PHYTOCHEMICAL SCREENING AND IN-VITRO ANTIDIABETIC ACTIVITY OF *ALLIUM SATIVUM* (GARLIC)

Vanitha NM[1] and Keshamma E[2*]
[1]Lecturer, Department of Microbiology, St. Joseph's College,
Lalbagh Road, Bengaluru, Karnataka, India.
[2]Assistant Professor, Department of Chemistry,
Government Boys College (Autonomous), Mandya, Mysore District, Karnataka, India.

***Corresponding Author**
Dr. Keshamma E,
Assistant Professor, Department of Chemistry,
Government Boys College (Autonomous),
Mandya – 571 401, Mysore District, Karnataka, India.
Email: keshamma.blr76@gmail.com

ABSTRACT

This study aimed for of phytochemical screening and evaluation of *in-vitro* antidiabetic activity of Garlic (*Allium sativum*). The garlic juice was prepared by adding 100 g of garlic with 250 mL of distilled water and crushed in a mixing machine. The resultant slurry was squeezed and filtered through a fine cloth and the filtrate was quickly frozen at -10°C until used. Results revealed that the *aq.* garlic extract at a concentration range of 25µg/mL, 50µg/mL, 100µg/mL, 200µg/mL, 300µg/mL, and 400µg/mL shown inhibition effect of 15.46%, 28.33%, 38.83%, 50.38%, 62.04%, and 75.26% respectively in alpha-amylase inhibition activity. The IC_{50} value of *aq.* garlic extract in alpha-amylase inhibition activity was found to be 27.29 µg/mL in comparison with the standard antidiabetic drug acarbose (51.89 µg/mL). Similarly, the *aq.* garlic extract at a concentration range of 25µg/mL, 50µg/mL, 100µg/mL, 200µg/mL, 300µg/mL, and 400µg/mL shown inhibition effect of 14.58%, 31.35%, 41.38%, 50.18%, 57.29%, and 75.95% respectively in alpha-glucosidase inhibition activity. The IC_{50} value of *aq.* garlic extract in alpha-glucosidase activity was found to be 30.56 µg/mL in comparison with the standard antidiabetic drug acarbose (41.52 µg/mL). In conclusion, anti-diabetic potential of aqueous garlic extract was demonstrated in this pilot study, and hence itcould be explored in the development of natural antidiabetic drugs.

Keywords: Garlic, *Allium sativum*, Diabetes mellitus, Antidiabetic, Alpha-amylase, Alpha-glucosidase

NeuroQuantology 2009; 4: 680-686

INTRODUCTION

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian books mention the use of plants in the treatment of various human ailments. India has about 45.000 species of plants and several thousand of them have been claimed to have medicinal properties.[1]Diabetes mellitus is a metabolic disturbance that progressively affects the functioning of various

body systems, and it is characterized by hyperglycemia, caused by an inherited and/or acquired deficiency in the production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. This insulin deficiency leads to an increase in the concentration of glucose in the blood and these damages many systems in the body, especially blood vessels and nerves. Hyperglycemia caused by a decrease in insulin production is called type 1 diabetes and hyperglycemia caused by insufficient use of insulin is called type 2 diabetes. Of these two types, type 2 diabetes is a major problem today and accounts for almost 95% of the total diabetic patients, and is expected to increase to 439 million by 2030.[1,2]

Treatment of type 2 diabetes is complicated by several factors: typically, insulin resistance, hyperinsulinemia, impaired insulin secretion, and reduced insulin-mediated glucose uptake and utilization. This type is treated with metformin, glibenclamide, α-glucosidase and α-amylase inhibitors.[3] In addition to the drugs currently available for the treatment of diabetes, traditional herbal medicines are also used worldwide for the treatment of diabetes. Inhibition of alpha-amylase enzymes elaborate in the digestion of carbohydrates can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet. Therefore, this can be a central strategy in the management of postprandial blood glucose level in type 2 diabetic patients and marginal patients.[4]

Studies have been conducted on oral antihyperglycemic agents from plants used in traditional medicine, and many plants have been found to have good activity.[5] The World Health Organization (WHO) has also recommended the evaluation of the efficacy of plants in conditions where we lack modern and safe medicines.[6] For this reason, scientific research has been directed towards natural anti-diabetic products that produce minimal or no side effects.

