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Research Article

SCREENING OF FIFTEEN INDIAN AYURVEDIC PLANTS FOR ALPHA-GLUCOSIDASE INHIBITORY ACTIVITY AND ENZYME KINETICS

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ABSTRACT

Alpha-glucosidase inhibitory activity of fifteen Indian medicinal plants has been evaluated by *in vitro* enzyme assay. Methanol extracts of *Cyperus rotundus* (tubers), *Plumbago zeylanica* (root), *Symplocos racemosa* (bark), and *Terminalia arjuna* (bark) had displayed 100% inhibition with the IC_{50} value of 3.98 µg/ml, 3.46 µg/ml, 8.16 µg/ml and 0.69 µg/ml, respectively. Bark extract of *Terminalia arjuna* is highly effective against alpha-glucosidase activity even at nanogram concentration. Plant parts of *Piper retrofractum* (stem), *Zingiber officinale* (rhizome), *Acorus calamus* (rhizome), *Picrorhiza kurroa* (rhizome), *Marsdenia tenacissima* (stem), *Clerodendron serratum* (root), and *Rubia cordifolia* (root) are not effective and they require high concentration to exhibit inhibition. Potential plants that show maximum inhibition at low concentration (<10 µg/ml) were subjected to kinetic analysis to determine the mode of inhibition of the enzyme. *Cyperus rotundus, Symplocos racemosa* and *Terminalia arjuna* exhibited uncompetitive inhibition and *Plumbago zeylanica* had displayed mixed inhibition to alpha-glucosidase enzyme activity. From the enzyme assay, we infer that *Cyperus rotundus, Plumbago zeylanica, Symplocos racemosa and Terminalia arjuna* contain potential alpha-glucosidase inhibitors that can be exploited for its use in the treatment of diabetes.

Keywords: Alpha-glucosidase inhibition, Kinetics, Medicinal plants, Diabetes

INTRODUCTION

The world prevalence of diabetes among adults (aged 20–79 years) was 6.4%, affecting 285 million adults (2010), and will increase to 7.7% and 439 million adults by 2030[1]. In India, the number of adults with diabetes was 50.8 million (2010) and will be expected to reach 87 million (2030), with a mean annual increment of 1.8 million[1]. Type 2 diabetes mellitus (T2DM) is a heterogeneous metabolic disorder, characterized by defects in insulin secretion and insulin sensitivity[2]. Absolute or relative deficiency of insulin results in hyperglycemia in diabetic individuals[3]. Delayed insulin secretion immediately after meal leads to sudden surge in blood glucose level known as 'hyperglycemic spikes'[4]. This 2-hour post-prandial plasma glucose will range from 140 to 199 mg/dL (impaired glucose tolerance) and then rise to greater than 200 mg/dL in the case of diabetics. Post-prandial phase is associated with macrovascular and microvascular diabetic complications and it is the major independent risk factor for cardiovascular disease (CVD) [5]. In the evolution of diabetic interventions, there is an effective preventive strategy to treat this risky post-prandial phase: Unabsorbed carbohydrates (oligosaccharides and disaccharides) are bound to the alpha-glucosidase enzymes located in the brush border of the enterocytes of the jejunum in small intestine and on cleavage to monosaccarides, they are immediately absorbed in the upper jejunum causing hyperglycemia[6]. On administering alpha-glucosidase inhibitors with carbohydrates, they compete with the binding of oligosaccharides and prevent their cleavage to monosaccharides, thereby slowing the digestion process and rise in post-prandial blood glucose level[7]. Acarbose, voglibose and miglitol are commerical alpha-glucosidase inhibitors that are considered as first-line treatment for diabetic individuals with post-prandial hyperglycemia. On giving them along with other oral hypoglycaemic agents like metformin, sulfonylurea improves glycemic control (reduced HbA1c). However, these alpha-glucosidase inhibitors have prominent gastrointestinal side effects like flatulence, diarrhoea, and abdominal discomfort[8]. This warrants the search for alternative natural herbal medicines that have fewer side effects than the available inhibitors for the treatment of diabetes. The current study aims to evaluate fifteen Indian Ayurvedic medicinal plants for alpha-glucosidase inhibitory activity *in vitro*. Plants used are *Aconitum heterophyllum* Wall, *Acorus calamus* Linn, *Clerodendron serratum* Linn, *Cyperus rotundus* Linn, *Marsdenia tenacissima* (Roxb.) Moon, *Mesua ferrea* Linn, *Nigella sativa* Linn, *Picrorhiza kurroa* Royle ex benth, *Piper retrofractum* Vahl, *Plumbago zeylanica* Linn, *Rubia cordifolia* Linn, *Saussurea lappa* C.B.C.L, *Symplocos racemosa* Roxb, *Terminalia arjuna* (Roxb.) Wight & Arn, *Zingiber officinale* Rosc. From this study we report that *Cyperus rotundus, Plumbago zeylanica, Symplocos racemosa and Terminalia arjuna* have the most potent alpha-glucosidase inhibiting components since they show maximum inhibition even at nanogram and microgram quantities. Kinetic analysis done on these plants showed that *Plumbago zeylanica* exhibited a mixed (non-competitive- uncompetitive) type of inhibition, whereas the other three plants inhibited the enzyme in an uncompetitive manner. This study shows that these plants contain potential alpha-glucosidase inhibitors which can be exploited further for the isolation of active components used in the treatment of diabetes.

