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In vitro anti-diabetic and anti-oxidant activities of *Oroxylum indicum* (Kurtz): A potent wild medicinal plant North-Eastern region in India

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Abstract
This study evaluated potential antioxidant and alpha (α)-amylase and alpha (α)-glucosidase inhibition properties of *Oroxylum indicum* (L.) Kurz, a medicinal plant growing in North-Eastern region in India. The plant extracts was examined for its antioxidant activity by using free radical 1, 1-dipheny l-2-picryl hydrazyl (DPPH) scavenging method, ABTS radical scavenging ability, reducing power capacity, estimation of total phenolic content, flavonoid content and flavone content. The study revealed that the different concentrations of the extract possessed promising amount of total phenolics, flavonoid and flavonol and exhibit potent radical scavenging activity using DPPH and ABTS as substrate. Water extract exhibited promising α-amylase and α-glucosidase inhibitory activities with an IC_{50} value 0.75 µg and 0.80 µg dry extract respectively and well compared with standard acarbose drug. Thus, it could be concluded that the plant extract has good potential for the management of hyperglycaemia, diabetes and the associated condition of oxidative stress due to the presence of antioxidant components. With the aid of this understanding, new effective anti-diabetic principles will be discovered from natural resources for the development of anti-diabetic medicines.

Keywords: *Oroxylum indicum*, water extract, Antioxidant activities, α-amylase inhibitory activity, α-glucosidase inhibitory activity

1. Introduction
Diabetes mellitus is a significant long-term metabolic condition that affects how proteins, fats and carbohydrates are metabolised. It comprises a class of metabolic illnesses known as hyperglycemia, in which blood sugar levels are raised due to either insufficient insulin production by the pancreas or improper cell response to the released insulin. Long-term complications of diabetes mellitus include heart disease, stroke, as well as the dysfunction and failure of other organs [1].

There are three forms of diabetes. Type 1, type 2, and gestational diabetes are the three primary kinds of the disease. Diabetes can strike either a woman or a man at any age. Type 1 diabetes comprises cases that have β-cell loss with an unidentified a etiology as well as those that can be attributed to an autoimmune process. The most prevalent primary form of diabetes, type 2, is brought on by abnormalities in insulin secretion or, more precisely, insulin resistance. A pregnant woman may develop gestational diabetes if her pancreas does not produce enough insulin during the pregnancy.

The only therapy of type 1 diabetes is the substitution of insulin. Many and diverse therapeutic strategies for the treatment of type 2 diabetes are known. The conventional treatments for diabetes include the reduction of the demand for insulin, stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues and the inhibition of degradation of oligo- and disaccharides [2-3].

The inhibitors of α-glucosidase are one class of medications used in the treatment of type 2 diabetes. The conversion of oligo- and/or disaccharides to monosaccharides is carried out by the enzymes collectively referred to as α-glucosidase. Due to the fact that monosaccharides are the type of carbohydrates that are absorbed via the mucosal border in the small intestine, the inhibitory activity of these enzymes results in a fall in blood glucose levels.

Another effective method to control diabetes is to inhibit the activity of α- amylase enzyme which is responsible for the collapse of starch to more simple sugars (dextrin, maltotriose, maltose and glucose) [4]. This is contributed by α-amylase inhibitors, which delays the glucose absorption rate thereby maintaining the serum blood glucose in hyperglycemic individuals [5].

Some inhibitors currently in clinical use are acarbose and miglitol which inhibit glycosidases such as α-glucosidase and α-amylase while others such as voglibose inhibit α-glucosidase.

However, many of these synthetic hypoglycemic agents have their limitations, are non-specific, produce serious side effects and fail to elevate diabetic complications. The main side effects of these inhibitors are gastrointestinal *viz*., bloating, abdominal discomfort, diarrhoea and flatulence [6]. Recently herbal medicines are getting more importance in the treatment of diabetes as they are free from side effects and less expensive when compared to synthetic hypoglycemic agents [7-8].

