

## BIOLOGICAL EVALUATION OF GARLIC LEAVES EXTRACTS FOR CARBOHYDRATE METABOLISM, *IN VITRO*

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### ABSTRACT

*Allium Sativum. Linn* (Garlic) is one of the widely used herbs in food and medicine. The cloves of garlic are investigated for anti-diabetic potential; however, no efforts have been made to explore the potential of leaves to modify carbohydrate metabolizing enzymes viz.  $\alpha$ -amylase and glucoamylase. The inhibitors of these enzymes are used to achieve greater control over hyperglycemia in Type II Diabetes Mellitus. The present work was designed to investigate the inhibitory potential of the different extracts of *Allium Sativum*, on enzymes  $\alpha$ -amylase and glucoamylase. Different concentrations of the extracts were used to study inhibition of enzymatic activity of those enzymes. The current study for the first time, revealed  $\alpha$ -amylase and glucoamylase inhibitory potential of *A. Sativum* leaves and the study could be helpful to isolate and characterize hypoglycemic compounds.

**KEY WORDS:**  $\alpha$ -amylase, glucoamylase, *Allium Sativum* leaves, hypoglycemic.

### INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetics drugs.<sup>[1]</sup> Diabetes Mellitus (hyperglycemia) is one of the most chronicle disease widely speeded in the population with increase in age and obesity level. One of the therapeutic approaches in Type II diabetes is to lower the corresponding postprandial blood glucose. Some inhibitors viz. acarbose, miglitol and voglibose are currently relevant in clinical to inhibit glycosidase.<sup>[2, 3]</sup> However, many of them have their limitations such as non-

specificity, producing serious gastrointestinal disorders viz. bloating, abdominal discomfort, diarrhea and flatulence.<sup>[4]</sup> One therapeutic approach to prevent postprandial hyperglycemia is to retard the digestion and absorption of carbohydrates in the gastrointestinal tract through inhibition of enzymes such as  $\alpha$ -amylase and glucoamylase.  $\alpha$ -amylases hydrolyse complex polysaccharides to produce oligosaccharides and disaccharides which are then hydrolysed by  $\alpha$ -glucosidase to monosaccharide which are absorbed through the small intestines into the hepatic portal vein. Inhibitors of both  $\alpha$ -amylase and glucoamylase delay digestion and subsequent absorption of carbohydrates thereby lowering postprandial glucose levels.

Garlic, a member of the Liliaceae family, is a common food for flavor and spice and it is one of the herbs most commonly used in modern folkloric medicine. It was an important medicine to the ancient Egyptians as listed in the medical text Codex Ebers (ca. 1550 BC) especially for the working class involved in heavy labor because it was an effective remedy for many ailments such as heart problems, headache, bites, worms and tumor. A lot of work has been done on garlic bulbs but we found no literature investigating the garlic leaves. Given this situation regarding the paucity of research in this area, we have devoted this study to investigate the effect of garlic leaves on carbohydrate metabolizing enzymes, aiming at its possible utilization in fortifying mankind.

In current scenario, phytochemicals have received much attention in the treatment of diabetes for various reasons and many researchers have focused on isolation of hypoglycemic agents from medicinal plants.<sup>[5]</sup> Plant polyphenols and flavonoids are some of the naturally occurring antidiabetic agents<sup>[6]</sup>, which are known to show an inhibitory effect on carbohydrate hydrolyzing enzyme inhibition, by virtue of their capability to bind with proteins. This phenomenon contributes to lower postprandial hyperglycemia in diabetes.<sup>[7]</sup> To the best of our knowledge, there is no scientific evidence on the inhibitory effects of the *Allium Sativum* leaves on carbohydrate hydrolyzing enzymes. The aim of the present study was to evaluate the anti  $\alpha$ -amylase and glucoamylase inhibitory potential of *Allium Sativum* leaves.

## MATERIALS AND METHODS

**Chemicals:** Porcine pancreatic  $\alpha$ -amylase and glucoamylase were purchased from Fluka Chemicals. The chemicals and reagents used were of Analytical grade, the water used was glass-distilled.