MATERIALS AND METHODS

Materials

The plant materials were purchased from Amman Ayurvedic shop, Vellore, India and ground to yield a powdered form for solvent extraction. *p*-Nitrophenyl-α-D-glucopyranoside (PNPG), Yeast alpha-glucosidase (EC 3.2.1.20), sodium phosphate salts and sodium carbonate were purchased from Sisco (SRL), India. The 96-well microplate reader was purchased from Bio Tek USA Inc. and Voglibose tablets (2 mg) were purchased from Bayer Pharma.

Plant background

Grounded plant parts (250 g) were extracted using methanol (250 ml) in Soxhlet apparatus for 8 hours. Then, the extract was evaporated to dryness and the final dry crude extract was stored in dark at -20°C until used for the experiments. The standard drug, Voglibose was dissolved in distilled water, centrifuged at 6000 rpm and the supernatant was taken and appropriately diluted.

Aconitum heterophyllum Wall, Ranunculaceae, Ativishaa (Sanskrit)

The plant is commonly found in alpine and sub-alpine region of the Himalayas at altitudes between 1,800-4,500 m. The roots of the plant have medicinal properties. Various butyrylcholinesterase and acetylcholinesterase inhibitors were extracted from the roots of the plant[9]. Norterpenoid alkaloids were extracted from the roots of these plants having antibacterial activity[10]. Ethanolic extracts of this plant stimulates phagocytic function while inhibiting the humoral component of the body's immune system, thus acting as an immunomodulator[11].

Acorus calamus Linn, Araceae, Vacha (Sanskrit)

Pectic polysaccharide obtained from this plant activates macrophages and stimulate Th1 response[12]. Ethanolic extract of the leaves promoted wound-healing activity in rats[13]. An inhibitory effect of the aqueous extract on water-bloom forming species of algae has been indicated[14]. Ethyl acetate extracts showed insulin releasing and alpha-glucosidase inhibitory activity[15] and they published IC_{50} value of 0.41 µg/ml.

Clerodendron serratum Linn, Verbenaceae, Bharangi (Sanskrit)

Ethanolic extract of the roots of *Clerodendron* has hepatoprotective activity against carbon tetrachloride induced toxicity in rats[15]. Alcoholic extract of the roots of this plant has anti-inflammatory activity[16]. It was also shown to have antinociceptive and antipyretic activity in animal model.

Cyperus rotundus Linn, Cyperaceae, Musta (Sanskrit)

The rhizome of this plant has been reported to play a major role in the protection of neurodegenerative disorders due to its antioxidant and free radical scavenging activity[17]. Ethanolic extract of this plant has analgesic properties[18]. Hydro-alcoholic extract of this plant displaying antiviral effect against Herpes Simplex[19]. Hydro-ethanolic extract of *Cyperus* reduces blood glucose level significantly in alloxan induced diabetic rats[20]. Ethanol extract of aerial parts of this plant showed marked protection against convulsions induced by chemo convulsive agents in mice[21].

Marsdenia tenacissima (Roxb.) Moon, Asclepiadaceae, Madhurasa (Sanskrit)

A significant antitumor effect of this herb has been reported in experimental and clinical applications[22]. Novel pregnane glycosides were isolated from roots of this plant[23]. Tenacigenin derived from this plant had shown to reverse multidrug resistance in cancerous cells[24].

Mesua ferrea Linn, Clusiaceae, Nagakesara (Sanskrit)

Estrogenic and progestational activity of this plant on mice and humans has been reported[25]. Recently it has been shown that Calophyllolide isolated from *Mesua* is effective in reducing the increased capillary permeability (induced in mice by Histamine, 5-HT and bradykinin). Main use of stamen has been described to control bleeding in menorrhagia and piles. Xanthones, a number of 4-phenylcoumarin derivatives, friedelin and triterpenes have been isolated from the plant. Xanthones are isolated from the heartwood; coumarin derivatives from the seeds; canophylial, canophyliol and canophyllic acid from the leaves.