Phytochemical constituents like saponin, phenols, flavonoids, etc studied in various plants such as *Proteus vulgaris*, *Euphorbia hirta*, *and Cassia glauca* showed potential α-amylase inhibitors [9]. The role of medicinal plants in disease prevention is attributed to its antioxidant properties due to the presence of bioactive constituents [10].

Oroxylum indicum (Kurtz) belongs to the family Bignoniaceae, has a long history of traditional medicinal use and modern research has shown that it contains a number of medically active compounds. The various parts of the plant are rich in flavonoids and glycosides and trials have shown various activities in the body. In particular, dichloromethane extracts of the stem bark and root have been shown to have antimicrobial activities against a range of both gram-positive and gram-negative bacteria and also the yeast *Candida albicans*. The isolated flavonoid baicalin has shown inhibitory effects against the human T cell leukaemia virus type 1, and the human immunodeficiency virus (HIV-1). The bitter bark of the root is astringent, blood purifier and tonic. It is used in the treatment of stomach complaints, diarrhoea and dysentery. An alcoholic maceration of the fresh bark is externally applied on allergic dermatitis. When mixed with turmeric, the bark is used for healing sores of animals. The root is credited with antirheumatic, ant dysenteric and diuretic properties. The seeds and bark are used medicinally for alleviating body pain, especially during fevers and as an antichloristic medicine. It is also applied to burns and wounds. The juice of the bark is taken internally to treat diarrhoea and dysentery. A decoction of the bark is refrigerant, used in the treatment of fevers and jaundice. A decoction of the leaves is drunk as a treatment for stomach-ache. Applied externally the leaves are employed in the treatment of cholera, fever, childbirth and rheumatic swellings. The boiled leaves are used as a poultice during and after childbirth and in dysentery as well as for an enlarged spleen. Leaf poultices may be further applied for toothache and headache. The seed is expectorant and laxative. A decoction is used in treating coughs, bronchitis and gastritis [11]. The seeds are applied externally to ulcers [12-13].

Thus, in this study, the antioxidant and anti-diabetic activities of the water extract from the seeds of *O. indicum* were carried out and a relationship between antioxidant and anti-diabetic activities was established. To determine the potential of *O. indicum* seed extract as anti-diabetic agents, we investigated the effect of extracts on the α-glucosidase and α-amylase inhibitory activities.

2. Materials and Methods

2.1 Plant materials

The seeds of *Oroxylum indicum* was collected from Gangtok, Sikkim, India and identification was authenticated in our office. The voucher specimens were preserved in our department under registry no BSITS 125. The plant parts were shed-dried, pulverized and stored in an airtight container for further extraction.

2.2 Chemicals

Alpha (α)-Glucosidase, porcine pancreas alpha (α)-amylase, *p*-nitrophenyl-α-D-glucopyranose (*p*-NPG), 3, 5-dinitrosalicylic acid (DNS), 1, 1-Dipheny l-2-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), butylated hydroxytoluene (BHT), gallic acid, rutin, quercetin, ascorbic acid and acarbose were purchased from Sigma Chemical Co. (St. Louis, MO, USA).Soluble starch, sodium potassium tartarate, sodium dihydrogen phosphate (NaH_2PO_4), Di-sodium hydrogen phosphate (Na_2HPO_4) sodium chloride, sodium hydroxide, butylated hydroxytoluene (BHT), potassium persulfate, sodium carbonate, Folin-Ciocalteu (FC) reagent, potassium ferricyanide, potassium per sulphate, aluminum chloride, ferric chloride ($FeCl_3$) were from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used including the solvents, were of analytical grade.

2.3 Extraction of plant material

The air-dried and coarse powdered plant sample of *O indicum* (10 g) was extracted with 100 ml water by maceration on an orbital shaker with agitation for 24hrs at room temperature. The extracts was filtered and residue was again extracted with same solvent for another 24 hrs and filtered. The filtered extracts were combined and concentrated using a rotary evaporator, under reduced pressure at approximately 40 °C and lyophilized to obtain the powdered extract. The powdered extract were analysed for their α-glucosidase and α-amylase inhibition assays. The total phenolic, flavonoid and flavonols content, reducing power and their free radical scavenging capacity of the water extract of the plant were also investigated.

