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Anti-diabetic activity of *Ginger* tuber proteins : *In vitro* studies

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ABSTRACT

The study was done to investigate the *in vitro* antidiabetic activity of proteins from Ginger tuber. Phytochemicals of Ginger tuber proteins was analyzed by using standard methods. *In vitro* antioxidant studies were carried out for the Ginger tuber proteins using DPPH model, *In vitro* anti-diabetic studies was done by alpha amylase enzyme, alpha glucosidases enzyme inhibition studies and Glucose uptake in Yeast cells studies. The phytochemical screening of Ginger tuber proteins showed that the extract contains more proteins and contains negligible amount of other phytochemicals. The *in vitro* antidiabetic potential of extract was confirmed through alpha amylase enzyme, alpha glucosidases enzyme inhibition studies and Glucose uptake in Yeast cells studies. The results of the present study concluded that the Ginger tuber proteins possess significant antioxidant and antidiabetic activity. The potential pharmacological activity of Ginger tuber proteins might be due to the presence of proteins.

INTRODUCTION

The pervasiveness of diabetes mellitus has reached outbreak proportions and has affected more of adults worldwide [1]. It is considered as the biggest threat throughout the world because of metabolic disorder with increasing incidence. The World Health Organization expected that, about 3% of the world population will suffering with diabetes and it may doubled by year 2025 [2]. The alpha-amylase and alpha-glucosidase inhibition activities would slowdown the degradation of carbohydrate, which leads to decrease in the absorption of glucose, and hence reduction of postprandial blood glucose level[3]. The treatment for diabetes spent vast amount of medicines, diets, physical training and there is a need for new natural and synthetic compounds to overcome diabetic problems [4]. (Syamsudin, 2010, Manikandan et al, 2013). *Ginger plant parts like leaves, flowers and tubers* are reported to have medicinal properties. The Ginger root is rich in polyphenols, vitamin C, beta carotene, flavonoids and tannins which showed good antioxidant activity [5-6]. It is also reported that, the plant leaves of Ginger are having antioxidant properties. The above results, reports encouraged us to study the antioxidant activity of Ginger root proteins.

MATERIALS AND METHODS:

The required chemicals were purchased from Hi-Media Pvt. Ltd., Loba Cheme and SRL. All other chemicals used in the study were obtained commercially and were of analytical grade.

Extraction:

10g of cleaned *Ginger tubers* collected from authentic source, cleaned with 0.1% $KMnO_4$ solution, followed with double distilled water, crushed, shade dried and powdered (British Pharmacopoeia 100 mesh) and stored in glass bottle. The tuber powder mixed with 200 ml of double distilled water and vortexed for 4 hours at 20C using magnetic stirrer. The vortexed mixture is centrifuged at 6000 rpm for 20 minutes, the supernatant was separated. The supernatant was subjected to 55% ammonium sulphate precipitation and vortexed over night. The mixture was centrifuged at 10000 rpm. The precipitated protein was collected and subjected to dialysis using 2.5kDa molecular cutoff biomembrane against water for 76 hours with an interval of 6 hours. The dialyzed precipitated was separated and stored at -10C for further analysis.

PHYTOCHEMICALANALYSIS:

The proteins of *Ginger* roots was subjected to phytochemical

analysis using following standard protocols.

The protein estimation was carried according to Bradford's method using BSA as standard. Absorbance was read at 535nm. Concentration of protein was calculated accordingly using standard graph. Total phenolics were determined according to the method of Folin Ciocalteu reaction using Gallic acid as a standard. Absorbance was read at 750 nm and the concentration was calculated using the standard graph accordingly. Ascorbic estimation was carried out according to Sadasivam S., Manickam. The absorbance was read against a reagent blank at 540nm. The concentration was calculated on the basis of the standard curve. Sugar estimation was done according to Dubois method. The absorbance was read at 520 nm. The amount of total sugar present in the given unknown sample solution was calculated using the standard calibration curve. Flavonoids estimation was done according to Cheon et al by using Quercetin as a standard. The absorbance was measured at 415 nm and the concentration was calculated accordingly [7-11].

Antioxidant activity:

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was assessed according to the method of Shimada et al. with minor modifications [12-13]. *Piper longum* at a concentration of 25µg each was mixed in 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M acetate buffer pH 5.5. The resulting solutions were then incubated at 37C for 30 min and measured spectro photometrically at 517 nm. BHA (400 µM), Ascorbic acid (400 µM) and α-tocopherol (400 µM) was used as positive control under the same assay conditions. Negative control was without any inhibitor or *Piper longum*. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The % DPPH radical scavenging activity of extracts of *Piper longum* was calculated from the decrease in absorbance at 517 nm in comparison with negative control.

In vitro methods employed in anti-diabetic studies

Inhibition of alpha amylase enzyme

A total of 500 µl of test samples and standard drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle [14-16].