Nigella sativa Linn, Ranunculaceae, Kalongi (Sanskrit)

Volatile oil extracted by hydro-distillation contains thymoquinone (3.8 %) which is involved in anti-inflammatory activities *in vivo* and *in vitro*[26] and the therapeutic potential of thymoquinone in pancreatic cancer has been proved[27]. It also showed its potential adjuvant effects to improve immunotherapy in the treatment of allergic Rhinitis[28]. Fixed oil and water extract of this plant (0.1% v/v) has shown to considerably reduce formation of sickle cells due to its calcium antagonistic and antioxidant activities[29]. Oral administration of the ethanol extract of *N. sativa* seeds to diabetic rats reduced hyperglycemia[30]. Methanolic extract of *Nigella* significantly inhibits glucose utilisation in the intestine of rats[31]. Petroleum ether extract of the seeds of this plant has shown to exert insulin sensitising action. *N. sativa* treatment in streptozotocin-induced diabetic rats reduced lipid peroxidation and serum nitric oxide levels to a significant level by increasing antioxidant enzyme activity[32]. Oil extracted from the plant blocks nitric oxide overproduction and ceases morphine-induced tolerance and dependence in mice[33]. Methanolic extracts of this plant possess potent CNS depressant and analgesic activity[34].

Picrorhiza kurroa Royle ex benth, Scrofulariaceae, Katuka (Sanskrit)

A glycoside, picroliv isolated from this plant is hepatoprotective. It has cholerectic and anti-cholestatic effects in rats and guinea pigs[35]. It also has anti-viral and immuno-modulatory effect. Picroliv restored cadmium induced abnormalities in the liver of male rats[36]. It has anti-inflammatory, anti-carcinogenic[37] and antioxidant effects[38]. Methanolic extract of the plant has healing potential in indomethacin induced stomach ulcers in mice[39]. Tannins derived from the plant inhibit cyclooxygenase and lipid peroxidation[40].

Piper retrofractum Vahl, Piperaceae, Chavya (Sanskrit)

Aqueous extracts of the plant have potent larvicidal activity[41]. Ethanolic extract of the plant has larvicidal and insecticidal properties[42]. Fruits of this plant have potent anti-bacterial and anti-fungal properties[43]. Phenolic amides obtained from the plant have potent antioxidant properties[44].

Plumbago zeylanica Linn, Plumbaginaceae, Chitraka (Sanskrit)

Plumbagin isolated from this plant possess significant anticancer activity[45] and anti-inflammatory effects[46]. Hexane and chloroform extract of this plant had significant larvicidal activity[47]. Acetone and ethanolic extracts of the leaves of this plant had reversible concentration dependent oestrogenic and anti-oestrogenic activity[48]. Methanolic extract of the root of this plant showed promising anti-oxidant activity [49].

Rubia cordifolia Linn, Rubiaceae, Manjistha (Sanskrit)

Anti-oxidative constituents were isolated from ethyl acetate extract of this plant[50]. Alcoholic extract of this plant increased brain gamma-amino-n-butyric acid levels and decreased brain dopamine and plasma corticosterone levels[51]. The extract also inhibited acidity and ulcer formation, decreased blood sugar level in alloxan treated animals[51].

Saussurea lappa C.B.C.L, Asteraceae, Kustha (Sanskrit)

Anti-hepatotoxic activity of the aqueous-methanol root extract has been reported in mice[52]. Ethanol extract of this plant inhibited *Streptococcus mutans* in a dose dependent manner[53]. The plant contains cholinergic and a calcium antagonist ingredient which is helpful for use in constipation and spasms[54]. Antifungal constituents were isolated from the roots of the plant[55].

Symplocos racemosa Roxb. Symplocaceae, Lodhra (Sanskrit)

Glycosides isolated from this plant are reported to inhibit thymidine phosphorylase whose overexpression is linked to angiogenesis[56]. New phenolic glycosides of salirepin series in the n-butanol fraction of the bark of *S. racemosa* has been isolated[57]. Ethyl substituted glycoside that inhibits lipoxygenase[58] and phenolic glycoside that inhibits human nucleotide pyrophosphatase phosphodiesterase has been identified[59].

Terminalia arjuna Roxb. Wight & Arn. Combretaceae, Arjuna (Sanskrit)

This plant is widely known to prove comprehensive relief to the people suffering from cardio-vascular diseases, especially hyperlipidemia and ischemic heart disease. Some important findings related to the above mentioned activity has been studied[60]. Anti-inflammatory, immunomodulatory and antinociceptive activity of the bark in mice and rats has been studied[61]. *T. arjuna* bark extract attenuated catecholamine-induced myocardial fibrosis and oxidative stress[62]. It has antimicrobial activity against multi drug resistant (MDR) strains of fungi and bacteria of clinical origin[63]. Oleanane-type triterpene glycosides have been isolated and they suppress the release of nitric oxide and superoxide from macrophages and also inhibit aggregation of platelets[64]. The effect of *T. arjuna* extract on adriamycin-induced micronuclei formation in cultured human peripheral blood lymphocytes has been studied[65]. *T. arjuna* has been found to be useful in diabetes associated with ischemic heart disease[66].

