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In vitro anti-diabetic activity of various indigenous medicinal plants of Junagadh region (Gujarat, India)

Harshad B Patel, Urvesh D Patel, Chirag M Modi, Punit R Bhatt and Kajal B Pandya

Abstract
An *in vitro* anti-diabetic activity of various indigenous medicinal plants was evaluated by α-amylase and α-glucosidase inhibition assay methods. Various extracts like chloroform, methanol and water extracts have been prepared by simple maceration of defatted plant powder using n-hexane. Qualitative phytochemical analysis was performed to evaluate the presence of active ingredients like flavonoids, alkaloids, glycosides, saponins, steroids, carbohydrates and tannins. Chloroform extracts of *Gymnema sylvestre* (Retz.) R.Br. ex Sm leaf, *Lepidium sativum* L. seed and *Pueraria tuberosa* (Willd.) DC. Tuber exhibited significantly higher ($p < 0.05$) inhibition of α-amylase activity as compare to standard drug acarbose. Water extracts of *Solanum xanthocarpum* Schrad. & H. Wendl. Leaf and *Centratherum anthelminticum* (L.) Gamble seed shown significantly higher ($p < 0.05$) inhibition of α-glucosidase followed by methanolic extracts of *Pueraria tuberosa* (Willd.) DC. Tuber and *Moringa oleifera* Lam. leaf as compared to acarbose. However, *Solanum xanthocarpum* Schrad. & H. Wendl. Leaf, *Centratherum anthelminticum* (L.) Gamble seed and *Moringa oleifera* Lam. leaf have shown quite lower activity against α-amylase enzyme. In conclusion, these extracts have shown the presence of active phytochemicals viz. flavonoids, alkaloids, glycosides, saponins and tannins, and showed significant *in vitro* anti-diabetic activity by inhibition of α-amylase and α-glucosidase enzyme activity.

Keywords: Anti-diabetic activity, phytochemical screening, α-amylase, α-glucosidase, *in vitro* inhibition assay, medicinal plants

1. Introduction
Diabetes mellitus is a chronic metabolic disease characterized by high blood sugar (glucose) level due to defective insulin production from beta cells of pancreas which is known as insulin-dependent diabetes mellitus (IDDM; type-I), or inappropriate action of insulin known as non insulin-dependent diabetes mellitus" (NIDDM; type-II), or combination of both [1]. Delayed and improper therapeutic regimens of diabetes mellitus results into long standing hyperglycemia which produces many complications viz. hyperglycemia, hyperlipidemia, oxidative stress, diabetic ketoacidosis, nephropathy, neuropathy and cardiovascular disorders [2]. High blood glucose level which generates reactive oxygen species (ROS) which in turn damage the cell membrane and cause oxidative stress, lipid peroxidation and destruction of β-cells [3]. Dietary starch compounds are the important sources of blood glucose, and pancreatic α-amylase and intestinal α-glucosidase are the main enzymes which govern the breakdown of starch and intestinal absorption of glucose, respectively [4]. Drugs have high intestinal α-glucosidases inhibitory activity and moderate action against pancreatic α-amylase maintain optimal blood glucose level after meal and that can be the most beneficial therapy for diabetes mellitus [5].

Indigenous medicinal plants have been identified and used effectively to maintain normal blood glucose level for a long period of time. Different mechanisms of actions that control the normal blood glucose level are mainly as insulin mimetic activity, reduction of insulin resistance and inhibition of α-amylase and α-glucosidase enzymes activity [6]. Both α-amylase and α-glucosidase inhibitors can effectively reduce the glucose absorption. Alpha-amylase inhibitors block the breakdown of long-chain carbohydrates into glucose, whereas, α-glucosidase inhibitors prevents the conversion of starch and disaccharides into glucose [7]. Inhibition of both the enzymes activity has become one of the vital therapeutic approach to retard glucose absorption and suppression of postprandial hyperglycemia [8].