2.4 Estimation of total phenolic content

The amount of total phenolic content of crude extracts was determined according to Folin-Ciocalteu procedure [14]. The tested extracts (100 µl) were introduced into test tubes. 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5 %) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (UV-visible spectrophotometer Shimadzu UV 1800).

2.5 Estimation of total flavonoids

Total flavonoids were estimated using the method of Ordonez *et al.*, 2006 [15]. To 0.5 ml of extracts, 0.5 ml of 2 % $AlCl_3$ ethanol solution was added. After one hour, at room temperature, a yellow color developed, indicated the presence of flavonoids and the absorbance was measured at 420 nm (UV-visible spectrophotometer Shimadzu UV 1800).

2.6 Estimation of total flavonols

Total flavones in the plant extracts was estimated using the method of Kumaran and Karunakaran, 2006 [16]. To 1.0 ml of extracts 1.0 ml of 2 % $AlCl_3$ ethanol and 3.0 ml (50 g/L) sodium acetate solutions were added. The absorption at 440 nm (UV-visible spectrophotometer Shimadzu UV 1800) was read after 2.5 h at 20 °C for the estimation of total flavonol content in the plant extract.

2.7 Measurement of reducing power

The reducing power of the extracts was determined according to the method of Oyaizu, 1986 [17]. The plant extracts (100µl) was mixed with phosphate buffer (2.5 ml, 0.2 M, *p*H 6.6) and 1 % potassium ferricyanide (2.5 ml).

The mixture was incubated at 50 °C for 20 min. Aliquots of 10 % trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml, 0.1 %). The absorbance was measured at 700 nm and reducing power is determined.

2.8 Determination of DPPH free radical scavenging activity

The free radical scavenging activity of the plant extract and mutilated hydroxyl toluene (BHT) as positive control was determined using the stable radical DPPH (1, 1-diphenyl-2-picrylhydrazyl) [18]. 100 µl of the tested extracts were placed in test tubes and 3.9 ml of freshly prepared DPPH solution (25 mg L^{-1}) in methanol was added in each test tube and mixed. 30 min later, the absorbance was measured at 517 nm (UV-visible spectrophotometer Shimadzu UV 1800). The capability to scavenge the DPPH radical was calculated, using the following equation:

DPPH scavenged (%) = {(Ac – At)/Ac} x 100

Where Ac is the absorbance of the control reaction and at is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration in mg of dry material per ml (mg / ml) that inhibits the formation of DPPH radicals by 50 %. Each value was determined from regression equation.

2.9 Scavenging activity of ABTS radical cation

The 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical action ($ABTS^{\cdot+}$)-scavenging activity was measured according to the method described by Re *et al.* [19]. ABTS was dissolved in water to a 7 mM concentration. The ABTS radicals were produced by adding 2.45 mM potassium persulphate (final concentration). The completion of radical generation was obtained in the dark at room temperature for 12-16 h. This solution was then diluted with ethanol to adjust its absorbance at 734 nm to 0.70 ± 0.02. To determine the scavenging activity, 1 ml of diluted $ABTS^{\cdot+}$ solution was added to 20, 40 & 80 µl of plant extracts, and the absorbance at 734 nm was measured 6 min after the initial mixing, using ethanol as the blank. The percentage of inhibition was calculated by the equation:

ABTS scavenged (%) = $(A_{cont} - A_{test}) / A_{cont} \times 100$

Where A_c and A_s are the absorbencies of the control and of the test sample, respectively. From a plot of concentration against % inhibition, a linear regression analysis was performed to determine the IC_{50} value of the sample.

