially increases as a function of development of the reducing power (Benzie and Strain, 1999). The FRAP (μmol/g, dwb) was the highest in 'kokti' (468.67) followed by medicinal items 'methi paste' (453.89), 'ghew simi' (400.56), 'panhelo dal' (359.61) and 'arhar dal' (332.76) (Fig. 2). The values were markedly higher than legumes, cereals and millets studied (Zia-Ul-Haq *et al.*, 2012; Talukdar, 2013a, b). The food item 'seto maysum dal' exhibited low to moderate levels of FRAP (Fig. 2).

The inhibition of β-carotene degradation activity (IBDA) was ranged between 98.65% (highest 'kokti') and 50.54% (lowest, seto maysum dal) with 'methi paste' 'ghew simi' and 'panhelo dal' in between 80% and 90% (Fig. 2), indicating significantly higher capacity than reference standard BHT value (52.11%, not in figure). The values were also considerably higher than *Lathyrus filiformis* (28%) (Pastor-Cavada *et al.*, 2009) and large black soybeans (25%), (Takahashi *et al.*, 2005) but was close to that of leafy vegetables (Gupta and Prakash, 2009). Compared with BHT, lower values for β-carotene assay were reported in methanolic extracts of pea, lentil and beans pods and leaves (Zia-Ul-Haq *et al.*, 2012), indicating superiority of present legume recipes to individual legume genotypes for inhibition of β-carotene oxidation.

Methanolic extracts of 'methi paste' and 'ghew simi' exhibited moderate levels of both α-amylase and α-glucosidase enzyme inhibition capacity, comparable to synthetic anti-diabetic drug acarbose (Fig. 3). Very low potential, however, was estimated in 'kokti', 'panhelo dal' and 'seto maysum dal'. Seeds of 'methi' (*Trigonella foenum-graecum* L.) have high potential to lower high blood sugar and bad cholesterol levels (Talukdar, 2011a). Raw seeds of beans alone may not be suitable for therapeutic use due to very low α-amylase but abnormally high α-glucosidase inhibition activity. Similarly, raw seeds of *Cajanus cajan* contained very high α-amylase (75.67%) and α-glucosidase (88.54%) inhibition activity (D Talukdar, unpublished). In the present preparations, the hypoglycemic effect of 'ghew simi'

presumably depends on the capacity of both beans and green vegetables cooked as mixed diet in inhibition of both enzymes, and the levels were comparable to acarbose. Similarly, in 'arhar dal' (directly cooked pigeon pea, *Cajanus cajan* seeds), α-amylase and α-glucosidase enzyme inhibition levels were assayed as 37.61% and 61.48%, respectively. Although, α-glucosidase enzyme inhibition level was still higher than that of acarbose (43.34%), α-amylase inhibition activity was very close to acarbose (Fig. 3). Quite remarkably, the α-amylase and α-glucosidase inhibition levels in these three recipes were adjusted to the levels which are comparable to those of the synthetic anti-diabetic drug acarbose. As high enzyme inhibition seems to be unsuitable for dietary integration of type II diabetes patients due to abnormal bacterial fermentation of undigested carbohydrate in the human colon (Kim *et al.*, 2011), moderate level of inhibition capacity observed in the present case has the potential to integrate into dietary items of type II diabetic patients as natural substitute of synthetic drug. Obviously, mode of preparation holds the key for effective pharmacological use of medicinal plants in particular disease.

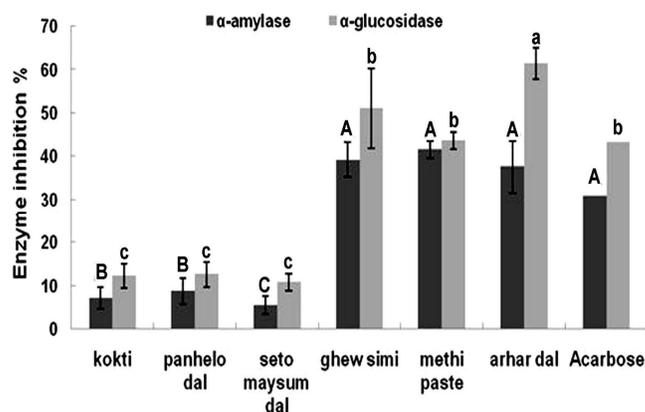


Fig. 3: α-amylase and α-glucosidase inhibition activities (%) of methanolic extract of six traditionally prepared food (kokti, panhelo dal, seto maysum dal) and medicinal items (ghew simi, methi paste, arhar dal) with acarbose as standard. Values are means ± standard error of five independent determinations (n = 5). Bars with different letters denote significant differences (P < 0.05) among the items by Duncan's Multiple Range Test.