Parenteral administration of insulin and oral use of hypoglycemic drugs like sulphonylureas, biguanides, thiozolidinediones, D-phenylalanine derivatives, meglitinides and α-glucosidase

inhibitors is the routine approach for the treatment of diabetes. However, use of these allopathic drugs for prolong period is associated with side effects like nausea, vomition, flatulence, gastrointestinal disturbance, headache, dizziness, weight gain, lactic acidosis, hyponatremia, pernicious anemia, dyspepsia and joint pain [9]. Herbal drugs are economic and possesses no or less adverse effects as compare to allopathic anti-hyperglycemic drugs, hence they are quite popular in developing countries [10]. Many medicinal plants are being consumed or used or suggested in Ayurveda for the treatment of diabetes like *Eugenia jambolana, Gymnema sylvestre, Momrodica charantia, Ocimum sanctum, Phyllanthus amarus, Pterocarpus marsupium, Trigonella foenum graecum* and *Tinospora cordifolia* [11]. However, *in vitro* and *in vivo* scientific validation of ethno medicinal plants is required for their further clinical use in diabetes.

A large number of medicinal plants are growing surrounding Junagadh area (Gujarat, India), and many of them might have an anti-diabetic potential. Hence, the present research work was planned to explore *in vitro* anti-diabetic activity of fifteen medicinal plants by α-amylase and α-glucosidase inhibition assays.

2. Materials and methods

2.1 Chemicals and reagents

Chloroform, methanol, dinitrosalicylic acid, sodium potassium tartrate were obtained from S. D. Fine Chem. Ltd, Mumbai, India. α-amylase, starch and p-Nitrophenyl α-D-Glucoside (pNPG) were purchased from Himedia Laboratories Pvt. Ltd, Mumbai, India. α-glucosidase and acarbose were procured from Sigma Aldrich, Bangalore, India. Sodium hydroxide, Dimethyl sulfoxide and sodium carbonate were procured from Merck India Ltd., Mumbai, India.

2.2 Collection and processing of plant material

All the plant materials (table 1) were collected from surrounding regions of Junagadh district, Gujarat (India). Collected plant materials were identified and authenticated by Mr. Punit Bhatt, pharmacognosist. A voucher specimen of each plant was deposited in the department. Collected plant materials were washed with tap water followed by shade drying. The material was used to make fine powder and stored in an air-tight container until use.

Table 1: List of selected medicinal plants used for evaluation of *in vitro* anti-diabetic activity

S. No.	Plant species	Family	Local name (Gujarati)	Part of plant used
1	*Cassia absus* L.	Liliaceae	Chimed	seed
2	*Carissa carandas* L.	Apocynaceae	Karamda	stem
3	*Cassia tora* L.	Calsalpiniaceae	Kuvadiyo	root
4	*Centratherum anthelminticum* (L.) Gamble	Asteraceae	Kalijiri	seed
5	*Enicostema littorale* Blume	Gentianaceae	Mamejvo	leaf
6	*Euphorbia nivulia Buch.-Ham.*	Euphorbiaceae	Dandaliyo thor	stem
7	*Ficus racemosa* L.	Moraceae	Umbaro	bark
8	*Gymnema sylvestre* (Retz.) R.Br. ex Sm.	Asclepiadaceae	Madhunashini	leaf
9	*Lepidium sativum* L.	Curciferace	Sheliyo	seed
10	*Moringa oleifera* Lam.	Moringaceae	Saragavo	leaf
11	*Pandanus tectorius* Parkinson ex Du Roi	Pandanaceae	Kevado	leaf
12	*Pueraria tuberosa* (Willd.) DC.	Fabaceae	Fagiyo	tuber
13	*Solanum xanthocarpum* Schrad. & H. Wendl.	Solanaceae	Bhoi-ringani	aerial part
14	*Syzygium cumini* (L.) Skeels	Myrtaceae	Kala jambu	leaf
15	*Tecomella undulata* (Sm.) Seem.	Bignoniaceae	Ragat rohido	bark

2.3 Preparation of extracts

Fine powders of plant material were defatted using n-hexane by soxhlet apparatus to remove chlorophyll and other non-polar debris. Defatted plant material was dried in oven at 45^0C for 1 to 2 hours. Aqueous, methanol and chloroform extracts were prepared by dissolving 50 grams of each powder in 500 mL of each solvent for 48 hours then contents were filtered through Whatmann filter paper no.1, and the filtrate was evaporated under reduced pressure using rotary vacuum evaporator below 50°C. The extracts were collected; yield was calculated and stored at 4°C for further use.

