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Comparative In vitro Evaluation of Anti-Diabetic Activity of Callus and Bulb Extract of *Allium sativum L.*

Babita Rana* and Rinku Patole
G.N. Khalsa College Matunga, Mumbai, Maharashtra-400019.

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***Corresponding Author Email:** babitarana2009@yahoo.com

Abstract
In the present research, comparative *in vitro* study has been made to evaluate the anti-diabetic potential of callus and bulb extract of *Allium sativum* L. Callus was induced from bulb explants in MS medium supplemented with 2, 4-D @ 0.5mg/l. sufficient amount of callus was obtained after 4 weeks of inoculation of explant. Callus was maintained by sub-culturing to have continuous supply throughout the project work. The extracts of callus and garlic bulb prepared in methanol were evaluated for their anti-diabetic activity by α-amylase and α-glucosidase inhibitory activities and were well compared with standard Acarbose drug. It could be concluded that due to the presence of anti-diabetic components the callus and plant extract have well prospective for the management of hyperglycemia. This knowledge will be helpful in isolating, characterization and purification of more potent anti-diabetic principle from callus culture to the clinical development of anti-diabetic therapeutics.

Keywords
Alium sativum, MS medium, α-amylase, α-glucosidase, anti-diabetic activity.

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INTRODUCTION
Diabetes is one of the major causes of premature death worldwide. As per WHO global report, in 2016 an estimated 1.6 million deaths were directly caused by diabetes. Diabetes prevalence has been rising more rapidly in developing countries like India. In next four years when India would surpass China to become the most populated country in the world it would also unfortunately become diabetic capital of the globe [1]. When diabetes is uncontrolled, it has dire consequences for health and well-being. It is a major cause of heart attacks, kidney failure, blindness, stroke and lower limb amputation [2]. In an effort to address this growing health challenge, scientists are focusing on herbal medicines as a source of hypoglycemic agents due to their more efficacy, fewer side effects and cost effectiveness [3]. The WHO has listed 21000 plant used for medicinal purposes worldwide. There are about 800 plants which have been reported to show anti-diabetic potential [4,5]. Anti-diabetic, anti-inflammatory, antiarthritic and chemotherapeutic role of medicinal plants could be attributed to occurrence of bioactive compound like flavonoids, phenolic compounds, terpenoids, coumarins and many other constituents [6].

Among different anti-diabetic plants, *Allium sativum* L. (garlic) is one of the major herbs used as spice worldwide. Phytochemical screening [7] revealed *Allium sativum* L. is a main source of sulfur containing compounds, predominantly S-alk-(en)yl-L-cysteine sulphoxides (Cs), being alliin the leading one. Volatile compounds such as allicin and lipid-soluble sulphur compounds such as diallyl disulphide, diallyl sulphide, ajoene, diallyl trisulphide associated with typical odour, taste, as well as biological and

therapeutic properties of *Allium sativum* L. Alliin (S-allyl cysteine sulfoxide) is the major active phytocomponent, localized in garlic cloves with hypoglycemic activities [8,9]. Inhibition of amylase and glucosidase enzyme involved in starch and glycogen digestion can be an important mechanism of action of phytocompounds which are arrtibuted to antihyperglycaemic effect [10]. Despite extensive investigations reported in literature regarding *in vitro* and *in vivo* anti-diabetic role of various medicinal plants including *Allium sativum* L. [11-14], there is no such report of *in vitro* anti-diabetic study of callus extract of *Allium sativum* L. so far. Thus, present study aimed at *in vitro* evaluation of anti-diabetic activity of the methanol extract of callus and bulb of *Allium sativum* L.

MATERIAL AND METHOD

Callus induction in garlic bulbs

Garlic bulbs were washed thoroughly in distilled water with 2 or 3 drops of liquid detergent such as 'Teepol' for 10-15 minutes and immediately transferred to laminar air flow for surface sterilization in 70% alcohol followed by treatment with 0.1% mercuric chloride sterilant for 10 minutes. The sterilant was decanted and bulbs were washed with autoclaved D/W to remove all the traces of sterilant

Murashige and Skoog medium [15] supplemented with 2,4-D (0.5, 2 and 5mg/l) was used to induce callus. The MS medium was autoclaved at 121ºC for 20 minutes and poured into culture tubes (25X150mm). Sterilized bulbs were inoculated onto MS medium containing 2,4-D at different concentrations. Culture tubes incubated in complete darkness at 27ºC for 10-12 days. Once the callus gets induced a photoperiod of 16 hrs light and 8 hrs dark with light intensity of 2500 lux provided by fluorescent lamps was maintained. Cultures were allowed to grow up to their maximum growth age i.e. 4 weeks which provided sufficient amount of callus to evaluate anti-diabetic activity.