Garlic (*Allium sativum*) is fragrant herb and tuber-derived spice (Figure 1), and it is one of the most popular herbs used worldwide to reduce various risk factors associated with cardiovascular diseases. Garlic, a member of the Liliaceae family, is a common food for flavour and spice and it is one of the herbs most commonly used in modern folkloric medicine. Garlic 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 labour because it was an effective remedy for many aliments such as heart problems, headache, bites, worms and tumours. Garlic is stated to possess many therapeutic benefits. Garlic's strong odour is largely due to sulphur-containing compounds (e.g. S-allylcysteine sulphoxide), which are believed to account for most of its medicinal properties.[7]



Figure 1. Showing Garlic (*Allium sativum*) bulbs

With this background in the current study, we aimed for phytochemical screening and evaluation of *in-vitro* antidiabetic activity of Garlic (*Allium sativum*) bulbs.

MATERIALS AND METHODS

Extract Preparation

The garlic bulbs were procured from local markets, peeled, washed, and chopped into small pieces. The garlic juice was prepared by adding 100 g of garlic with 250 ml of distilled water and crushed in a mixing machine. The resultant slurry was squeezed and filtered through a fine cloth and the filtrate was quickly frozen at -10°C until used.[8]

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the aqueous (*aq.*)garlic extract was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, and its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[9]The phenolic content of the extract was determined from calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water and was expressed in mg gallic acid equivalent (GAE)/g of extract powder (mg GAE/g).

Total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination in *aq.* garlic extract.[10] The flavonoid content was determined from extrapolation of calibration curve which was made by preparing quercetin solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg quercetin equivalent (QE)/g of extract powder (mg QE/g).

Alpha-amylase inhibitory assay

The alpha-amylase inhibition assay of *aq.* garlic extractwas carried out by the method of Miller, (1959).[11]*Aq.* garlic extractand standard drug acarbose (25µg/mL, 50µg/mL, 100µg/mL, 200µg/mL, 300µg/mL, and 400µg/mL) were incubated for 10 minutes at 25°C with 500 µL of 20 mM sodium phosphate buffer (pH 6.8) with 20 µL of amylase (1U/mL). After pre-incubation, each tube was added with 1 mL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) and incubated for 15 min. One mL DNS was added to arrest the reaction. After that, the tubes were kept in a boiling water bath for 5 min and cooled to room temperature. After that, distilled water (10mL) was added to the reaction mixture, and the absorbance was measured at 540 nm. The test compound was not used in the preparation of the control samples. The following formula was used to determine the percent inhibition of alpha-amylase activity;

% Inhibition = (Abs control – Abs test) / (Abs control)

Alpha-glucosidase inhibition assay

The alpha-glucosidase inhibition assay of *aq.* garlic extractwas carried out as described by Matsui et al (1996) with slight modifications.[12]The different concentrations of *aq.* garlic extractand standard drug acarbose (25µg/mL, 50µg/mL, 100µg/mL, 200µg/mL, 300µg/mL, and 400µg/mL) were prepared. Phosphate buffer (1 mL; 100mM, pH 6.8) and 80 µL of test *aq.* garlic extract/ acarbose of concentrations (25µg/mL, 50µg/mL, 100µg/mL, 200µg/mL, 300µg/mL, and 400µg/mL) were added to 20 µL of alpha-glucosidase and incubated at 37°C for 10 minutes. Later, pNPG- 50µL (5mM) was added to the assay mixture to initiate the reaction. Then, the reaction mixture was incubated at room temperature for one hour and arrested the reaction by adding 2.5mL of 0.1 M Na_2CO_3. The absorbance was measured at 400nm to determine the activity of alpha-glucosidase activity. The following formula was used to determine the percent inhibition of alpha-glucosidase activity;

% Inhibition = (Abs control – Abs test) / (Abs control)

RESULTS

The results of quantitative estimation of phytochemicals in *aq.* garlic extract revealed that the total flavonoid quantity was found to be highest (23.58 mgQE/g extract) in *aq.* garlic extract when compared with total phenolic quantities (12.63 mgGAE/g extract) (Table 1).

Table 1: Quantitative estimation of phytochemicals in*aq.* garlic extract

Phytochemicals	***Aq.* garlic extract**
Total Phenolics	12.63 mgGAE/g extract
Total flavonoids	23.58 mgQE/g extract

Values are expressed mean; n=3

The results of effect of *aq.* garlic extract on alpha-amylase inhibition activity was represented in Table 2. The *aq.* garlic extractat a concentration range of 25µg/mL, 50µg/mL, 100µg/mL, 200µg/mL, 300µg/mL, and 400µg/mL showed inhibition effect of 15.46%, 28.33%, 38.83%, 50.38%, 62.04%, and 75.26% respectively in alpha-amylase inhibition activity. The IC_{50} value of *aq.* garlic extractin alpha-amylase inhibition activity was found to be 27.29µg/mL in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 51.89µg/mL.