### Plant Material and Extract Preparation

Garlic leaves were purchased from local market in Mumbai. They were washed properly with distilled water. The garlic leaves (100 g) were extracted with 300 ml petroleum ether, chloroform, ethyl acetate and ethanol, respectively by maceration on an orbital shaker at room temperature. Solvent was replenished every 24 h for 5 days to ensure that all possible compounds could be extracted. The resulting extracts were filtered and concentrated under reduced pressure. The filtered plant extracts were combined and Stored at 4 ° C until further study.

**Table 1 : Percentage yields of the extracts obtained.**

Extract	% Yield
Petroleum ether	1.64
Chloroform	2.67
Ethyl Acetate	9.15
Ethanol	11.65

### $\alpha$ -amylase assay

0.5 mL of the reaction mixture containing 0.1 mL (20-100 $\mu$ g/mL) modulator, 0.3 mL of 20mM phosphate buffer (pH 4.5), and 0.1 mL of  $\alpha$ -amylase (1.3 $\mu$ g) were incubated at 37° C for 30 minutes. Then 0.5 mL of Starch solution (10mg/mL prepared in 20mM phosphate buffer pH 7.0) was added and incubated further at 37° C for 30 minutes. The reaction was then terminated keeping the test tubes in boiling water bath for 1-2 minutes, cooled under running tap water. 1mL of DNS (3,5-dinitrosalicylic acid) was added and the test-tubes were kept in boiling water bath for 15 minutes. The test-tubes were cooled and diluted with 7mL distilled water. Acarbose was used as a standard. The absorbance was recorded at 530nm using spectrophotometer and liberated glucose was estimated. A unit activity (U) is defined as the mg of glucose liberated per mg of protein per minute. The maximum inhibition was determined from plots of percent inhibition versus modulator and calculated as below.

% activity= (enzyme activity of test / enzyme activity of control) x 100;

% inhibition = (100 -% activity)

### Glucoamylase assay

0.5mL of the reaction mixture containing, 0.1mL modulator (20-100 $\mu$ g/mL), 0.3mL of 100mM acetate buffer (pH 4.5), and 0.1mL of glucoamylase (1.3 $\mu$ g) were incubated at 37°C for 30 min. Then, 0.5mL of starch solution (5mg/mL prepared in 100mM acetate buffer pH 4.5) was added and incubated further at 37°C for 30 min. The reaction was terminated by

keeping the test-tubes in boiling water bath for 1-2 minutes, cooled under running tap water. The test-tubes were cooled and diluted with 7mL of distilled water. Acarbose was used as a standard. The absorbance was recorded at 530nm using spectrophotometer and liberated glucose was estimated. A unit activity (U) is defined as the mg of glucose liberated per mg of protein per minute. The maximum inhibition was determined from plots of percent inhibition versus modulator and calculated.

### Statistical analysis

The results are expressed as mean  $\pm$  standard error of mean. Experiments were performed in triplicate.

## RESULTS AND DISCUSSION

Diabetes is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin<sup>[8]</sup>. Recent decades have experienced a sharp increase in the incidence and prevalence of diabetes mellitus. Plant extracts have long been used for the ethno medical treatment of diabetes in various systems of medicine and are currently accepted as an alternative for diabetic therapy<sup>(9)</sup>. Recent advances in understanding the activity of intestinal enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase both are important in carbohydrate digestion and glucose absorption) have led to the development of newer pharmacological agents. As part of this research, we investigated the inhibitory effects of *A.Sativum* L. leaves against  $\alpha$ -amylase and glucoamylase compared to standard acarbose. We have established the inhibitory activity of *Allium Sativum* leaves against  $\alpha$ -amylase and glucoamylase. The activity displayed by each extract is shown in Figure.1, which revealed that chloroform and ethyl acetate extracts shows prominent decrease in the activity of glucoamylase enzyme at a concentration 1000 $\mu$ g/mL, whereas, ethyl acetate and ethanol extracts exhibits decrease in the activity of  $\alpha$ -amylase.