Inhibition of alpha glucosidases enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1ml with 0.2 M Tris buffer pH 8.0 and various concentration of *Ginger* root proteins for 5 min at 37°C. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37°C. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method [17-18].

Glucose uptake in Yeast cells

The procured yeast cells (commercial baker's yeast) was washed in distilled water by repeated centrifugation till the supernatant clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of *Ginger tuber* proteins (15 mg) were added to 1mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37°C. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged and glucose was estimated in the supernatant. Metformin was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated. All the tests were performed in triplicate [19-20].

STATISTICAL ANALYSIS

Statistical analysis was done in SPSS (Windows Version 10.0.1 Software Inc., New York) using a one-sided student's t-test. All results refer to means ± SD. $P < 0.05$ was considered as statistically significant when compared to relevant controls.

RESULTS

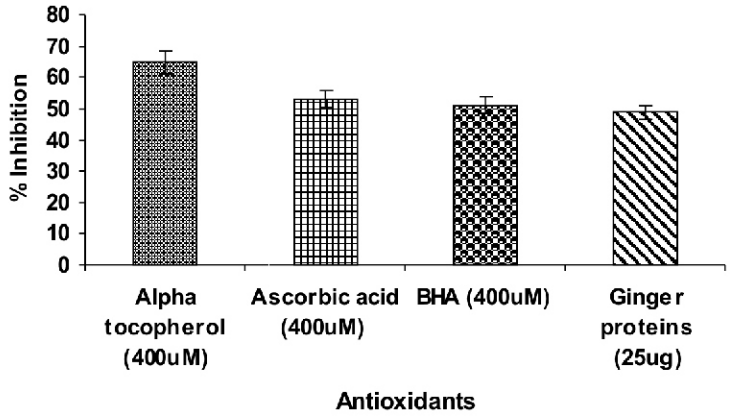

Figure 1. : DPPH radical scavenging activity of Ginger proteins

The results are mean ± S.D (n = 5)

Table 1. : Proximate analysis of dialyzed ammonium sulphate protein precipitate of Ginger extract

Phytochemicals	g%
Proteins	06.30
Carbohydrates	00.60
Polyphenols	00.04
Flavonoids	00.21
Ascorbic acid	00.15

DISCUSSION

The p*roteins* were isolated from Ginger as explained in materials and methods. The isolated dialyzed proteins were subjected to proximate analysis to estimate the phytochemicals presents. The analysis result showed in Table-1 that, the dialyzed protein extract rich in proteins and contains very negligible amount of free sugars, polyphenols, flavonoids and ascorbic acid. The proteins were analyzed for their antioxidant activity by DPPH radical scavenging activity where, lipid soluble α-tocopherol, water soluble Ascorbic acid and BHA were used as standard antioxidants at a maximum dosage of 400µM and proteins of *Ginger proteins* used at a dosage of 25µg. Fig-1 showed that, α-tocopherol, Ascorbic acid, BHA and the *Ginger proteins* showed inhibition of DPPH radicals. The inhibition of the *Ginger proteins* is comparable with standard proteins. This means, the *Ginger proteins* have good antioxidant activity when

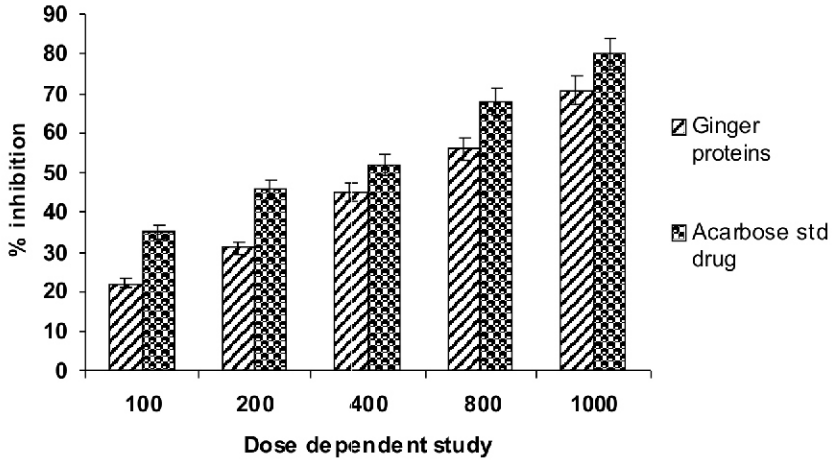

Figure 2. : α-amylase inhibitory activity by Ginger proteins

The results are mean ± S.D (n = 5)