Polyphenol content (TP + TF) in all six recipes was significantly (P < 0.05) correlated with TEAC, DPPH, SRSA, and H₂O₂-scavenging capacity and FRAC and IBDA (Table 1). However, relationship between polyphenols and FRAP and IBDA was rather weak in three food items than medicinal items. The association between polyphenol level and enzyme inhibition activity in 'kokti', 'panhelo dal' and 'seto maysum dal' was significantly negative due to their high polyphenol content but low level of enzyme inhibition capacity, but strongly positive for 'methi paste', 'ghew simi' and 'arhar dal' (Table 1). This indicates significant contribution of polyphenols in conferring enzyme inhibition capacity in these three indigenous recipes, traditionally used by hill folks suffering in diabetes (Talukdar and Talukdar, 2012a), and can be ideal hypoglycemic formulations in pharmacological use against high diabetes.

Table. 1: Correlations of total polyphenol content (TP + TF) with TEAC, DPPH, SRSA, H₂O₂-scavenging capacity, FRAP and IBDA, and inhibition of α -amylase and α -glucosidase enzymes in methanolic extracts of six recipes using Pearson correlation coefficient (r), n = 10.

Recipes	TEAC	DPPH	SRSA	H ₂ O ₂	FRAP	IBDA	α -amylase	α -glucosidase
Kokti	0.775 ^b	0.818 ^b	0.867 ^c	0.906 ^c	0.615 ^a	0.637 ^a	-0.811 ^b	-0.895 ^c
Panhelo dal	0.745 ^b	0.984 ^c	0.699 ^b	0.723 ^b	0.696 ^a	0.648 ^a	-0.786 ^b	-0.737 ^b
Seto maysum dal	0.765 ^b	0.734 ^b	0.831 ^b	0.819 ^b	0.591	0.475	-0.851 ^c	-0.779 ^b
Methi paste	0.878 ^c	0.913 ^c	0.932 ^c	0.885 ^c	0.754 ^b	0.703 ^b	0.934 ^a	0.985 ^a
Ghew simi	0.698 ^b	0.737 ^b	0.787 ^b	0.864 ^c	0.698 ^a	0.705 ^b	0.956 ^c	0.923 ^c
Arhar dal	0.876 ^c	0.945 ^c	0.769 ^b	0.823 ^b	0.734 ^b	0.679 ^a	0.894 ^c	0.812 ^b

TEAC-Trolox equivalent antioxidant capacity, DPPH-1,1-diphenyl-2-picrylhydrazyl, SRSA-superoxide radical scavenging activity, H₂O₂-hydrogen peroxide scavenging, FRAP-ferric reducing antioxidant power, IBDA-inhibition of β -carotene degradation, a,b,c in superscript indicate significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, n = 10

CONCLUSION

For the first time, antioxidant potential and type II diabetes-related enzyme inhibition capacity was tested in six popular legume-based recipes of Indian Himalayan region. Methanolic extract of all the six preparations contained moderate to high phenolics and flavonoid content with richest natural antioxidants were found in 'kokti', followed by 'panhelo dal, ghew simi', 'methi paste' and 'arhar dal'. Moderate to high level of TEAC, radical scavenging capacity, FRAP and inhibition capacity of β -carotene degradation in these four preparations and their significant correlations with TP + TF content indicated positive role of polyphenols in conferring protection against different radicals and development of reducing power.

Present results also indicated high potential of mixed sprouts ('kokti') and directly cooked ('panhelo dal', 'arhar dal', 'ghew simi') traditional food preparations to be developed as functional foods. The effectiveness of methanol extract of 'methi paste, 'ghew simi' and 'arhar dal' against type II diabetes was established by balanced changes in both α -amylase and α -glucosidase inhibition capacity and their significantly positive association with polyphenol levels.

Present results revealed potential of plant-based medicinal formulations instead of synthetic agent in management of type II diabetes and supported traditional wisdoms with scientific results towards development of functional and therapeutic foods for effective pharmacological use in diabetes and diabetes-related disease after proper clinical trial.

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