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Research Article

IN VITRO ANTIDIABETIC AND ANTIOXIDANT ACTIVITIES OF THE METHANOLIC EXTRACT OF *ALPINIA PURPURATA* STEM

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ABSTRACT

Objective: In the present study the methanolic extract of stem of *Alpinia purpurata* was studied for alpha amylase and alpha glucosidase inhibition using an *in vitro* model.
Methods: The methanolic extract was also examined for its antioxidant activities by using free radical 1,1-diphenyl-2-picryl hydrazyl (DPPH) scavenging method.
Results: The study revealed that the different concentration of extracts exhibit potent radical scavenging activity using DPPH as substrate. The stem of *Alpinia purpurata* exhibited significant α-amylase (70%) and α-glucosidase (87.8%) inhibitory activities at the concentration 100 µg/ml respectively and well compared with standard acarbose drug.
Conclusion: Therefore, it is suggested that the methanolic extract of stem of *Alpinia purpurata* is a potential source for natural antidiabetic and antioxidant compounds and could have potential use in the management of diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that affects the metabolism of carbohydrates, fat and protein. It is characterized by hyperglycemia, in which blood sugar levels are elevated either because the pancreas do not produce enough insulin or cells of the body do not respond properly to the insulin produced [1]. Type 1 diabetes results from inadequate synthesis of insulin by β-cells of the pancreas, while type II diabetes is characterized primarily by insulin resistance (a condition in which peripheral cells do not respond normally to insulin) or β-cell dysfunction [2]. The treatments for diabetes is reduction of the demand for insulin, stimulation of insulin secretion, enhance the mode of action of insulin at the target tissues and inhibition of degradations of oligo- and disaccharides [3,4]. The enzymes alpha glucosidase are responsible for the breakdown of oligo- and disaccharides to monosaccharides. The inhibitory action of these enzymes leads to a decrease of blood glucose level. The activity of alpha amylase enzymes which is responsible for the collapse of starch to more simple sugars (dextrin, maltotriose, maltose and glucose). The alpha amylase inhibitors delays the glucose absorption rate thereby maintaining the serum blood glucose in hyperglycemic individuals [5]. Inhibitors of α-amylase and α-glucosidase delay the breaking down of carbohydrates in the small intestine and diminish the postprandial blood glucose excursion [6]. Antioxidants such as ascorbic acid, carotenoids and phenolic compounds are more effective which possess free radical chain reaction breaking properties [7]. They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions [8]. 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 [9,10].

Alpinia purpurata (Vieill.) K. Schum (red ginger) is a herbacious perennial plant, internationally known in the ornamental plant market as potted plant, landscape accent and cut flower [11]. The rhizome has sharp odour, which could improve appetite, taste and voice. It is also used for headache, rheumatism, sore throat and renal disease [12]. The plant possesses moderate antibacterial and anticancer activities, which may be due to the presence of secondary metabolites in

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the leaves of *A. purpurate* [13]. In addition to the proposed anti-inflammatory activity, its phytomedicinal potential to treat tuberculosis is also described [14]. The chemical constituents shows that the presence of volatile oil, chiefly sesquiterpene, hydrocarbons, sesquiterpene alcohols, gingerole., starch, tannins flavonoids like galangin [15-18]. Therefore, in the present study, the antidiabetic and antioxidant activities of the methanolic extract of stem of *Alpinia purpurata* were evaluated employing in vitro assay methods.

MATERIALS AND METHODS

Collection of plant material

The stem of *Alpinia purpurata* were collected in the month of May from the mullipatti, pudukkottai, Tamil Nadu, India. The plant was identified and rhizomes of *Alpinia purpurata* were authenticated and confirmed from Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirapalli, and Tamil Nadu for identifying the plants. The voucher specimen number SGP001 (27.11.2018).

Chemicals and reagents

Alpha (α)-Glucosidase, porcine pancreas alpha (α)-amylase, *p*-nitrophenyl-α-D-glucopyranose (*p*-NPG), 3,5-dinitrosalicylic acid (DNS), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 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 ($Na2HPO_4$) sodium chloride, sodium hydroxide, potassium ferricyanide, ferric chloride ($FeCl_3$) were from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used including the solvents, were of analytical grade.

Preparation of methanol extracts

The stem of *Alpinia purpurata* were washed in running water, cut into small pieces and then shade dried for a week at 35-40ºC, after which it was grinded to a uniform powder of 40 mesh size. The methanol extracts were prepared by soaking 100 g each of the dried powder plant materials in 1 L of methanol using a soxhlet extractor continuously for 10 hr. The extracts were filtered through what mann filter paper No. 42 (125mm) to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure. The final dried samples were stored in labeled sterile bottles and kept at -20ºC. The filtrate obtained was used as sample solution for the further isolation [19]

Antioxidant activity (DPPH free radical scavenging activity) determination

The antioxidant activity of the stem of *Alpinia purpurata* was examined on the basis of the scavenging effect on the stable DPPH free radical activity [20]. Ethanolic solution of DPPH (0.05 mM) (300 µl) was added to 40 µl of rhizomes of *Alpinia purpurata* with different concentrations (20 - 100 µg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 540 nm. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation [21].