Table 2: Effect of *aq.* garlic extracton alpha-amylase inhibition activity

Conc. of *aq.* garlic extract (µg/mL)	**Inhibition (%)**	**Conc. of Acarbose (µg/mL)**	**Inhibition (%)**
25	15.46	25	26.45
50	28.33	50	52.12
100	38.83	100	64.03
200	50.38	200	72.83
300	62.04	300	84.42
400	75.26	400	98.89
IC_{50} (µg/mL) = 27.29		IC_{50} (µg/mL) = 51.89	

Values were expressed Mean; n=3

The results of effect of *aq.* garlic extract on alpha-glucosidase inhibition activity was represented in Table 3. The *aq.* garlic extract at a concentration range of 25µg/mL, 50µg/mL, 100µg/mL, 200µg/mL, 300µg/mL, and 400µg/mL shown inhibition effect of 14.58%, 31.35%, 41.38%, 50.18%, 57.29%, and 75.95% respectively in alpha-glucosidase inhibition activity. The IC_{50} value of *aq.* garlic extract in alpha-glucosidase activity was found to be 30.56 µg/mL in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 41.52 µg/mL.

Table 3: Effect of *aq.* garlic extracton alpha-glucosidase inhibition activity

Conc. of *aq.* garlic extract (µg/mL)	**Inhibition (%)**	**Conc. of Acarbose (µg/mL)**	**Inhibition (%)**
25	14.58	25	21.34
50	31.35	50	42.49
100	41.38	100	53.57
200	50.18	200	58.29
300	57.29	300	73.28
400	75.95	400	85.70
IC_{50} (µg/mL) = 30.56		IC_{50} (µg/mL) = 41.52	

Values were expressed Mean; n=3

DISCUSSION

Ayurvedic medicine has long employed a number of herbs to treat a variety of ailments. Medicinal plants are the best source of bioresources for chemical entities for synthetic drugs, food supplements, modern and traditional medicine, nutraceuticals, and pharmaceutical intermediates.[13,14]Diabetes is an important human ailment afflicting many from various walks of life in different countries. A more practical approach would involve a series of *in-vitro*prescreens before testing a potential new hypoglycaemic agent in animals. This is because there are no perfect models for type 2diabetes, and there are social and financial constraints on the extensive use of animals in experimentation. Furthermore, alpha-amylase and alpha-glucosidase inhibitors have become a new treatment strategy to combat diabetes mellitus.[15] Hence the present study was conducted with the main purpose of phytochemical screening and evaluation of *in-vitro* antidiabetic activity of Garlic (*Allium sativum*).

Alpha-amylase and alpha-glucosidase are the enzymes responsible for digestion of carbohydrates and increasing the postprandial glucose levels in diabetic patients. Inhibiting their activity could help in controlling postprandial hyperglycemia.[16]Results of our study showedIC_{50} value of 27.29 µg/mL for *aq.* garlic extractin alpha- amylase inhibition activity which was comparable with that of IC_{50} value standard antidiabetic drug acarbose (51.89 µg/mL). Similarly, IC_{50} value of *aq.* garlic extractshowed 30.56 µg/mLin alpha-glucosidase inhibition assay, and which was also comparable with that ofstandard antidiabetic drug acarbose (41.52 µg/mL). These findings were comparable with the literature findingsreported by various other research investigators.

In a study by Mathew et al., ingestion of garlic juice resulted in better utilization of glucose in glucose tolerance tests performed in rabbits, while allicin at a dose of 250 mg/kg was 60% as effective as tolbutamide in alloxan-induced diabetic rabbits.[17] Garlic has been found to be effective in lowering serum glucose levels in STZ-induced as well as alloxan-induced diabetic rats and mice. Most of the studies showed that garlic can reduce blood glucose levels in diabetic mice, rats and rabbits.[18]Previous research studies consistently showed that S-allyl cysteine sulphoxide, (allicin), a sulphur-containing amino acid in garlic (200 mg/kg body weight), had a potential to reduce the diabetic condition in rats almost to the same extent as did glibenclamide and insulin.[19,20] Aged garlic

extract was also effective in preventing adrenal hypertrophy, hyperglycaemia and elevation of corticosterone in mice made hyperglycaemic by immobilization stress.[21] In addition, Liu and co-workers reported that both garlic oil and diallyl trisulphide improved glycaemic control in STZ-induced diabetic rats.[22]

CONCLUSION

In conclusion, theanti-diabetic potential of aqueous garlic extractswas demonstrated in this pilot study. Hence, aqueous extract of Garlic *(Allium sativum)*could be explored in the development of natural antidiabetic drugs.