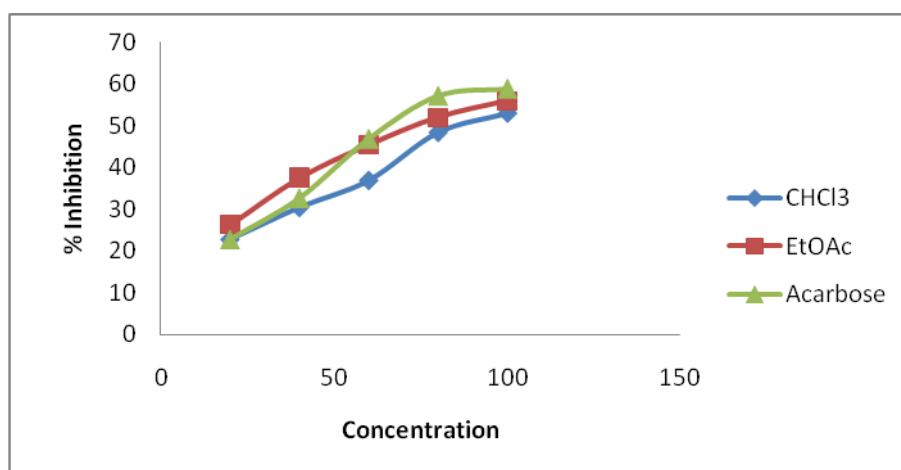
**Table 2: Effect of extracts on glucoamylase and  $\alpha$ -amylase activity**

S.No	Extract	Glucoamylase Activity	$\alpha$ -Amylase Activity
1	Acarbose	3.27	18.98
2	Petroleum ether	10.61	34.79
3	Chloroform	3.48	33.95
4	Ethyl Acetate	3.71	19.05
5	Ethanol	11.42	20.12

Concentration of the extracts = 1000 $\mu$ g/mL.

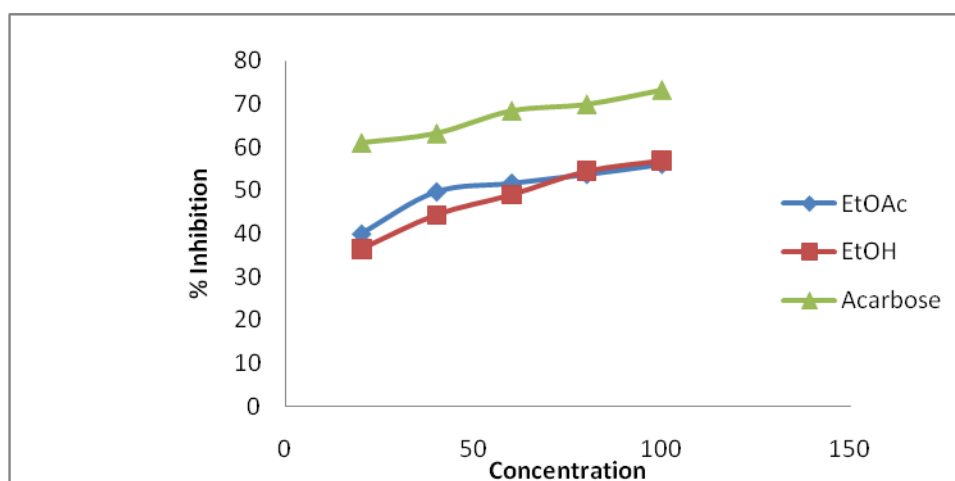
The extracts showing significant inhibition against  $\alpha$ -amylase and glucoamylase were selected to study effects of varying concentration ranging from 20 $\mu$ g/mL to 100 $\mu$ g/mL.

The percentage of inhibition ranged from 53.02% to 22.89%. in case of chloroform extract and 55.92% to 26.31% in the case of ethyl acetate extract. The standard drug acarbose exhibited inhibition from 58.94% to 22.89%. The  $IC_{50}$  value of chloroform and ethyl acetate extracts were  $89.77\mu\text{g/mL}$  and  $77.85\mu\text{g/mL}$  respectively, whereas the standard acarbose showed  $IC_{50}$  values,  $72.81\mu\text{g/mL}$ , Fig.1.