Zingiber officinale Rosc. Zingiberaceae, Sunthi (Sanskrit)

Fractions obtained from the rhizome of this plant show anti-microbial and antioxidant effects[67]. A formulation of the plant was effective in controlling alcohol hangover symptoms[68]. Dried fermented ginger improved intestinal function[69]. Ethanolic extract of

this plant had remarkable inhibitory activity against multi-drug resistant bacterial and fungal strains[70]. The plant has larvicidal constituents[71] and anti-invasive property of the constituents of the plant in hepatocarcinoma cells has been studied[72]. Ginger promoted glucose transport in muscle cell line[73]. The protective action of ginger oil on gastric ulcer induced by aspirin in rats has been investigated[74]. Ethanolic extract of ginger has protective effect against renal damage induced by alcohol[75]. Aqueous extract of ginger rhizome produced significant increase in insulin levels and decrease in fasting glucose levels in diabetic rats[76]. Treatment with the extract also caused reduction in serum cholesterol levels, blood pressure and serum triglycerides in diabetic rats.

Inhibition assay for alpha-glucosidase activity

Alpha-glucosidase inhibition was analysed using kinetic end-point assay described by Pistia-Brueggeman with few modifications. Alpha-glucosidase inhibitory activity was performed following the modified method of Pistia Brueggeman and Hollingsworth[77]. In a 96-well plate reader, a reaction mixture containing 50 µl of phosphate buffer (50 mM; pH 6.8), 10 µl of alpha-glucosidase (1 U/ml) and 20 µl of plant extract of varying concentrations was pre-incubated for 5 min at 37°C, and then 20 µl of 1 mM PNPG was added to the mixture as a substrate. After further incubation at 37°C for 30 min, the reaction was stopped by adding 50 µl of sodium carbonate (0.1M). All the enzyme, inhibitor and substrate solutions were made using the same buffer. Voglibose was used as a positive control and water as negative control. The yellow colour produced (due to *p*-nitrophenol formation) was quantitated by colorimetric analysis and reading the absorbance at 405 nm. Each experiment was performed in triplicates, along with appropriate blanks.

The % inhibition has been obtained using the formula:

% inhibition = {Absorbance (control) –Absorbance (sample)}/ Absorbance (control)

IC_{50} value is defined as the concentration of extract inhibiting 50% of alpha-glucosidase activity under the stated assay conditions. In case of significant inhibition, IC_{50} values were determined by linear regression by fitting to a sigmoidal dose-response equation with variable slope. All values are represented as Mean ± Standard Deviation.

Kinetics of alpha-glucosidase inhibition

The mode of inhibition of plant extracts against alpha-glucosidase activity was measured with increasing concentrations of PNPG (0.125, 0.25, 0.5 and 1 mM) as substrate in the absence or presence of the plant extracts at two different concentrations for each plant extract. The enzyme reaction was performed by incubating the mixture at 37°C for 30 min and optimal doses of the plant extracts were determined based on the results from inhibitory activity assay as described earlier. Mode of inhibition of plant extracts was determined by Lineweaver-Burk plot analysis of the data calculated following Michaelis-Menten kinetics[78,79]. Experimental inhibitor constant (K_i) values were determined by secondary plots. The theoretical value of K_i

$K_i = V_{max}' * I / (V_m - V_{max}')$

RESULTS AND DISCUSSION

Extracts obtained from several Ayurvedic plants have been reported for their anti-diabetic activity. But these plants have not been evaluated for the mechanism of action to control blood glucose level. In the present study, fifteen Indian Ayurvedic medicinal plants were evaluated for their alpha-glucosidase inhibitory potential. Plants used in this study along with the parts used and inhibition values are listed in Table 1. Before our study, except *Acorus*, none of the other plants have been studied for their alpha-glucosidase inhibitory effect *in vitro*. On screening 15 plant extracts, 4 extracts exhibited 100 % inhibition, while 10 extracts showed 10-100% AGI activity (10-100%). Only one extract (*Acorus calamus*) showed less than 10% inhibition. Voglibose, an available drug for alpha-glucosidase inhibition showed IC_{50} value of 21.98±2.91 µg/ml.

Extracts with 100% inhibitory effect on the alpha-glucosidase inhibition at 100 µg/ml

The extracts of *Terminalia arjuna*, *Plumbago zeylanica*, C*yperus rotundus*, *Symplocos racemosa* had displayed 100% inhibition at the concentration of 100 µg/ml (Table 1 and Fig. 1). The bark extract of *Terminalia arjuna* was the most potential inhibitor of the enzyme based on the IC_{50} value (0.69 µg/ml). On comparison to the control drug Voglibose with the IC_{50} value of 21.98±2.91 µg/ml, all these four plants displayed maximum inhibition at lower concentrations.