2.10 *In Vitro* α-amylase inhibitory assay

A starch solution (1 % w/v) was prepared by stirring 1g starch in 100 ml of 20 mM of phosphate buffer (pH 6.9) containing 6.7mM of sodium chloride. The enzyme solution was prepared by mixing 27.5 mg of porcine pancreatic amylase α-amylase (PPA) in 100 ml of 20 mM of phosphate buffer (PBS, pH 6.9) containing 6.7mM of sodium chloride. To 100 µl of (2, 4, 8, 10, 15 µg/ml) plant extracts, 200 µl porcine pancreatic amylase was added and the mixture was incubated at 37 ºC for 20 min. To the reaction mixture 100 µl (1 %) starch solution was added and incubated at 37 ºC for 10 min. The reaction was stopped by adding 200 µl DNSA (1g of 3,5 di nitro salicylic acid, 30g of sodium potassium tartarate and 20 ml of 2N sodium hydroxide was added and made up to a final volume of 100 ml with distilled water) and kept it in a boiling water bath for 5 minutes. The reaction mixture diluted with 2.2 ml of water and absorbance was read at 540 nm.

For each concentration, blank tubes were prepared by replacing the enzyme solution with 200 µL in distilled water. Control, representing 100 % enzyme activity was prepared in a similar manner, without extract. The experiments were repeated thrice using the same protocol [20].

2.11 *In Vitro*α -Glucosidase Inhibition Assay

The inhibition of α-glucosidase activity was determined using the modified published method [21].One mg of α-glucosidase was dissolved in 100 ml of phosphate buffer (pH 6.8). To 100 µl of (2, 4, 8, 10, 15 µg/ml) plant extracts, 200 µl α -glucosidase were added and the mixture was incubated at 37ºC for 20 min. To the reaction mixture 100 µl 3mM *p* -nitrophenyl α-D-glucopyranoside (*p*-NPG) was added and incubated at 37 ºC for 10 min. The reaction was terminated by the addition of 2ml Na_2CO_3 0.1 M and the α-glucosidase activity was determined spectrophotometrically at 405 nm on spectrophotometer UV-VIS (Shimadzu UV-1800) by measuring the quantity of *p*-nitrophenol released from *p*-NPG.

Acarbose was used as positive control of α-amylase and α-glucosidase inhibitor. The concentration of the extract required to inhibit 50 % of α-amylase and α -glucosidase activity under the assay conditions was defined as the IC_{50} value.

a. Method for calculation of α-amylase and α -glucosidase inhibitory concentration (IC50)

The concentration of the plant extracts required to scavenge 50 % of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by:

I % = (Ac-As)/Ac X 100

Where Ac is the absorbance of the control and as is the absorbance of the sample [22].

Values are presented as mean ± standard error mean of three replicates. The total phenolic content, flavonoid content, flavonols content, reducing power and IC_{50} value of each plant material was calculated by using Linear Regression analysis.

3. Results and Discussion

The results showed that the extract exhibited good antioxidant property and dose dependent α-amylase and α- glucosidase inhibitory activities by *in vitro* assay using starch as substrate.

3.1 Total phenol, flavonoid and flavonols content of the extracts

Polyphenols have been said to be important phytochemicals with significant antioxidant capacities and other important medicinal characteristics. Total phenolic content in the plant extract was determined by the FC method and the calibration curve developed using Gallic acid. A regression equation was obtained from the standard curve and the amount of Gallic acid in the water extract of *O. indicum* was calculated from

the regression equation: y = 0.0013x + 0.0498, R^2 = 0.999 where y was the absorbance and x was the Gallic acid equivalent (mg/g).The investigation showed that the plant under study contain a very good amount of total phenolics (17.65 ±0.25 mg GAE/g dry plant material) which is well compared with the methanol extract of *Terminalia arjuna* (20.862 mg GAE/g dry plant material) [23].

Total flavonoid contents in the plant extracts were calculated as rutin equivalent (mg/g) using the equation based on the calibration curve: y = 0.0182x - 0.0222, R^2 = 0.9962, where y was the absorbance and x was the Rutin equivalent (mg/g) and the flavonols content in the plant was estimated as quercetin (mg/g) equivalent using the equation based on the calibration curve: y = 0.0049x + 0.0047, R^2 = 0.9935, where y was the absorbance and x was the quercetin equivalent (mg/g). The experimental result showed the presence of appreciable amount of flavonoid 5.12 ± 0.005 mg/gm) and flavonols (4.31 ± 0.012 mg/gm) in the water extract of *O. indicum*.

