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Inhibitory effect of *C. esculenta* on α-Amylase and α-glucosidase enzyme activity: A LIBS based study

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Abstract
The present study deals with the evaluation of inhibitory activity of *Coloasia esculenta* for α-amylase and α-glucosidase enzymes. Correlation of activity with LIBS based phytoelemental profile of *C. esculenta* is uniqueness of the study. Varied concentrations ranging from 1 to 10 mg/ml of aqueous extract of *C. esculenta* were screened for their α-amylase and α-glucosidase inhibitory activities *in vitro*. Appreciable α-glucosidase and α-amylase inhibitory efficacies were observed in a concentration dependent manner. *C. esculenta* showed maximum α-glucosidase and α-amylase inhibition of 56.92 and 73.85% at 10mg/ml concentration with IC_{50} value at 6.05 and 6.19 mg/ml respectively. However, standard drug, acarbose showed maximum α-glucosidase and α-amylase inhibition of 95.52 and 98.17% at 1 mg/ml concentration with IC_{50} value at 1.83 and 0.25 mg/ml respectively. LIBS analysis reveals the presence of phytoelements viz. Mg, Ca, K and Na which are well known antidiabetic elements and hence could be responsible for inhibitory activities of both the carbohydrate hydrolyzing enzymes. Thus, it could be stated that, the plausible mechanism for antidiabetic efficacy of *C. esculenta in vivo,* could be due to its α-glucosidase and α-amylase enzyme inhibition activity and as such it can be explored as a potential candidate for the management of diabetes.

Keywords: *Coloasia esculenta*, Corms, *in vitro*, α-amylase, α-glucosidase

Introduction
Diabetes is a metabolic disorder related with glucose metabolism. A number of pharmacological approaches are used to control diabetes by different modes of action but inhibition of conversion of carbohydrate in glucose and its absorption from the intestine play a vital role in keeping a check on postprandial hyperglycemia which has been recognized as the most primitive metabolic abnormality [1]. There are two important enzymes of carbohydrate metabolism viz. α-amylase and α-glucosidase. α-amylase acts as a key enzyme in the carbohydrate metabolism and responsible for hydrolysis of complex starch to oligosaccharides. α-glucosidase converts oligosaccharides, trisaccharides and disaccharides into monosaccharides [2]. Inhibition of these two enzymes delays the degradation of dietary carbohydrates as well as intestinal absorption of glucose resulting into fall of high postprandial blood glucose level [3]. Various inhibitors are currently in use clinically such as acarbose, miglitol and voglibose. These potent inhibitors are generally associated with side effects such as distended stomach, diarrhoea etc, due to bacterial infection of undigested carbohydrates present in the colon [4]. Therefore, natural α-amylase and α-glucosidase inhibitors put forward a striking stratagem for controlling postprandial hyperglycaemia.
Colocasia esculenta belongs to the Araceae family and commonly known as 'Taro' in English and 'Arvi' in Hindi. *C. esculenta* is also reported to display antidiabetic, antiinflammatory, antioxidant and anticancer activities [5]. *C. esculenta* corms extract showed considerable antibacterial and antioxidant activities [6, 7].
Present research is an extension of our previous study in order to validate our earlier results and correlate corms antidiabetic attribute with the presence of certain set of trace elements present in the plant. Various trace elements have been found to play a role in regulating or potentiating insulin action [8, 9, 10]. Many medicinal plants have been studied for the presence of their trace elements to find out a possible correlation between them and their medicinal properties [11, 12]. LIBS is an atomic emission spectroscopic technique, it is fast and direct analytical technique for elemental analysis of solid, liquid, or gaseous materials, with no or very little sample preparation [13] and hence LIBS have inherent quality of no chemical contaminations at the time of analysis.

Hence, the present study was aimed to evaluate the LIBS based antidiabetic elemental profile of *C. esculenta* corms based on their inhibitory activity against α-amylase and α-glucosidase *in vitro*.

Material and Methods

Plant material

Corms (1 Kg) of *C. esculenta* (Araceae) were purchased from the local market of Allahabad, U.P., India and identified by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, Allahabad, India. A voucher specimen has been submitted to the University herbarium (No. MRL/CE/02).