Extracts with 40-100% inhibitory effect on the alpha-glucosidase inhibition

The extracts of *Mesua ferrea* and *Aconitum heterophyllum* had shown moderate potency of 45.9% and 40.3% at 100 µg/ml, respectively.

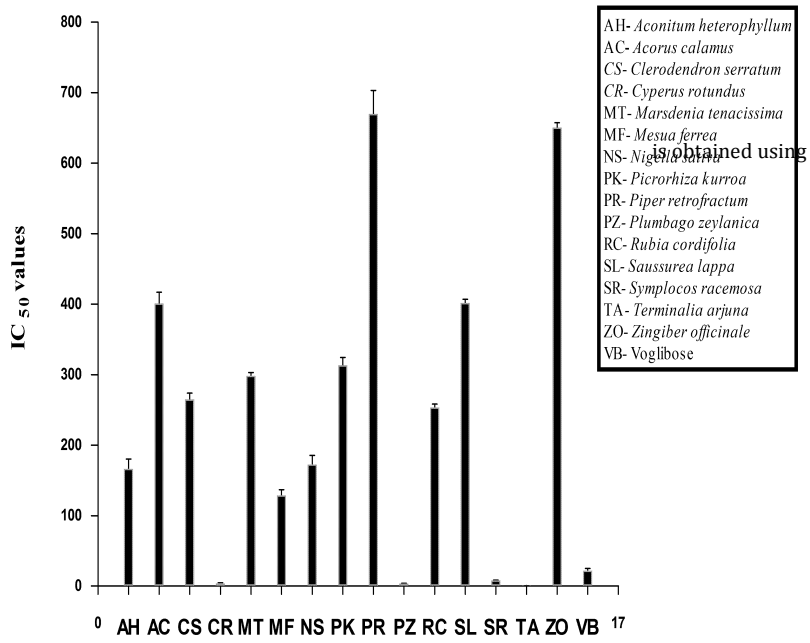
Extracts with 10-40% inhibitory effect on the alpha-glucosidase inhibition

The extracts of the following plants showed inhibition against alpha-glucosidase at 100 µg/ml. *Nigella sativa* (28.7%), *Rubia cordifolia* (29.7%), *Clerodendron serratum* (32.3%), *Marsdenia tenacissima* (22.1%), *Picrorhiza kurroa* (26.2%), *Saussurea lappa* (19.9%), *Zingiber officinale* (16.9%), and *Piper retrofractum* (15%).

Table 1: Inhibitory effect of plant extracts on alpha-glucosidase (Positive control: Voglibose (21.98±2.91 µg/ml)

Botanical name	Family	Parts used	Percentage inhibition at 100 µg/ml	IC_{50} (µg/ml)
Aconitum heterophyllum	Ranunculaceae	Rhizome	40.3	166.5±13.63
Acorus calamus	Araceae	Rhizome	7.5	401.06±15.96
Clerodendron serratum	Verbenaceae	Root	32.3	264.66±8.92
*Cyperus rotundus***	Cyperaceae	Tubers	100	3.98±0.55
Marsdenia tenacissima	Asclepiadaceae	Stem	22.1	298.43±4.79
Mesua ferrea	Clusiaceae	Dried buds	45.87	128.75±7.9
Nigella sativa	Ranunculaceae	Seeds	28.7	172.81±12.6
Picrorhiza kurroa	Scrofulariaceae	Rhizome	26.2	313.53±10.84
Piper retrofractum	Piperaceae	Stem	15.0	670.24±32.97
*Plumbago zeylanica***	Plumbaginaceae	Root	100	3.46±0.53
Rubia cordifolia	Rubiaceae	Root	29.7	253.42±4.88
Saussurea lappa	Asteraceae	Rhizome	19.9	401.76±5.17
*Symplocos racemosa***	Symplocaceae	Bark	100	8.16±0.28
*Terminalia arjuna***	Combretaceae	Bark	100	0.69±0.08
Zingiber officinale	Zingiberaceae	Rhizome	16.9	650.33±7.1

** Potential plants showing 100% inhibition.



is obtained using the formula:

Fig. 1: Graphical representation of the IC_{50} values for the plant extracts and standard (Voglibose)