The result of investigation also showed a very good reducing power (4.91±0.006 mg/gm dry plant material) with the water extract of the plant. The reducing power of the extract was evaluated in ascorbic acid equivalent (AAE) in milligram per gram (mg/g) of dry extract using the following equation based on the calibration curve: y = 0.0023x - 0.0063, R^2 = 0.9955 where y was the absorbance and x was the ascorbic acid equivalent (mg/g).

It has been established that phenolic compounds are the major plant compounds with antioxidant activity and this activity is due to their redox properties. Phenolic compounds are a class of antioxidant agents which can be adsorbed and neutralize the free radicals [24].

Flavonoids and flavonols are regarded as one of the most widespread groups of natural constituents found in the plants. It has been recognized that both flavonoids and flavonols show antioxidant activity through scavenging or chelating process [25].

The results strongly suggest that phenolics are important components of these plants. The other phenolic compounds such as flavonoids, flavonols, which contain hydroxyls are responsible for the radical scavenging effect in the plants. Thus after determining the total phenol content of the plants, the antioxidant activities of the plants were estimated for their free radical scavenging activity.

3.2 DPPH free radical scavenging activity

The evaluation of anti-radical properties of the methanol extract of *O indicum* was executed by DPPH radical scavenging assay. The 50 % inhibition of DPPH radical (IC_{50}) by the plant extract was determined (Table 1), a lower value would reflect greater antioxidant activity of the sample. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [26]. The antioxidant effect is proportional to the disappearance of the purple colour of DPPH in test samples. Thus antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colourless stable molecule 2, 22-diphenyl-1-hydrazine is formed and as a result of which the absorbance (at 517 nm) of the solution is decreased.

Hence the more potent antioxidant, more decrease in absorbance is seen and consequently the IC_{50} value will be minimum. In the present study the potent radical scavenging activity (IC_{50} = 0.28 ± 0.008 mg/gm dry extract) was shown by the water extract of *O. indicum*.

Table 1: Anti-oxidant activities of the water extract of *O. indicum*

Name of the plant	Total phenolic content (GAE mg / g dry extract)	Total flavonoid content (Rutin equivalent mg / g dry extract)	Total flavonols content (Quercetin equivalent mg / g dry extract)	Reducing power (Ascorbic acid equivalent mg / g dry extract)	DPPH Free radical scavenging ability (IC_{50} mg / g dry extract)	ABTS Free radical scavenging ability (IC_{50} mg / g dry extract)
O. indicum	17.65 ± 0.25	5.12 ± 0.005	4.31 ± 0.012	4.91 ± 0.006	0.28 ± 0.008	0.27 ± 0.001

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

3.3 Scavenging activity of ABTS radical action

The antioxidant activity of the methanol extract of *O. indicum* using ABTS assay was also carried out. The antioxidant effect is proportional to the disappearance of the colour of ABTS in test samples. Concentration of sample that could scavenge 50 % free radical (IC_{50}) was used to determine antioxidant capacity of sample compared to standard. Sample that had IC_{50} < 50 ppm, it was very strong antioxidant, 50-100 ppm strong antioxidant, 101-150 ppm medium antioxidant, while weak antioxidant with IC_{50} > 150 ppm. A strong inhibition was observed (IC_{50} =0.27 ± 0.001 mg/gm dry ext.) with the water extract of the plant under investigation.

3.4 *In Vitro* α -amylase inhibitory assay

α –amylase is one of the key enzymes that play a role in digestion of starch and glycogen and carbohydrate metabolism. Its inhibition is one of the strategies for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity. It is involved in carbohydrate metabolism and thus inhibiting it would lead to reduced post prandial blood sugar [27].

A number of medicinal plants and their formulations are used for treating diabetes in the traditional Indian Ayurvedic system as well as in ethno medicinal practices as their principal bioactive components showed good α –amylase inhibitory and antioxidant properties [28].