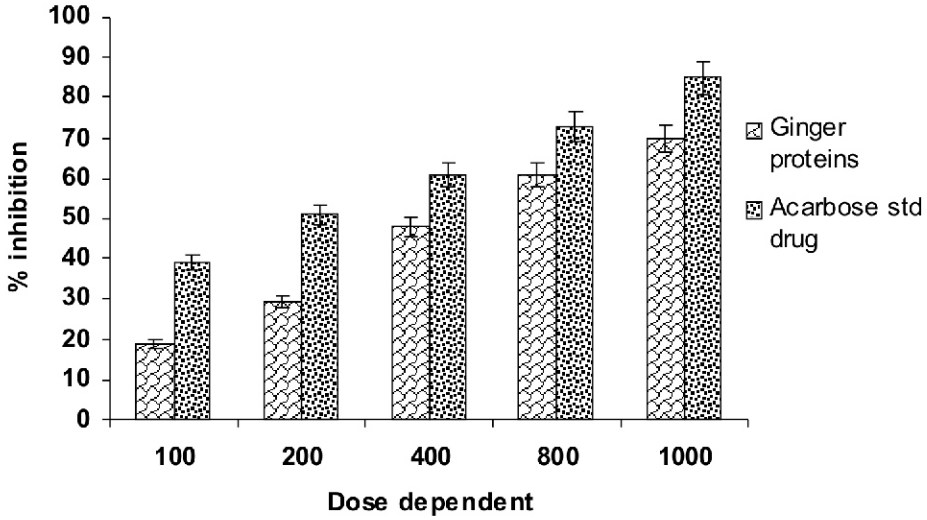

Figure 3. : The in vitro α-glucosidase inhibitory activity of Ginger proteins

The results are mean ± S.D (n = 5)

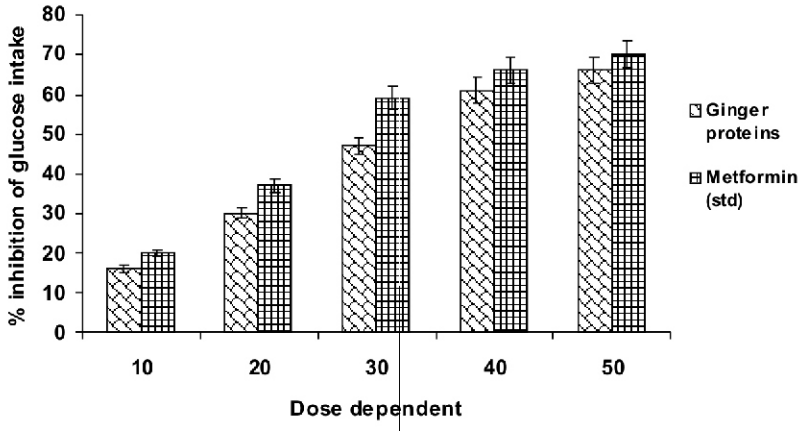

Figure 4. : Glucose uptake in yeast cells by Ginger proteins

The results are mean ± S.D (n = 5)

compared to standards.

The dose-dependent *in vitro* α-amylase inhibitory activity of *Ginger proteins* was done as explained in methods. It was found that, there is increase in percentage inhibitory activity with the increase in dosage against α-amylase enzyme. As a standard drug, Acarbose was used with similar dosage to compare inhibitory capacity of the *Ginger proteins*. Figure-2 showed that, the % inhibitory activity of *Ginger proteins* ranges a minimum of 22.11±0.03 (at 100µg/ml) to a maximum of 71.02±0.04 (at 1000µg/ml) where as the standard drug Acarbose showed % inhibitory activity ranges from 36.03±0.11 (at 100µg/ml) to a maximum of 79.21±0.01 (at 1000µg/ml).

The *in vitro* α-glucosidase inhibitory activity of *Ginger* proteins was studied as explained in methods. It was found that, there is increase in percentage inhibitory activity with the increase in dosage against α-glucosidase. As a standard drug, Acarbose was used with similar dosage to compare inhibitory capacity of the *Ginger* proteins. Figure-3 showed that, the % inhibitory activity of *Ginger* proteins ranges a minimum of 19.10±0.21 (at 100µg/ml) to a maximum of 71.23±0.31 (at 1000µg/ml) where as the standard drug Acarbose showed % inhibitory activity ranges from 39.11±0.01 (at 100µg/ml) to a maximum of 81.23 (at 1000µg/ml).

The rate of glucose transport across cell membrane in yeast cells system is as presented in Figure-4. In Yeast (*Saccharomyces cerevisiae*) glucose transport takes place via diffusion. After the treatment of the yeast cells with these *Ginger* proteins in a dose dependent manner, the glucose uptake was found to increase and the % increase in glucose uptake by the yeast cell at different glucose concentrations i.e. 25mM, 10mM and 5mM respectively was found. The *Ginger* proteins exhibited significant activity at all glucose concentrations in comparison with Standard drug Metformin.

CONCLUSION

The results of the present study demonstrated that the phytochemical analysis of crude *Ginger* proteins are rich in proteins and contains negligible amount of other phytochemicals. The *in vitro* antidiabetic potential of *Ginger* proteins was confirmed through different model systems and to compare the activity standard drugs are used. Hence, further purification and in vivo studies needed.

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