2.4 Phytochemical screening

Qualitative phytochemical screening was performed for each extract as per standard procedures described by Harborne [12].

2.4.1 Tests for alkaloid

Test solution was prepared by trituration of 40 to 50 mg extract with dilute acid (10 % acetic acid or 1 to 5 % hydrochloric acid). After filtration, 0.5 to 1 mL filtrate was added with 1 to 2 mL of following reagents.

Mayer's test

Mayer's reagent (Solution I: Dissolve 1.36 g $HgCl_2$ in 60 mL water; Solution II: Dissolve 5 g potassium iodide in 10 mL water. Combine this two solutions and add water upto 100 mL). Test solution (0.5 to 1 mL) was added with 1 to 2 mL of Mayer's reagent and development of white or buff colour precipitates indicates the presence of alkaloid.

Drangendorff's test

Drangendorff's reagent (Solution I: Dissolve 0.85 g basic bismuth nitrate in 10 mL glacial acetic acid with 40 mL water under heating; Solution II: Dissolve 13.33 g potassium iodide in 30 mL water. Mix the solutions I and II). Test solution (0.5 to 1 mL) was added with 1 to 2 mL of Drangendorff's reagent and development of bright orange red precipitates indicates the presence of alkaloid.

Wagner's test

Wagner's reagent **(**Dissolve 1.27 g sublimed iodine (I_2) and 2 g potassium iodide in 20 mL water, and add water upto 100 mL). Test solution (0.5 to 1 mL) was added with 1 to 2 mL of Wagner's reagent and development of brown precipitates indicates the presence of alkaloid.

Hager's test

Hager's reagent (Dissolve 1.3 g picric acid in distilled water).

Test solution (0.5 to 1 mL) was added with 1 to 2 mL of Hager's reagent and development of yellow precipitates indicates the presence of alkaloid.

2.4.2 Test for flavonoid

Test solution was prepared by dissolution of 50 to 100 mg extract in 10 mL methanol/water.

Shinoda test

Test solution (1 to 2 mL) was added with a pinch of magnesium metal powder and a few drops of concentrated hydrochloric acid. Presence of flavones, flavonols, the corresponding 2, 3-dihydro derivatives, xanthones indicated with development of orange, pink, red to purple colours.

Test solution (1 to 2 mL) was added with zinc metal powder and a few drops of concentrated hydrochloric acid. Presence of flavanonols indicated with development of deep-red to magenta colour and flavanones and flavonols gave weak pink to magenta colour or no colour.

Sulfuric acid test

Test solution (1 to 2 mL) was added with few drops of concentrated sulfuric acid from the side wall of test tubes. Flavones and flavonols dissolve into concentrated H_2SO_4, producing a deep yellow coloured solution. Chalcones and aurones produce red or red-bluish solutions. Flavanones give orange to red colour.

2.4.3 Test for saponin

Take 0.1 to 0.2 g of extract was added in 10 mL distilled water, and shake vigorously. Stabilize frothing for 10 to 15 minutes indicates presence of saponin.

2.4.4 Test for sterol

Salkowaski's test

The crude extract (about 50 to 100 mg) was shaken with 2 mL of chloroform and added with of 2 mL of concentrated H_2SO_4 along the side of the test tube. Development of reddish brown colour at the interface indicates the presence of terpenoid or sterol.

2.4.5 Test for sugars

Molisch's Test

Molisch's reagent (Dissolve 1 g of α- naphthol in 10 mL of methanol or isopropyl alcohol). Test solution (1 to 2 mL) was mixed in a test tube containing 0.5 mL of water, and added with two drops of Molisch's reagent followed by 1 mL of concentrated sulphuric acid from the side of the inclined test tube. Appearance of red brown/violet ring at the interface of acid and aqueous solution indicates the presence of sugars.

2.4.6 Test for tannins

Ferric Chloride Test

Prepare 5 % solution of ferric chloride in 90 % methanol. Test solution (1 to 2 mL) was added with few drops of ferric chloride solution and development of dark green or deep blue colour indicates the presence of tannins.