The result of experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. The water extract (2-15µg/ml) of *O. indicum* exhibited potent α -amylase inhibitory activity in a dose dependent manner. The water extract of the plant showed inhibitory activity from 3.28 ± 0.04 to 20 ± 0.02 % with an IC_{50} value of 0.75 µg dry extract (Table 2). Acarbose is a standard drug for α –amylase inhibitor. Acarbose at a concentration of (2-15 µg/ml) showed α –amylase inhibitory activity from 6.99 ± 0.03 to 56.17 ± 0.05 % with an IC_{50} value 0.32 µg dry extract. A comparison of α–amylase inhibitory activity between the standard drug and plant extracts has been depicted in fig. 1. So the plant extract might be used as starch blockers since it prevents or slows the absorption of starch in to the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltriose and other simple sugars. In our study, water extract of the plant showed maximum α- amylase inhibitory activity (IC_{50} = 0.75 µg dry extract) which could be attributed to the presence of polyphenols (21.34 ± 0.25 mg/g)

and flavonoids (0.35±0.31) because polyphenols are not only capable of reducing oxidative stress but also of inhibiting carbohydrate hydrolysing enzymes because of their ability to bind with proteins [1].

Our results are in accordance with the previous study wherein, there is a positive relationship between the total polyphenol and flavonoid content and the ability to inhibit intestinal α-glucosidase and pancreatic α-amylase [29-30].

Table 2: *In vitro* anti-diabetic activity of the water extract of *O. indicum* using alpha amylase method and comparison with standard drug acarbose

SL No	Plant extract/ standard drug	Concentration µg/ml	% of Inhibition	IC $_{50}$ µg dry extract
1	Water extract of *O. indicum*	2 4 8 10 15	3.28±0.04 5.14±0.06 10.71±0.03 14.32±0.05 20.00±0.02	0.75
2	Acarbose	2 4 8 10 15	6.99±0.03 11.58±0.05 26.45±0.02 41.64±0.06 56.17±0.05	0.32