Following these results, kinetic study has been performed on the four extracts *(C. rotundus, P. zeylanica, S. racemosa* and *T. arjuna)* showing 100% inhibition against alpha-glucosidase activity. Lineweaver-Burk (LB) plot has been used to determine the mode of inhibition (Fig. 2A-2D) and experimental inhibitor constant (K_I) values were determined by secondary plots (Fig. 3A-3D). The tuber extract of *C. rotundus* contains uncompetitive inhibitor(s) of alpha-glucosidase (Fig. 2A and 3A) and there is a reduction of V_{max} from 1.54mM.min^{-1} to 0.67mM.min^{-1} and K_m from 2.68 mM to 1.16 mM. The root extract of *P.zeylanica* has mixed inhibitor(s) of alpha-glucosidase (Fig. 2B and 3B) and there is a reduction in V_{max} from 1.82 mM.min^{-1} to 1.28 mM.min^{-1} and K_m from 3.37 mM to 2.49 mM. The bark extract of *S. racemosa* (Fig. 2C and 3C) contains uncompetitive inhibitor(s) of alpha-glucosidase and there is a reduction of V_{max} from 0.41 mM.min^{-1} to 0.23 mM.min^{-1} and the K_m from 0.73 mM to 0.40 mM. The bark extract of *T. arjuna* contains uncompetitive inhibitor(s) of alpha-glucosidase (Fig. 2D and 3D) and the V_{max} reduced from 1.19 mM.min^{-1} to 0.48 mM.min^{-1} and the K_m from 2.14 mM to 0.87 mM. The extracts of *S. racemosa* and *T. arjuna* requires low substrate concentration to reach half the maximal velocity.

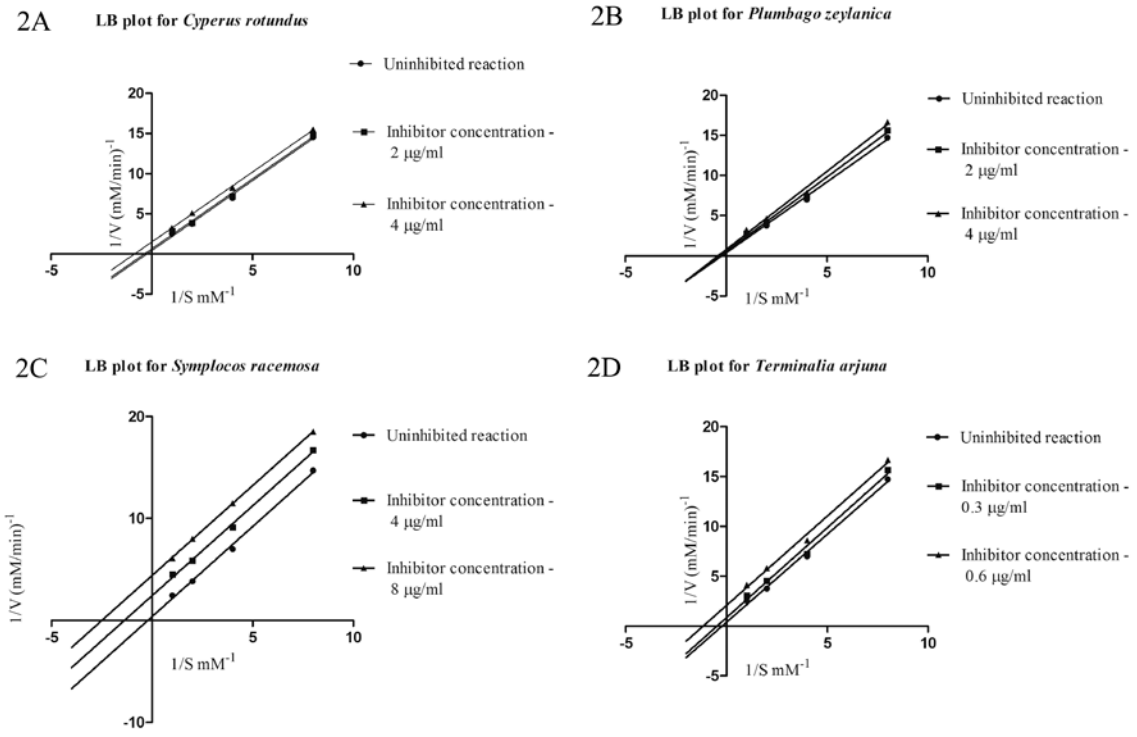


Fig. 2A-2D: Lineweaver-Burk plot for kinetic analysis of alpha-glucosidase inhibition by plant extracts

Yeast alpha-glucosidase was treated with various concentration of PNPG (0.125-1 mM) in the absence or presence of the extract *(Cyperus, Plumbago, Symplocos)* at the concentration range 2 µg/ml and 8 µg/ml. In the case of *Terminalia*, the extract at a concentration of 0.3 µg/ml and 0.6 µg/ml has been used. The enzyme reaction was performed by incubating the mixture at 37°C for 30 min.