Lead Acetate Test

Prepare 10 % solution of basic lead acetate in distilled water. Test solution (1 to 2 mL) was added with few drops of lead acetate solution and development of white or buff coloured precipitate indicates the presence of tannins.

2.5 α-amylase inhibition by dinitro salicylic acid (DNS) assay

The stock solution (1 mg/mL) was prepared by dissolving 30 mg extract in 30 mL Milli-Q water. Chloroform extract was dissolved by using 5 to 10 % DMSO as a solvent. Suitable dilutions were made from this stock solution with Milli-Q water only. DNS reagent was prepared as follow; Solution-1: 438 mg DNS in 20 mL distilled water, solution-2: 12 g sodium potassium tartrate in 8 mL 2M NaOH. Heat both the solutions separately and add in to bottle containing 12 mL distilled water.

Five hundred micro liter of test (extract) or standard solution (Acarbose 1 mg/mL in distilled water) was added with 500 µL α-amylase solution (1U/mL in PBS, pH 6.7) and 1 mL starch solution (1% in PBS, pH 6.7). After proper mixing, the reaction mixture was kept in incubator for 10 minutes. After incubation, 1 mL DNS reagent was added in to reaction mixture and immediately put the whole test-tubes in boiling water for 3 minutes. The test tubes were cooled at room temperature and added with 6 mL distilled water. Absorbance of mixture was read at 540 nm in double Beam UV Visible Spectrophotometer (Fusiontek, UV2900). Control solution was prepared by adding all the reagents except test or standard [13].

2.6 α-glucosidase inhibition Assay

One hundred and twenty micro litres of different extract solutions or acarbose dilutions prepared in buffer solution (0.2 M potassium phosphate buffer, pH 6.8) were taken in 96-well plate having a transparent and flat bottom. Twenty microliter of α-glucosidase enzyme solution (1U/mL) was added and incubated for 15 min. After incubation, to initiate the reaction, 20 µL of 5 mM p-Nitrophenyl α-D-Glucoside (pNPG) solution was added and again incubated for 15 min till development of yellow colour in the reaction mixture. Reaction was terminated with addition of 80 µL of 0.1 M Na_2CO_3 solution [14]. Absorbance was read at 405 nm wavelength in UV/Vis micro plate spectrophotometer (Multiskan GO, Thermo Fisher Scientific India Pvt Ltd, India).

2.7 Data and statistical analysis

Anti-diabetic activity of test or plant extracts was measured by calculating % inhibition of enzymatic activity for different range of concentrations with following equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

All the data were presented as means ± standard error (SE) for analysis of each sample in in triplicates. Data were analyzed statistically by one way ANOVA and different group means were compared by Duncan's Multiple Range Tests to observe difference among the treatments ($p<0.05$) [15].

3. Results & Discussion

Qualitative analysis of different phytochemical compounds in various plant extracts is given in table 2. Extracts of *Gymnema sylvestre*, *Lepidium sativum*, *Moringa oleifera* and *Pueraria tuberosa* have shown the presence of various phytochemical ingredients like flavonoids, alkaloids, tannins and glycosides which might be responsible for *in vitro* anti-diabetic effects. Fenercioglu *et al.* [16] found that the plants containing natural antioxidants like tannins, flavonoids,

Vitamin C and Vitamin E can inhibit the lipid peroxidation and generation of diabetes-induced reactive oxygen species (ROS), and prevent the further destruction of β-cells of pancreas. Administration of tannins and phenolic compounds results into phosphorylation of insulin receptors and translocation of glucose transporter proteins which reduces the blood glucose level [17]. Kotadiya *et al.* [18] have observed the *in vivo* antidiabetic effect of commonly used flavonoid compound quercetin and *Opuntia elatior* fruit juice in rats. The combined treatment has significantly (p<0.05) prevented a steep onset of hyperglycemia after STZ administration in test group compared to diabetic control rats. The hypoglycemic effect might be due to presence of soluble fibers which absorb the sugar molecules from the intestine and prevent their entry in to systemic circulation.