REFERENCES

1. Hawkey CJ, Langman MJ. Non-steroidal anti-inflammatory drugs: overall risks and management. Complementary roles for COX-2 inhibitors and proton pump inhibitors. Gut. 2003;52(4):600-8.
2. Tripathi BK, Srivastava AK. Diabetes mellitus: complications and therapeutics. Med Sci Monit. 2006;12(7):130-47.
3. Bnouham M, Legssyer A, Mekhfi H, Ziyyat A. Medicinal plants used in the treatment of diabetes in Morocco. International Journal of Diabetes and Metabolism. 2002;10(1):33-50.
4. Subramanian R, Asmawi MZ, Sadikun A. In vitro α-glucosidase and α-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. Acta biochimica polonica. 2008;55(2):391-8.
5. Kesari AN, Kesari S, Singh SK, Gupta RK, Watal G. Studies on the glycemic and lipidemic effect of *Murraya koenigii* in experimental animals. Journal of Ethnopharmacology. 2007;112(2):305-11.
6. World Health Organization. WHO monographs on selected medicinal plants. World Health Organization, 1999; Vol. 2.
7. Augusti KT. Therapeutic values of onion (*Allium cepa* L.) and garlic (Allium sativum L.). Indian J Exp Biol 1996; 34: 634-640.
8. El-Demerdash, F.M., Yousef, M.I., El-Naga, N.I.A., 2005.Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats.Food and Chemical Toxicologiy, 43(1), 57–63.
9. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidative substrates by means of Folin-Ciocalteau reagent, Packer L. Methods in Enzymology. 1999; 299:152-78.
10. Ordonez AAL, Gomez JD, Vattuone MA, Lsla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chemistry. 2006;97(3):452-8.
11. Miller GL. Use of dinitro salicylic acid reagent for determination of reducing sugar. Analytical chemistry. 1959;31: 426-428.
12. Matsui T, Yoshimoto C, Osajima K, Oki T, Osajima Y. In vitro survey of *α-glucosidase* inhibitory food components. Bioscience, biotechnology, and biochemistry. 1996;60(12):2019-22.
13. O'Byrne KJ, Dalgleish AG, Browning MJ, Steward WP, Harris AL. The relationship between angiogenesis and the immune response in carcinogenesis and the progression of malignant disease. European journal of cancer. 2000;36(2):151-69.
14. O'Byrne KJ, Dalgleish AG. Chronic immune activation and inflammation as the cause of malignancy. British journal of cancer. 2001;85(4):473-83.
15. Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Nkeh-Chungag BN, Iputo JE. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J Med Plants Res. 2009;4(9):789-95.
16. Rackova L, Oblozinsky M, Kostalova D, Kettmann V, Bezakova L. Free radical scavenging activity and lipoxygenase inhibition of *Mahonia aquifolium* extract and isoquinoline alkaloids. Journal of inflammation. 2007; 4:1-7.
17. Mathew PT, Augusti KT. Studies on the effect of allicin (diallyl disulphide-

oxide) on alloxan diabetes I. Hypoglycaemic action and enhancement of serum insulin effect and glycogen synthesis. Indian J Biochem Biophys 1973; 10: 209–212.

18. Jamison JR. Garlic (*Allium sativum*). In: Clinical Guide to Nutrition and Dietary Supplements in Disease Management. London: Churchill Livingstone, 2003: 541-546.
19. Sheela CG, Augusti KT. Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic *Allium sativum* Linn. Indian J Exp Biol 1992; 30: 523–526.
20. Sheela CG, Kumud K, Augusti KT. Anti-diabetic effects of onion and garlic sulfoxide amino acids in rats. Planta Med 1995; 61: 356-357.
21. Kasuga S, Ushijima M, Morihara N, et al. Effect of aged garlic extract (AGE) on hyperglycemia induced by immobilization stress in mice. Nippon Yakurigaku Zasshi 1999; 1999: 191-197.
22. Liu C-T, Hse H, Lii C-K, Chen P-S, Sheen L-Y. Effects of garlic oil and diallyl trisulfide on glycemic control in diabetic rats. Eur J Pharmacol 2005; 516: 165-173.