Preparation of extract

The corms of *C. esculenta* were first peeled off, washed well and shade dried. Dried Corms were then boiled in distilled water for 48 hours. The extract of corms was filtered through Whatmann filter paper no. 45 and the filtrate was concentrated and dried in lyophilizer to obtain dry powder. The finally prepared *C. esculenta* corms extract powder was a dark brown solid material (9.7g) which was dissolved in distilled water for further experiments for evaluating its enzyme inhibitory activity.

Chemicals

All the chemicals and solvents used in these assays were of high purity (99%). Sodium acetate, sodium chloride, sodium hydroxide, sodium phosphate, sodium potassium tartrate, sodium carbonate, 3,5-dinitrosalicylic acid (DNSA) and starch were obtained from HiMedia, New Delhi, India. Enzymes viz. α-glucosidase, porcine pancreatic α-amylase (PPA) and para-nitrophenyl α-D-glucopyranoside (pNPG) were purchased from Sigma Aldrich, New Delhi, India. Acarbose was purchased from Bayer Scientific.

Antidiabetic Assays - *in vitro*

α-amylase inhibition assessment

The effect of the plant extract on α-amylase inhibitory activity was carried out using a modified procedure[14] of McCue and Shetty, 2004. A total of 250µL of varied concentrations of sample ranging from 1 to 10 mg/ml was placed in a tube and 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution was added. The content of the tubes were pre-incubated at 25 ºC for 10 minutes, after which 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added. The reaction was terminated by adding dinitrosalicylic acid reagent. The content of each test tube was diluted with distilled water and the absorbance measured at 540 nm in a spectrophotometer. A control was also prepared using the same procedure. The α-amylase inhibitory activity was calculated by the following equation:

α-Amylase % Inhibition = {(Ac – As)/Ac}×100

Where, Ac and As are the absorbance of the control and sample, respectively

α-glucosidase inhibition assessment

The effect of the plant extract on α-glucosidase activity was determined by the method [15] of Kim *et al*., 2005. A total of 50µL of different concentrations of sample ranging from 1 to 10 mg/ml was pre-incubated with 0.02M sodium phosphate buffer (pH 6.9) containing α-glucosidase at 25 °C for 10 minute, after which 3.0mM substrate solution of p-nitropheynyl glucopyranoside in 20 mM phosphate buffer (pH 6.9) was added. The reaction mixture was incubated at 37 ºC for 20 minutes and then quenched by adding 0.1M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow colored para-nitrophenol released from p-nitropheynyl glucopyranoside at 405 nm. A control was also prepared using the same procedure. The α-glucosidase inhibitory activity was calculated by the following equation:

α-Glucosidase % Inhibition = {(Ac – As)/Ac}×100

Where, Ac and As are the absorbance of the control and sample, respectively

LIBS set up for Element detection

The LIBS spectra of aqueous extract of *C. esculenta* corms were recorded for identifying the presence of best set of elements responsible for their antidiabetic efficacy. A pulsed laser beam from a Q-switched Nd: YAG laser (Continuum Surellite III-10) was focused on the sample surface using a converging lens (Quartz) of 30 cm focal length, the temperature of the locally heated region rose rapidly and resulted in plasma formation on sample surface. The emitted light from micro-plasma was collected using an optical fiber tip placed in the vertical plane at 45º with respect to the laser beam and finally fed into an entrance slit of the spectrometer (Ocean Optics LIBS2000+) equipped with CCD. The spectrometer has resolution of 0.1 nm in wavelength range from 200-500 nm and 0.75 nm in wavelength range 550-1100 nm, respectively [16].

Statistical Analysis

The entire group of data was statistically evaluated using one-way ANOVA, followed by a post hoc Scheffe's test using the SPSS computer software, version 7.5. The values were considered significant when $P<0.05$. Experiments were done in triplicate and the mean value was reported as mean±S.D.

Results

α-amylase inhibitory activity

Fig. 1 and Fig. 2 illustrate the α-amylase inhibitory activity of *C. esculenta* corms and standard drug, acarbose respectively. Results in both the cases were observed in a dose dependent manner. In case of *C. esculenta* corm maximum inhibition of 73.85% was observed at the concentration of 10mg/ml. Whereas, standard drug, acarbose showed maximum inhibition of 98.17% at 1mg/ml concentration. The IC_{50} values calculated from the linear regression of the percent inhibition activity versus sample concentrations was found to be 6.19 and 0.25mg/ml for *C. esculenta* corms and the standard drug, acarbose respectively. Since, the enzyme inhibitory activity is inversely proportional to IC_{50} value therefore, a lower IC_{50} means better enzyme inhibitory activity.