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

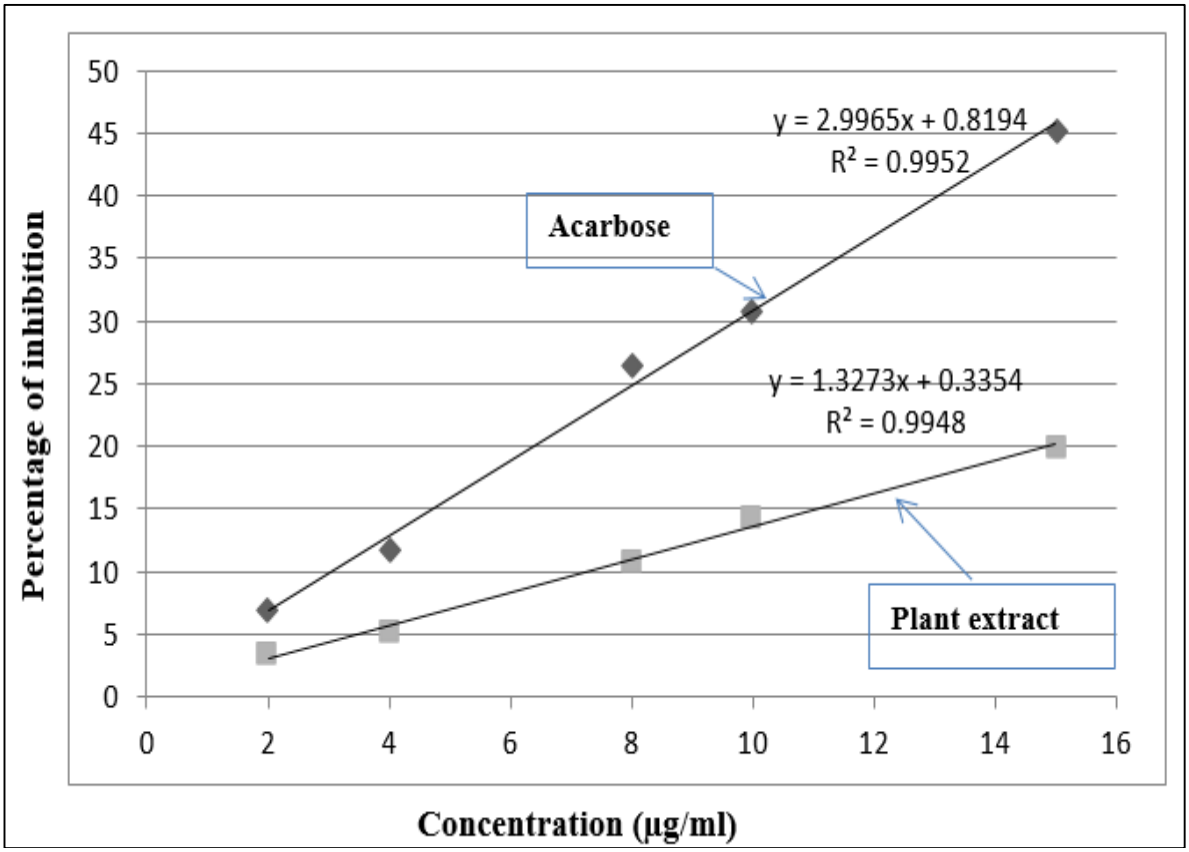

Fig 1: *α*-Amylase inhibitory activity of Acarbose vs. water extract of *O. indicum*

3.5 *In Vitro α* -glucosidase inhibitory assay

α-glucosidase is a glucosidase located in the brush border of the small intestine. It breaks down starch and disaccharides to glucose. *α*-glucosidase inhibitors are oral anti-diabetic drugs used for diabetes mellitus type 2 that work by inhibiting the digestion of carbohydrates (such as starch and table sugar). Carbohydrates are normally converted into simple sugars (monosaccharides), which can be absorbed through the intestine. Hence, *α*-glucosidase inhibitors reduce the impact of carbohydrates on blood sugar. *A-glucosidase* inhibitors from natural sources have received tremendous attention because of the highly abundant compounds in nature and their promising biological activities. On the basis of literatures published worldwide, it has been established that natural products isolated from medicinal plants showed potent *α*-glucosidase inhibitory activity. Structurally these natural product inhibitors includes terpene, alkaloid, quinine, flavonoid, phenol, phenylpropanoid, and steride frameworks rich in organic acid, ester, alcohol, and allyl functional groups. A majority of the compounds reported contain flavonoid, terpene, and phenylpropanoid ring structures [31].

The results of anti-diabetic activity using *α*-glucosidase inhibitory assay of water extracts of *O. indicum* stem are shown in Table 3. The extract revealed a significant inhibitory action of *α*-glucosidase enzyme. The percentage inhibition at 2-15 µg/ ml concentrations of *O. indicum* extract showed a dose dependent increase in percentage inhibition. The percentage inhibition varied from 19.62 % - 5.74 % for highest concentration to the lowest concentration.

Thus the inhibition of the activity of *α*-glucosidase by *O. indicum* would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as

a result the reduction of postprandial blood glucose level elevation [32]. A comparison of *α*–glucosidase inhibitory activity between the standard drug and plant extracts has been depicted in fig. 2.

In this study acarbose was also used as a standard drug for α-glucosidase inhibitor. Acarbose at a concentration of (2-15 µg/ml) showed α-glucosidase inhibitory activity from 29.57 ± 0.14 to 41.82 ± 0.08 % with an IC_{50} value 0.46 µg dry extract.

This indicates that the water extract of *O. indicum* is very potent α-amylase and α-glucosidase inhibitor in comparison with acarbose. This could be justified that the nature of some extract constituents (phenols, flavonoids saponins, steroids, alkaloids, terpenoids) present in the extract could be responsible as being effective inhibitors of α-amylase and α-glucosidase.