Table 2: Phytochemical screening of various plants used for evaluation of *in vitro* anti-diabetic activity

Type of phytochemical	Extracts	Name of plants
Alkaloid	CE	*E. littorale, L. sativum, M. oleifera, S. xanthocarpum*
	ME	*C. absus, E. littorale, L. sativum, M. oleifera, S. xanthocarpum*
	WE	*C. absus, C. carandas, C. anthelminticum, E. littorale, L. sativum, M. oleifera, P. tuberosa, S. xanthocarpum*
Glycoside	CE	*P. tectorius*
	ME	*C. absus, C. carandas, C. anthelminticum, E. littorale, F. racemosa, G. sylvestre, M. oleifera, P. tectorius, P. tuberosa, S. xanthocarpum, T. undulata*
	WE	*C. absus, C. tora, C. anthelminticum, E. littorale, E. nivulia, F. racemosa, G. sylvestre, L. sativum, M. oleifera, P. tectorius, P. tuberosa, S. xanthocarpum, S. cumini*
Saponin	CE	*P. tectorius*
	ME	*C. tora, E. nivulia, F. racemosa, P. tuberosa, S. cumini*
	WE	*C. absus, C. carandas, C. tora, C. anthelminticum, E. littorale, E. nivulia, F. racemosa, G. sylvestre, L. sativum, M. oleifera, P. tectorius, P. tuberosa, S. xanthocarpum, S. cumini, T. undulata*
Flavonoid	CE	*C. absus, C. carandas, C. tora, G. sylvestre, P. tectorius, P. tuberosa, S. xanthocarpum, S. cumini*
	ME	*C. absus, C. carandas, C. tora, E. littorale, E. nivulia, G. sylvestre, L. sativum, M oleifera, P. tectorius, P. tuberosa, S. cumini, T. undulata*
	WE	*C. absus, C. carandas, C. anthelminticum, E. littorale, E. nivulia, F. racemosa, M. oleifera, P. tectorius, S. xanthocarpum, S. cumini, T. undulata*
Steroid	CE	*C. absus, C. carandas, C. tora, E. littorale, E. nivulia, F. racemosa, G. sylvestre, M. oleifera, P. tectorius, S. xanthocarpum, S. cumini, T. undulata*
	ME	*C. absus, C. carandas, C. anthelminticum, E. littorale, F. racemosa, G. sylvestre, P. tectorius, S. xanthocarpum*
	WE	----------
Carbohydrate	CE	*C. carandas, S. cumini*
	ME	*C. absus, C. carandas, G. sylvestre, L. sativum, M. oleifera, P. tectorius, P. tectorius, P. tuberosa, S. xanthocarpum, S. cumini, T. undulata*
	WE	*C. absus, C. carandas, C. tora, C. anthelminticum, G. sylvestre, L. sativum, P. tuberosa, S. xanthocarpum*
Tannin	CE	*F. racemosa, M. oleifera*
	ME	*C. anthelminticum, F. racemosa, G. sylvestre, T. undulata*
	WE	*C. absus, C. carandas, C. tora, F. racemosa, G. sylvestre, L. sativum, M. oleifera, P. tectorius, P. tuberosa, S. xanthocarpum, S. cumini, T. undulata*

Out of 15 medicinal plants and their three different extracts, some extracts have shown good inhibitory activity against α-amylase and some have against α-glucosidase or both. Per cent inhibitions of α-amylase and α-glucosidase enzymatic activity by different extracts of medicinal plants are shown in table 3 and 4, respectively. An α-amylase is responsible for the conversion of starch into mono and disaccharides which ultimately increases the blood sugar level. Chloroform extracts of *Lepidium sativum* seed, *Gymnema sylvestre* leaf and *Pueraria tuberosa* tuber at the concentration of 200 µg/mL have shown good per cent inhibition of α-amylase activity of 56.65 ± 0.81, 55.94 ± 0.80 and 55.06 ± 0.58 %, respectively which were significantly higher (p < 0.05) than 46.43 ± 0.26 % of acarbose (standard drug). Doke *et al.* [19] have reported some higher 80 ± 0.10 % inhibition of intestinal α-amylase activity by *Lepidium sativum* seed coat phenolic extract at the concentration of 17 µg/mL. *Gymnema sylvestre* leaves are used traditionally as anti-diabetic in the Indian traditional medicine. It contains the active principle gymnemic acid, a triterpenoid joined with sugar moiety which is very potent anti-diabetic agent [20]. Shaul *et al.* [9] also reported that administration of polyherbal extract mixture containing the hydro-alcoholic extract of leaves of *G. sylvestre* resulted into reduced blood glucose level and ameliorating effect against the biochemical and pathological alterations in streptozotocin induced diabetic rats.