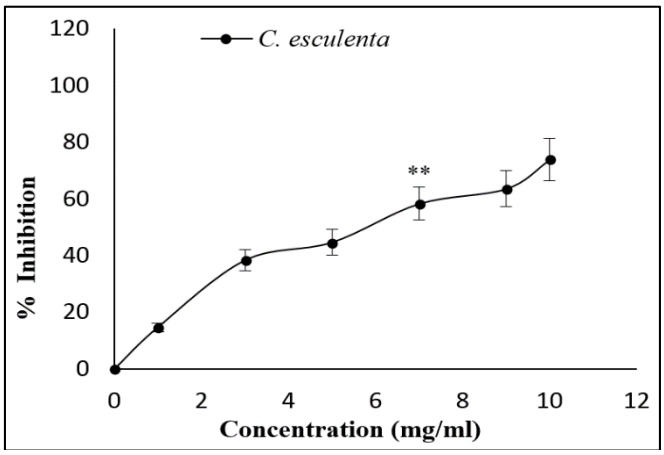

Fig 1: Inhibition of α-amylase enzyme activity of *C. esculenta*. **P<0.01 as compared with control

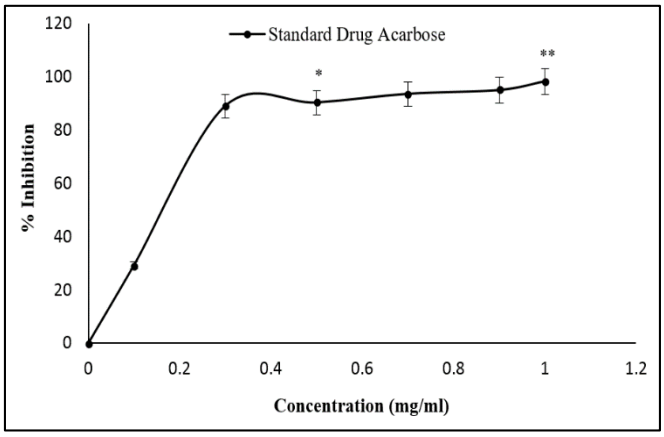

Fig 2: Inhibition of α-amylase enzyme activity of standard drug, acarbose. **P<0.01, *P<0.05 as compared with control

α-glucosidase inhibitory activity

Fig. 3 demonstrates α-glucosidase inhibitory activity of *C. esculenta* corms and standard drug, acarbose. Just like α-amylase inhibitory activity the results of α-glucosidase inhibitory activity were also found to be in a dose dependent manner in addition to a significant percent inhibition of 56.92% in *C. esculenta* corm at 10 mg/ml concentration. Similarly the IC_{50} value was also found to be 6.05 mg/ml, confirming thereby that the enzyme inhibitory activity of *C. esculenta* corm was more for α-glucosidase than for α-amylase. At the same concentration standard drug, acarbose showed 95.52% maximum inhibition and its IC_{50} value was found to be 1.83 mg/ml.

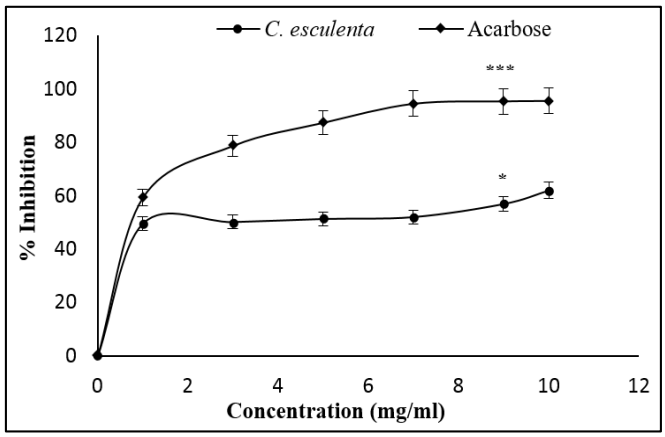

Fig 3: Inhibition of α-glucosidase enzyme activity of *C. esculenta* and acarbose. ***P<0.001, *P<0.05 as compared with control