Percent (%) inhibition of DPPH activity = [(A - B) / A] x 100
Where B and A are the absorbance values of the test and of the blank sample, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined.

Alpha-Amylase Inhibitory Assay

This assay was carried out using a modified procedure of McCue and Shetty, 2004. A total of 250μL of stem of *Alpinia purpurata* (20-100 µg/ml) was placed in a tube and 250 μL of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5mg/mL) was added. This solution was preincubated at 25ºC for 10 min, after which 250 μL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at for 25ºC for 10min. The reaction was terminated by adding 500 μL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5min and cooled to room temperature. The reaction mixture was diluted with 5mL distilled water and the absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water [22].
The α-amylase inhibitory activity was calculated as percentage inhibition:

%Inhibition = [(Abs control - Abs extract) / Abs control] x 100

Alpha-Glucosidase Inhibitory Assay

The effect of the stem of *Alpinia purpurata* on α-glucosidase activity was determined according to the method described by Kim *et al*., 2005 using α-glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophenyl glucopyranoside (pNPG) was prepared in 20mM phosphate buffer, and pH 6.9. 100 μL of α- glucosidase (1.0U/mL) was preincubated with 50 μL of the different concentrations (20-100 µg/ml) of the extracts for 10min. Then 50 μL of 3.0mM (pNPG) as a substrate dissolved in 20mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37ºC for 20min and stopped by adding 2mL of 0.1M Na_2CO_3.The α-glucosidase activity was determined by measuring the yellow-colored paranitrophenol released from pNPG at 405 nm. The results were expressed as percentage of the blank control [23].

The α-glucosidase inhibitory activity was calculated as percentage inhibition:
%Inhibition = [(Abs control - Abs extract) / Abs control] x 100

Statistical Analysis

All assays were conducted in triplicate. Statistical analyses were performed with SPSS 16.0 for an analysis of variance (ANOVA) followed by Duncan's test. Differences at $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Antioxidant activity of stem of Alpinia purpurata by DPPH method

The result showed that the stem of *Alpinia purpurata* had better percentage antioxidant activities at high concentrations when compared with ascorbic acid (Table 1). The compound showed 85 % activity at concentration 100 µg/ml while ascorbic acid gave 94.69 % at the same concentration (fig. 1). The previous study suggested that the lupeol has antioxidant properties by scavenging free radicals, decreasing lipid peroxidation and increasing the endogenous blood antioxidant enzymes levels [25].

Table 1 Antioxidant activity of stem of *Alpinia purpurata* by DPPH activity

S.No	Concentrations	Scavenging Effect (%)	
		rhizomes of *Alpinia purpurata*	Ascorbic acid
1	20 (µg/ml)	57±1.56	41.60±1.33
2	40 (µg/ml)	80.7±1.24	66.85±1.37
3	60 (µg/ml)	82.4±1.35	76.74±1.42
4	80 (µg/ml)	83.3±1.42	82.34±1.47
5	100 (µg/ml)	95±1.20	94.69±1.50

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

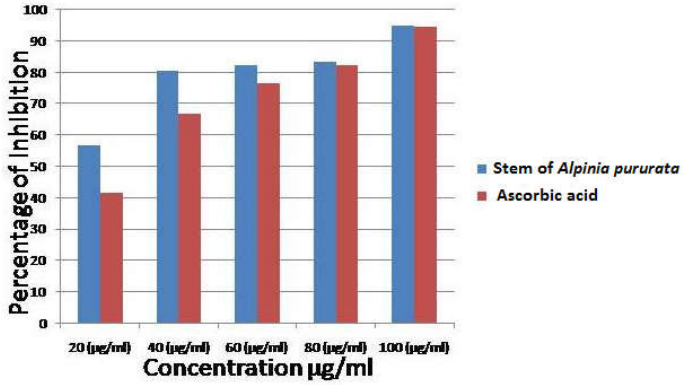

Fig 1 Antioxidant activity of stem of *Alpinia purpurata* by DPPH activity

In vitro alpha amylase inhibitory assay

In this study the *in vitro* alpha amylase inhibitory activities of the methanolic extract of stem of *Alpinia purpurata* was investigated. The result of experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against alpha amylase enzyme. The stem of *Alpinia purpurata* (20-100 µg/ml) of the various concentrations exhibited potent α-amylase inhibitory activity in a dose dependent manner. The stem of *Alpinia purpurata* showed inhibitory activity from 28±0.25 to 70±0.37% at concentration 100 µg/ml (Table 2). Acarbose is a standard drug for α-amylase inhibitor. Acarbose at a concentration of (20-100 µg/ml) showed α-amylase inhibitory activity from 39.85±0.24 to 85.97±0.37% at the same concentrations 100 µg/ml. A comparison of α-amylase inhibitory activity between the standard drug has been depicted in fig. 2. 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 [26]. The isolated compounds were tested for their antidiabetic potential in vitro by inhibition of α-amylase enzyme. Total saponins, Lupeol and stigmasterol showed higher alpha amylase inhibitory activity which confirms its antidiabetic potential was reported [27].