Table 3: Comparison of *in vitro* anti-diabetic activity of various extracts of selected medicinal plants by α-amylase inhibition method

Name of plant	Type of extract	Percent inhibition at Concentration (µg/mL)					
		10	25	50	100	200	500
Acarbose	Std.	37.64±0.61^{c}	39.61±0.17^{b}	40.63±0.47^{b}	43.62±0.69^{a}	46.43±0.26^{a}	52.69±2.16^{a}
Carissa carandas stem	ME	33.12±0.58^{b}	36.58±0.69^{b}	39.64±0.70^{b}	42.56±0.46^{a}	43.76±0.46^{a}	46.95±0.46^{a}
Cassia tora root	WE	37.91±0.58^{c}	40.17±0.46^{b}	41.10±0.58^{b}	42.29±0.58^{a}	43.62±0.58^{a}	44.95±0.69^{a}
Euphorbia nivulia stem	ME	23.64±0.58^{a}	27.58±0.58^{a}	36.02±0.57^{b}	36.56±0.69^{a}	39.33±0.92^{a}	48.74±0.58^{a}
Gymnema sylvestre Leaves	CE	43.32±0.69^{c}	49.00±0.50^{c}	50.55±0.69^{c}	52.00±0.81^{b}	55.94±0.80^{d}	63.01±0.81^{d}
Lepidium sativum seed	CE	7.86±0.83^{a}	9.99±0.70^{a}	12.25±0.69^{a}	55.33±0.69^{c}	56.65±0.81^{d}	57.32±0.69^{c}

	ME	36.84±0.93[b]	39.50±0.58[b]	40.70±0.58[b]	42.96±0.69[a]	44.42±0.81[a]	52.40±1.03[a]
Moringa oleifera Leaves	WE	25.01±0.69[a]	30.20±0.69[a]	33.92±0.81[b]	35.25±0.70[a]	52.00±0.81[b]	53.86±0.81[a]
Pueraria tuberosa tuber	CE	45.43±0.58[c]	48.28±0.58[c]	50.14±0.69[c]	54.53±0.46[b]	55.06±0.58[c]	56.12±0.46[b]
Syzygium cumini leaves	WE	29.93±0.70[b]	35.38±0.69[b]	39.77±0.69[b]	43.49±0.81[a]	45.88±0.81[a]	52.93±0.92[a]
Tecomella undulata bark	ME	16.23±0.46[a]	20.22±0.46[a]	26.87±0.58[a]	39.37±0.46[a]	50.14±0.69[a]	50.80±0.70[a]

CE: Chloroform extract; ME: Methanol extract; WE: Water extract
Values with different superscripts in each column are significantly different from each other (p<0.05).

Table 4: Comparison of *in vitro* anti-diabetic activity of various extracts of selected medicinal plants by α-glucosidase inhibition method