Analysis of glycemic elemental profile of *C. esculenta* corms

LIBS spectra of the plant extract *C. esculenta* corms were recorded in different spectral ranges for their elemental screening in order to identify the trace elements responsible for their bioactivities. Figs. 4a and 4b shows the LIBS spectra of the aqueous extract of *C. esculenta* corms in two spectral ranges viz. 200-500 and 600-850 nm respectively. Spectral studies reveal that the plant extract contain several trace elements viz. Mg, Ca, Na and K in addition to C, O, H and N in significant concentrations. It is important to identify the specific concentration ratios of these elements in order to define their roles in diabetes and other chronic diseases. According to Boltzmann distribution law, the intensity of a spectral line of a particular element is directly proportional to its concentration, therefore evaluation of the concentration ratios of these elements by taking the ratio of intensity of these elements to the intensity of a reference line is necessary.

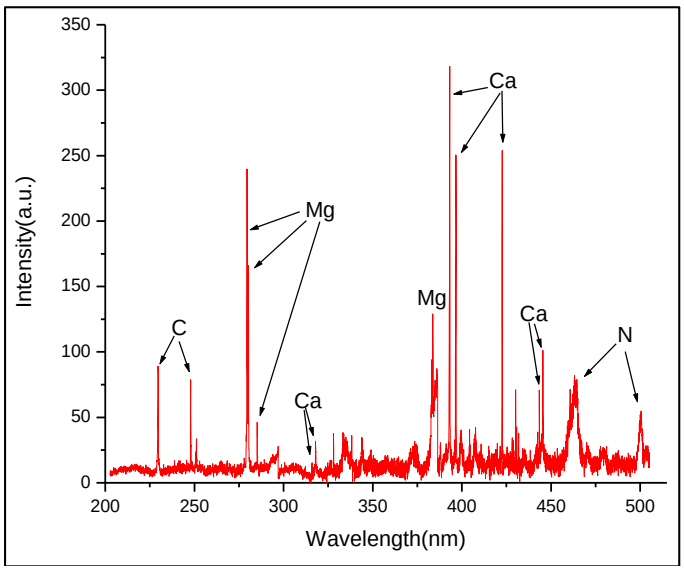

Fig 4a

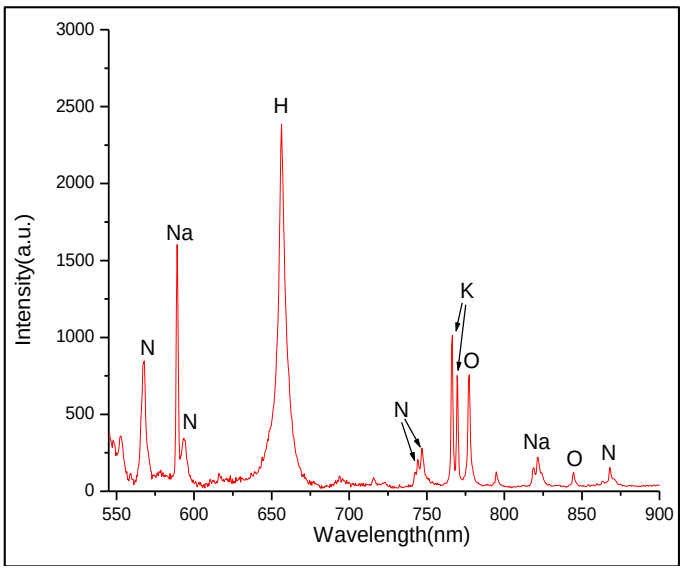

Fig 4b

Fig 4a and 4b: LIBS spectra of *C. esculenta* corms in the range of 200-500 & 600-850nm

To ascertain the concentration of Mg, Ca, Na and K elements in the plant extract, the relative concentrations of these essential elements have been evaluated by measuring the intensity of the selected lines in triplicate from the LIBS spectra of the sample based on Boltzmann distribution law. The relative intensity ratios of different constituent elements of the plant extract are given in Table 1. Table 1 clearly shows the relative intensities of elements present in *C. esculenta* corms with respect to C (247.8 nm) and it indicates that the concentration of Ca is greater than Mg (Ca >Mg). While, the relative intensities of elements present in *C. esculenta* corms with respect to O (777.4 nm) is shown in Table 2 which indicates that the concentration of Na is greater than K (Na > K).