**Fig.1**Effect of  $\text{CHCl}_3$  and EtOAc extracts on glucoamylase in varying concentrations

Fig.1. shows the percentage inhibition of glucoamylase activity of chloroform and ethyl acetate leaves extract compared to standard, acarbose. The ethyl acetate and ethanol extracts of *A.Sativum* leaves showed moderate  $\alpha$ -amylase inhibition at varying concentration 20-100 $\mu\text{g/mL}$  with  $IC_{50}$   $58.21\mu\text{g/mL}$  and  $52.8\mu\text{g/mL}$  respectively. The standard Acarbose exhibited  $IC_{50}$   $50.19\mu\text{g/mL}$ , Fig.2.



**Fig.2:** Effect of EtOAc and EtOH extracts on  $\alpha$ -amylase in varying concentrations

## CONCLUSION

The treatment goal of diabetes patients is to maintain near normal levels of glycemic control, in both the fasting and post-prandial states. Many natural resources have been investigated with respect to suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine<sup>[10]</sup>. In this study, we have investigated the anti-diabetic potential of the *A. Sativum* leaves, which is used in traditional ayurvedic medicine for the treatment of several diseases. This valuable herb was not previously investigated for its invitro anti-diabetic activity. However, our study clearly established the anti-diabetic potential of *A. Sativum* leaves. Plants are natural reservoir of bioactive compounds that may be sources of lead compounds with glucoamylase and  $\alpha$ -amylase inhibition activity.

We observed that amongst the four extracts, chloroform and ethyl acetate extracts of *A. sativum* Leaves showed good  $\alpha$ -amylase inhibition whereas the ethyl acetate and ethanol extracts revealed a promising glucoamylase inhibition. The leaves of *A. Sativum* essentially contain herbal bioactive compounds inhibiting carbohydrate regulatory enzymes and therefore further structural characterization need to be carried out in order to identify the bioactive constituents. The present study was restricted to the preliminary screening of enzyme inhibition of the *Allium Sativum* leaves extracts.

## ACKNOWLEDGEMENT

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## REFERENCES

1. Hammer K.A, Carson C.F, Riley T.V, Antimicrobial activity of essential oils and other plant extracts, *J. Appl. Microbiol.*,1999, 86(6), 985.
2. Fernandes B, Sagman U, Auger M, Demetrio M, Dennism J.W, Beta 1-6 branched oligosaccharides as a marker of tumor progression in human breast and colon neoplasia , *Cancer Res*, 1991, 51, 718-23.
3. Matsuo T, Odaka H, Ikeda H, Effect of an intestinal disaccharide inhibitor (AO-128) .on obesity and diabetes, *Am. J. of Clin. Nutr.*, 1999, 55, 3145-3175.
4. Cheng Y.Y, Fantus I.G, Oral antihyperglycemic therapy for Type 2 DM, *Canadian Medicinal Association Journal*, 2005, 172, 213-226.
5. Pradhan R, DineshKumar, Mitra A, Some salient points in Type 2 Diabetes prevalence in rural Bengal, *Ethnomed*, 2009, 3 127-31.

6. Mai T.T, Thu N.N, Tien P.G, Vanchuyen N, Alpha glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents, *J. Nutr. Sci. Vitaminol* (Tokyo), 2007, 53, 267-76.
7. Harborne JB, *Methods in Plant Analysis Phytochemical Methods*, London Chapman and Hal, 1984, 1-32.
8. ADA, 2007, Diagnosis and classification of diabetes mellitus, *Diabetes Care*, 30: S42-S7.
9. Gupta A.D, Pundeer V, Bandle G, Dhar S, Ranganath I R, Kumar G.S, *Pharmacology*, 2009, 1, 200-208.
10. Hara Y, Honda M, The inhibition of  $\alpha$ -amylase by tea polyphenols, *Agricultural and Biological Chemistry*, 1990, (54)8, 1939-45.