Table 2 *In vitro* antidiabetic activity of the stem of *Alpinia purpurata* using alpha amylase method and comparison with standard drug acarbose.

S.No	Concentrations	Alpha amylase (%)	
		Stem of *Alpinia purpurata*	Acarbose
1	20 (µg/ml)	28±0.25	39.85±0.24
2	40 (µg/ml)	32.8±1.24	60.21±1.37
3	60 (µg/ml)	51.4±1.35	67.20±1.42
4	80 (µg/ml)	61.4±1.42	77.25±1.47
5	100 (µg/ml)	70±0.27	85.87±0.37

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

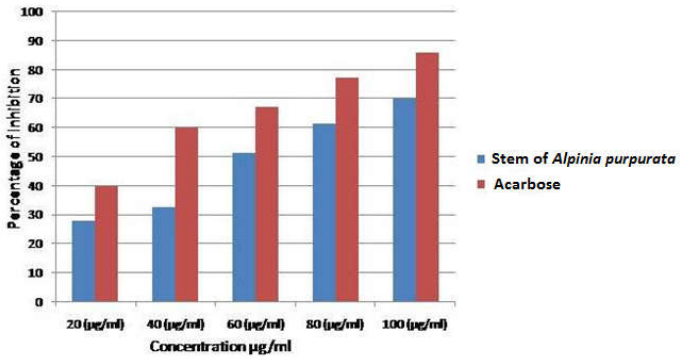

Fig 5 α-Amylase inhibitory activity of Acarbose vs stem of *Alpinia purpurata*

In Vitro α-glucosidase inhibitory assay

The results of antidiabetic activity using α- glucosidase inhibitory assay of the methanolic extract of stem of *Alpinia purpurata* are shown in Table 3. The extracts revealed a significant inhibitory action of α-glucosidase enzyme. The percentage inhibition at 20-100 µg/ ml concentrations of extracts showed a dose dependent increase in percentage inhibition.

The percentage inhibition varied from 65±0.49 -87.8±0.91 for highest concentration to the lowest concentration. Thus the inhibition of the activity of α-glucosidase by extracts 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. A comparison of α-glucosidase inhibitory activity between the standard drug has been depicted in fig. 3.

In this study acarbose was also used as a standard drug for α-glycosidase inhibitor. Acarbose at a concentration of (20-100 µg/ml) showed α-glucosidase inhibitory activity from 72.70 ±1.40 to 95.68±1.38 % with an IC50 value 45.03±1.03 µg/ml. This indicates that the stems of *Alpinia purpurata* is very potent α-amylase and α-glucosidase inhibitor in comparison with acarbose [27]. The hypoglycemic activity of crude extracts and isolated compounds (lupeol acetate, cis-p-coumaric acid, lupeol, β-sitosterol, trans-p-coumaric acid, linoleic acid, (+)-catechin, afzelin and quercitrin) was assessed by the ability to inhibit α-amylase and α-glucosidase enzymes [28].

Table 3 *In vitro* antidiabetic activity of the stem of *Alpinia purpurata* using alpha glycosidase method and comparison with standard drug acarbose.

S.No	Concentrations	Alpha glycosidase (%)	
		Stem of *Alpinia purpurata*	Acarbose
1	20 (µg/ml)	68±0.49	72.70 ±1.40
2	40 (µg/ml)	69.4±0.92	62.34±1.37
3	60 (µg/ml)	80.2±0.55	75.48±1.42
4	80 (µg/ml)	85.1±1.90	84.54±1.47
5	100 (µg/ml)	87.8±0.91	95.68±1.38

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

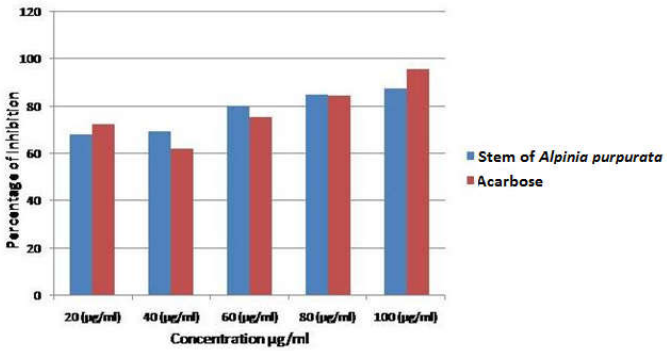

Fig 3 **α**- glycosidase inhibitory activity of Acarbose vs stem of *Alpinia purpurata*

CONCLUSION

The plant *Alpinia purpurata* stem showed significant enzyme inhibitory activity, so the compound which are responsible for inhibiting activity, have to be done for the usage of antidiabetic agent. To investigate the biological activities of extracts, the antioxidant and antidiabetic activities of the methanolic extract of rhizomes of *Alpinia purpurata* has been analysed. As a result, we found that the extracts 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 hyperglycemia in the treatment of type 2 diabetes mellitus.

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Author Contribution

All authors contribute equally to this manuscript.

Conflicts of Interests

The authors declare that they have no conflict of interest. It has not been published elsewhere. That it has not been simultaneously submitted for publication elsewhere. All authors agree to the submission to the journal.

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