Table 3: *In vitro* anti-diabetic activity of the water extract of *O. indicum* using alpha glucosidase method and comparison with standard drug acarbose

SL No	Plant extract/ standard drug	Concentration µg/ml	% of Inhibition	IC $_{50}$ µg dry extract
1	Water extract of *O. indicum*	2	5.74 ± 0.09	0.80
		4	8.13 ± 0.07	
		8	13.88 ± 0.12	
		10	17.70 ± 0.09	
		15	19.14 ± 0.11	
2	Acarbose	2	29.57 ± 0.14	0.46
		4	31.58 ± 0.17	
		8	34.93 ± 0.12	
		10	38.28 ± 0.09	
		15	41.82 ± 0.08	

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

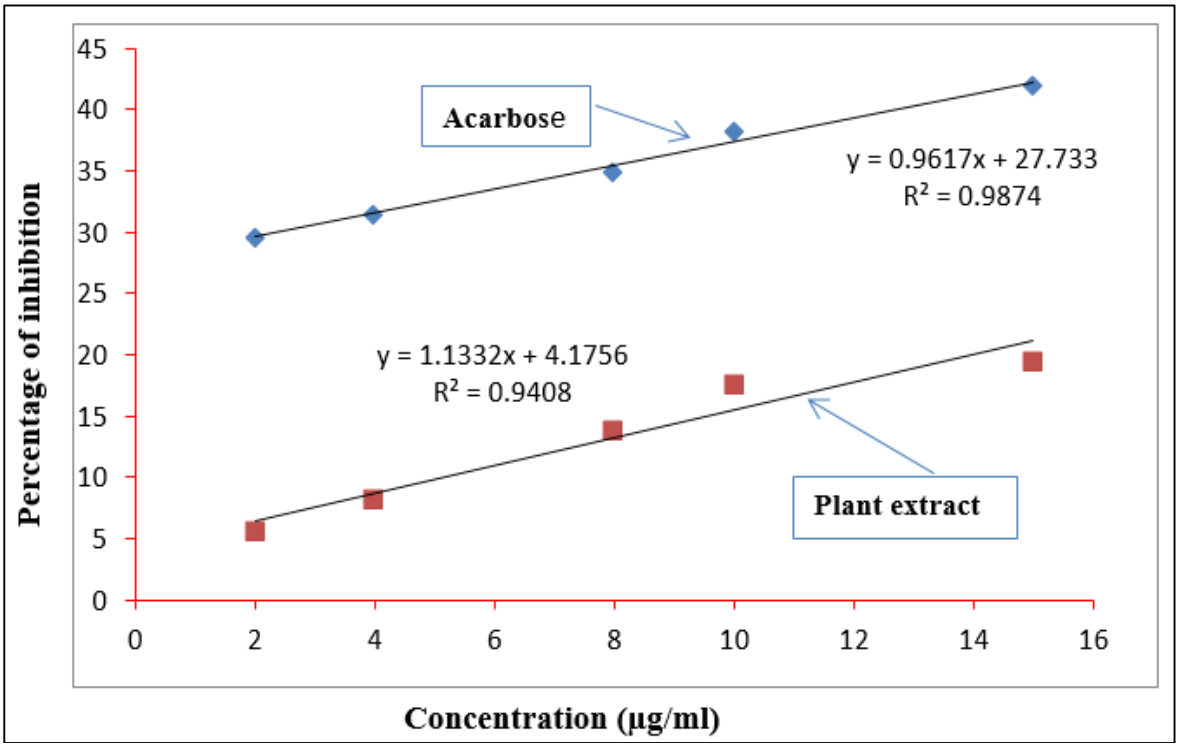

Fig 2: *α* -Glucosidase inhibitory activity of Acarbose vs. water extract of *O. indicum*

4. Conclusion

To investigate the biological activities of *O. indicum* seeds, the antioxidant and anti-diabetic activities of the water extract of the plant has been analysed. As a result, we found that the extract of *O. indicum* have free radical scavenging activity and inhibitory activity against α-amylase and α-glucosidase and this therapeutic potentiality could be exploited in the management of post prandial hyperglycaemia in the treatment of type 2 diabetes mellitus. Although the effects of *O. indicum* extract have been established *in vitro*, these results indicate that *O. indicum* has potential as a crude drug and a dietary health supplement.

The plant showed significant enzyme inhibitory activity, so the compound isolation, purification and characterization which are responsible for inhibiting activity, have to be done for the usage of anti-diabetic agent. Further studies are also required to elucidate whether the plant have anti-diabetic potential by *in vivo* for corroborating the traditional claim of the plant.

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