Name of plant	Type of extract	Percent inhibition at Concentration (µg/mL)				
		200	400	600	800	1000
Acarbose	Std	33.51±0.10[a]	34.08±0.15[a]	39.32±0.15[a]	59.41±0.46[a]	76.91±0.47[d]
Carissa carandas stem	WE	53.66±0.30[a]	54.00±0.30[a]	54.86±0.35[a]	55.15±0.40[a]	61.08±0.40[a]
Centratherum anthelminticum seed	CE	61.83±0.29[c]	62.35±0.19[b]	66.84±0.29[d]	70.87±0.35[c]	72.94±0.25[d]
	ME	44.39±0.29[a]	45.31±0.25[a]	46.06±0.25[a]	59.70±0.25[a]	63.56±0.36[a]
	WE	50.43±0.19[a]	69.72±0.25[d]	70.52±0.30[e]	77.26±0.40[f]	77.84±0.35[e]
Enicostemma littorale leaves	WE	46.75±0.25[a]	48.01±0.29[a]	48.93±0.15[a]	56.13±0.29[a]	60.10±0.299[a]
Lepidium sativum seed	ME	59.01±0.15[b]	59.24±0.19[b]	61.83±0.29[b]	64.02±0.35[b]	66.67±0.29[b]
	WE	61.95±0.25[c]	62.46±0.25[b]	63.67±0.45[b]	65.92±0.35[b]	67.13±0.30[b]
Moringa oleifera leaves	CE	38.92±0.25[a]	43.35±0.26[a]	48.88±0.20[a]	60.85±0.25[a]	63.27±0.25[a]
	ME	67.18±0.19[d]	68.80±0.25[d]	70.6±0.17[e]	72.42±0.20[d]	74.04±0.20[d]
	WE	69.20±0.35[e]	70.06±0.25[e]	71.16±0.19[e]	72.42±0.25[d]	73.81±0.25[d]
Pandanus tectorius leaves	WE	66.49±0.19[d]	67.07±0.25[c]	67.65±0.15[d]	69.43±0.19[c]	76.05±0.30[d]
Pueraria tuberosa tuber	CE	50.78±0.36[a]	54.00±0.20[a]	59.07±0.36[a]	66.61±0.45[b]	71.68±0.29[c]
	ME	70.98±0.29[f]	72.02±0.39[f]	72.37±0.36[f]	73.58±0.39[e]	76.86±0.39[d]
	WE	58.20±0.29[b]	64.59±0.39[b]	65.57±0.39[b]	67.07±0.39[b]	70.35±0.39[c]
Solanum xanthocarpum Leaves	CE	64.65±0.25[c]	64.88±0.25[b]	66.61±0.35[c]	71.27±0.25[c]	75.30±0.29[d]
	WE	72.19±0.19[f]	73.46±0.35[f]	77.37±0.29[g]	78.70±0.35[g]	79.22±0.35[f]

CE: Chloroform extract; ME: Methanol extract; WE: Water extract
Values with different superscripts in each column are significantly different from each other (p<0.05).

Water extracts of *Solanum xanthocarpum* leaf and *Centratherum anthelminticum* seed at the concentration of 200 µg/mL shown significantly higher (p < 0.05) inhibition of α-glucosidase activities up to 72.19 ± 0.19 and 50.43 ± 0.19 %, respectively which were higher than the respective value (33.51 ± 0.10 %) of acarbose. Methanolic extract of *Pueraria tuberosa* tuber and *M. oleifera* leaf have also shown α-glucosidase inhibitory action of 70.98 ± 0.29 and 67.18 ± 0.19 %, respectively. In agreement to this, presence of condensed tannins in leaves of *M. oleifera* exhibited anti-diabetic effects mainly by inhibiting the α-amylase and α-glucosidase activities [21]. Higher enzyme inhibitory action of these plant extracts might be due to the presence of flavonoids, alkaloids, glycosides, saponins and tannins which are known for their antioxidant and anti-diabetic activities. Some researchers have reported that administration of antioxidant compounds in diabetic mice showed beneficial effect and it can provide protection to β-cells against glucose toxicity [22]. *Solanum xanthocarpum* leaf, *Centratherum anthelminticum* seed *and Moringa oleifera* leaf extracts have shown comparatively high inhibitory effect against α-glucosidase and quite lower against α-amylase enzyme. These is a desirable characteristics of any compound which helpful for delay the availability of dietary carbohydrate substrate for glucose production in the gut [23].

Conclusion

In conclusion, extracts of *Lepidium sativum* seed, *Moringa oleifera* leaf, *Pueraria tuberosa* tuber and *Gymnema sylvestre* leaf contain active phytochemicals viz. flavonoids, alkaloids, glycosides, saponins and tannins, and showed significant *in vitro* anti-diabetic activity by inhibition of both α-amylase and α-glucosidase enzyme activity. These natural plant metabolites might have therapeutic potential for the control of postprandial blood glucose levels and development of novel and effective anti-diabetic drugs in future.

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