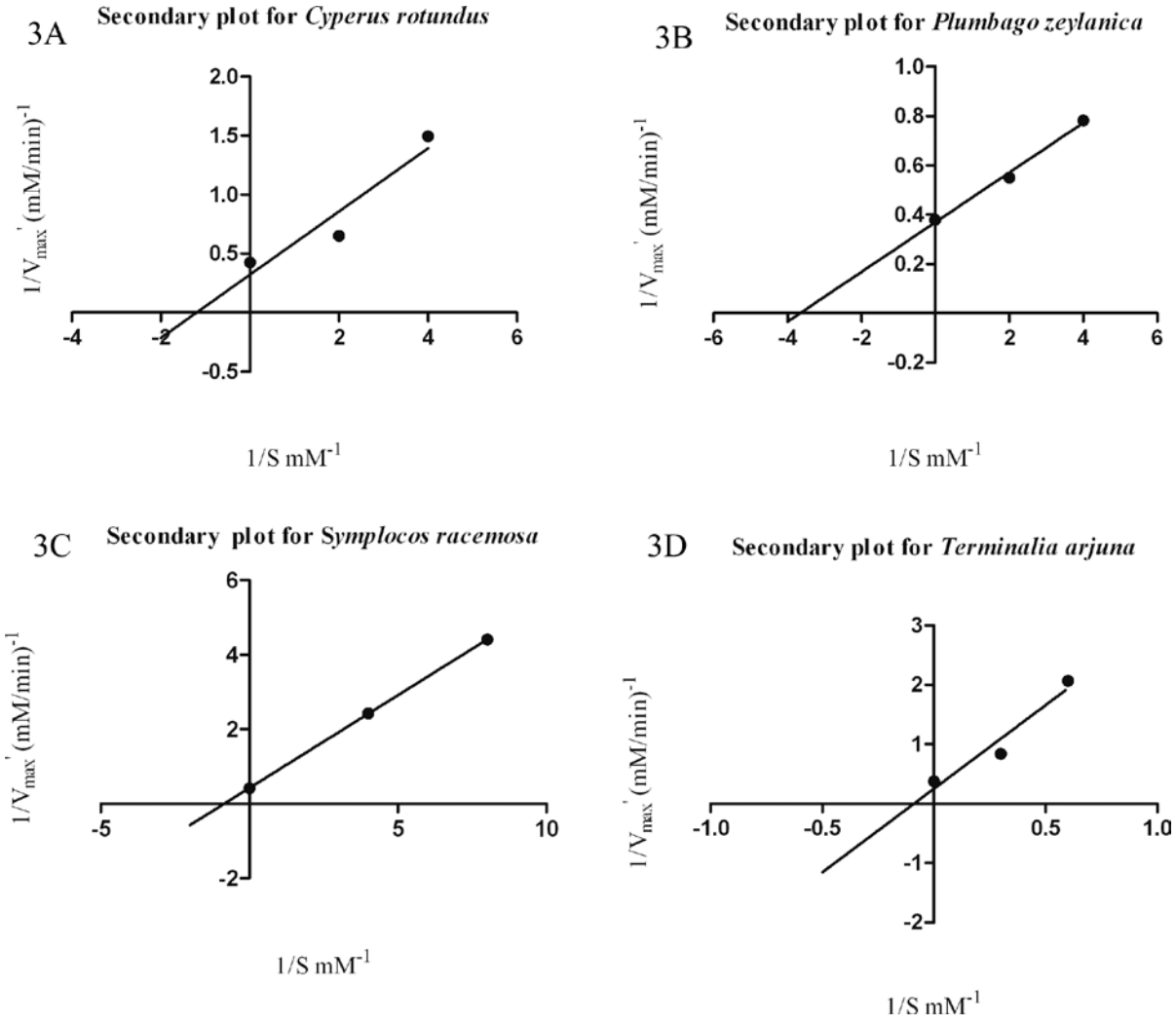


Fig. 3A-3D: Secondary plots for the kinetic analysis of alpha-glucosidase by plant extracts

Table 2: Kinetic analysis of alpha-glucosidase inhibition by the plant extracts

Extract concentration (µg/ml)	*C. rotundus*		*P. zeylanica*		*S. racemosa*		*T. arjuna*	
	K_m (mM)	V_{max} (mM.min^{-1})	K_m (mM)	V_{max} (mM.min^{-1})	K_m (mM)	V_{max} (mM.min^{-1})	K_m (mM)	V_{max} (mM.min^{-1})
0	4.099	2.353	4.654	2.639	4.709	2.666	4.654	2.639
2	2.676	1.536	3.373	1.821	0.726	0.412	2.145	1.189
4	1.165	0.669	2.488	1.279	0.400	0.227	0.866	0.484

Inhibitor concentration (µg/ml) was plotted on the X-axis and 1/V (mM/min)$^{-1}$ values obtained from the Lineweaver-Burk plot was plotted on the Y axis. The point on the X-axis where the line intersects the axis is the experimental inhibitor constant (K_i).