Table 1: Intensity Ratio of elements of *C. esculenta* corms with respect to C (247.8 nm) Spectral range 200-500nm

Element	Wavelength	Intensity	Intensity ratio
C	247.856	177.5	1
C	229.689	396.5	2.233802817
Ca	422.673	501	2.822535211
Ca	393.366	1149	6.473239437
Ca	396.847	808	4.552112676
Mg	285.213	93	0.523943662
Mg	280.271	387	2.18028169

Table 2: Intensity Ratio of elements of *C. esculenta* corms with respect to O (777.4 nm) Spectral range 600-850nm

Element	Wavelength	Intensity	Intensity ratio
H	656.273	36303	16.45648
N	868.028	254	0.115141
N	567.956	6821.5	3.092248
O	777.417	2206	1
O	844.636	204	0.092475
Na	589.592	4603	2.086582
K	766.49	2909	1.318676
K	769.896	2411.65	1.093223

Discussion

The observed significant inhibitory activity of *C. esculenta* corms against α-amylase and α-glucosidase enzymes suggests that the plant could be explored further as an effective and safe promising therapeutic agent for treating diabetes by controlling the enzyme activity during carbohydate metabolism. Thus, *C. esculenta* corms could also be employed for formulations preparations associated with better efficacy and free from toxicity and side effects as compared to syntheic drugs. Evaluation of antidiabetic activity *in vitro* with possible phytoconstituents and mechanisms for blood glucose control in diabetes is well reported [17].

Bioactivities of medicinal plants are not only due to the presence of certain natural products as biomarkers but also phytoelements dependent, as trace elements present in plants also play an important role in glucose metabolism. Thus, curative properties of the *C. esculenta* corms extract for hyperglycemia may be due to a specific set of trace elements present. LIBS analysis of the *C. esculenta* corms extract showed appreciable concentrations of Mg, Ca, Na and K. All these four elements play a vital role in maintaining proper glucose levels.

The fourth most abundant mineral in the body is Mg which is involved in a number of biochemical reactions[18] and plays an important role in the transport mechanism of glucose in the cell membrane. It is also involved in the secretion, and binding activity of insulin[19]. Normal concentrations of K are required for optimal secretion of insulin [20]. Hypokalemia reduces the capacity of the pancreas to secrete insulin and therefore is recognized as a reversible cause of glucose intolerance [21]. Some studies suggest that the low levels of serum K than the optimum level can cause insulin resistance [22, 23]. Na is diuretic in nature and plays an important role in the transport of metabolites. Hence, Na/K ratio for food is an important factor in the prevention of hypertension and arteriosclerosis, where Na enhances and K depresses blood pressure [24].

Sufficient levels of Ca are required for release of insulin [25] as it is reported to play an important role in glucose tolerance factor, which decreases the blood glucose level by utilizing insulin [8]. Mg and Ca manage the blood glucose levels and also restore the antioxidant activity in type 2 diabetic rats [26]. Accordingly, the use of *C. esculenta* as an oral drug supplement might be beneficial for diabetic patients having abnormal levels of Mg and Ca.

Finally, it could be summarized that *C. esculenta* corms possess significant pancreatic *α*-amylase as well as *α*-glucosidase inhibitory activities. Potent antidiabetic activity *in vivo* of *C. esculenta* corms has already been done in diabetic rats by our research group. Therefore, it could be suggested that *in vivo* antidiabetic action of the corms extract may be due to inhibition of α-amylase and α-glucosidase enzymes. Moreover, these results of *in vitro* studies scientifically supports *in vivo* antidiabetic activity of *C. esculenta* corms as well.

Conclusion

Conclusively it could be stated that *C. esculenta* corms could be explored further as an effective α-glucosidase inhibitor which retards the digestion of carbohydrates and slows down the absorption of glucose. Hence, it proved itself as a novel therapeutic agent for reducing postprandial glucose specially in diabetic patients.

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