Table 2 shows the kinetics of alpha-gluosidase inhibition by the plant extracts. In all the four plants, on increasing the substrate concentration of the extract (from 2 µg/ml to 4 µg/ml), there is a decrease in the values for maximum velocity (V_{max}) and the substrate concentration required to reach half the maximum velocity (K_m).

In *C. rotundus*, K_m value of PNP-glycoside for alpha-glucosidase was 2.49 mM and the experimental K_i value was 1.21 µg/ml. The extract of *P. zeylanica* had shown the K_m value of 1.17mM and the experimental K_i value was 3.68 µg/ml. In *S. racemosa*, K_m value of PNP-glycoside for alpha-glucosidase was 0.40 mM and the experimental K_i value was 0.77 µg/ml. This K_i value is close to that of the calculated K_i value (0.74 µg/ml).

The extract of *T. arjuna* had shown the K_m value of 0.87 mM and the experimental K_i value of 0.09 µg/ml. The commercial alpha-glucosidase inhibitor, Voglibose is a competitive inhibitor of the enzyme and it is required at higher concentration to reduce the post-prandial glucose level. The uncompetitive inhibitors bind to

the enzyme-substrate complex, lowering the K_m and the maximum enzyme activity (V_{max}).

CONCLUSION

Diabetes mellitus is a progressive metabolic disorder affecting majority of the population across the world. There are various measures to manage and treat this aggravating disease. The main effect of diabetes is increase in glycemic level and therefore to reach normoglycemic level, along with insulin and other oral hypoglycemic agents like sulfonylureas, biguanides[80], Thiazolidinediones (TZD), alpha-glucosidase inhibitors (AGI) and incretin mimetics (GLP-1, GIP, DPP-4 inhibitors)[81] are being used. Alpha-glucosidase inhibitors delay the action of alpha-glucosidases to break complex carbohydrates in to simple sugars, thereby lowering the absorption of glucose. These inhibitors play a vital role in reducing the post-prandial hyperglycemia. As a consequence of their pharmacological action, alpha-glucosidase inhibitors also cause a concomitant decrease in post-prandial plasma insulin and gastric inhibitory polypeptide (GIP) and a rise in late post-prandial plasma glucagon-like peptide 1 (GLP-1) levels[82]. In individuals with normal or impaired glucose tolerance with hyperinsulinemia, alpha-glucosidase inhibitors decrease hyperinsulinemia and improve insulin sensitivity[83]. Post-prandial hyperglycemia contributes to raise in glycated haemoglobin (HbA1c) levels, which as an indicator of total glycemic load, is tightly correlated with the incidence of micro and macroangiopathy in Type 2 diabetes. It can induce or deteriorate fasting hyperglycemia and be associated with coagulation activation and/or lipid metabolism abnormalities[84]. Epidemiological data from the United Kingdom Prospective Diabetes Study (UKPDS) also showed that there is a 14–16% decrease in macrovascular complications for every 1% absolute reduction in glycated haemoglobin[85]. Alpha-glucosidase inhibitors like acarbose, miglitol and voglibose are used in conjunction with other anti-diabetic drugs. But these inhibitors have some side effects like flatulence and diarrhea. This indicates that newer AGI's with lesser side-effects needs to be discovered.

Therefore, we have screened potential anti-diabetic plants for alpha-glucosidase inhibition. From the study we have identified *Cyperus rotundus*, *Plumbago zeylanica*, *Symplocos racemosa* and *Terminalia arjuna* for potential alpha-glucosidase inhibitory activity compared to the standard (voglibose). Particularly, *Terminalia arjuna* is effective even in nanogram quantities. Therefore, our plan is to investigate its efficiency *in vivo* using animal models. Kinetic studies performed on these effective plants exhibit uncompetitive inhibition for *Cyperus rotundus*, *Symplocos racemosa* and *Terminalia arjuna*. This indicates that the substrate binding could cause a conformational change to take place in the enzyme such that it reveals an inhibitor binding site. *Plumbago zeylanica* exhibited non-competitive-uncompetitive inhibition. This indicates that the inhibitor constant for free enzyme is lesser than the inhibitor constant for enzyme-substrate complex. Also, the binding of the inhibitor to the free enzyme or the enzyme-substrate complex is mutually independent.

Competing interests

The authors have no conflicting interests.

Authors' contribution

AB carried out the *in vitro* enzyme assay, kinetic analysis, prepared figures, and drafted the manuscript. MSS has contributed in standardizing the assay and figure preparation. KT conceived of the study, designed, involved in preparing the manuscript and interpretation. All authors read and approved the final manuscript.

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