Preparation of callus and bulb crude extract

Callus and bulbs were cleaned, dried at 50°C, grinded using motor and pestle and extracted with methanol using Soxhlet apparatus.

In vitro α-amylase inhibitory assay

Different concentrations (2, 4, 8, 10 and 20 mg/ml) of callus and bulb extract were prepared in 0.02M phosphate buffer of pH 6.9. To 100µl of each concentration 200 µl α-amylase enzyme solution was added [enzyme solution was prepared by mixing 27.5 mg of porcine pancreatic α -amylase in 100 ml of 20 mM of phosphate buffer (pH 6.9) containing 6.7mM sodium chloride] followed by incubation for 10 min at 25ºC. After pre incubation 100 µl of 1% starch solution (1g starch in 100 ml of 20mM of phosphate buffer of pH 6.9 containing 6.7mM of sodium chloride) was added. The reaction mixture was incubated at 37ºC for 10 min. The reaction was stopped by adding 200 µl of DNSA (1g of 3, 5 di nitro salicylic acid, 30g of sodium potassium tartarate and 20 ml of 2N sodium hydroxide was added and made up to a final volume of 100 ml with distilled water) and kept it in a boiling water bath for 5 minutes and cooled to room temperature The reaction mixture was diluted with 4 ml of water and absorbance was read at 540 nm. A blank showing 100% enzyme activity was prepared by replacing plant extract with 100 µl of buffer. For each concentration, blank tubes were prepared by replacing the enzyme solution with 200 µl distilled water. Positive control was prepared in a similar manner replacing plant or callus extract with standard Acarbose. Experiment was repeated thrice using the same assay [16].

Percentage inhibition of α-amylase activity was calculated as follow:

% α-amylase inhibition = (Ac-As)/Ac X 100

Where Ac is the absorbance of the control and As is the absorbance of sample. Values were presented as mean ± standard error of three replicates.

In vitro α -glucosidase Inhibition Assay [17]

To 100 µl of (2, 4, 8, 10, 20 mg/ml) callus and bulb extract, 200 µl of α -glucosidase (1mg of α -glucosidase dissolved in 100 ml of phosphate buffer of pH 6.8) was added and the mixture was incubated at 37ºC for 20 min to the reaction mixture 100 µl of 3mM p-nitro phenyl α -D-glucopyranoside (p-NPG as substrate) was added and incubated at 37 ºC for 10 min. The reaction was terminated by the adding 2ml of 0.1M Na_2CO_3 following which absorbance was measured at 405 nm by measuring the quantity of nitro phenol released from p-NPG. Acarbose was used as positive control for α-glucosidase inhibition assay. Experiment was repeated thrice using the same assay [16].

Percentage inhibition of α-glucosidase activity was calculated as follow:

% α-glucosidase inhibition =(Ac-As)/Ac X 100

Where Ac is the absorbance of the control and As is the absorbance of sample. Values were presented as mean ± standard error of three replicates.

RESULT AND DISCUSSION

Callus induction

Callus induction and establishment was best reported on MS medium supplemented with 2,4-D @2mg/l using bulb explants. These static cultures were allowed to grow up to their maximum growth age i.e. 4 weeks

Figure 1. Three-week old callus derived from garlic bulb explant

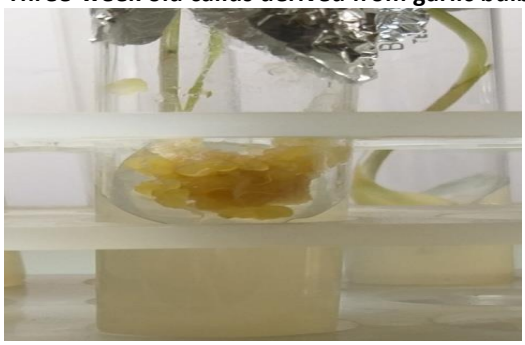


Figure 2. Four-week old callus derived from garlic bulb explant

In vitro *α*-amylase inhibitory assay

In vitro α -amylase inhibitory activity of the methanolic callus and plant extract of *Allium sativum* and their comparison with standard drug Acarbose is tabulated in **Table 1.** Results of experiment **(Graph 1)** showed that the methanol extract (2- 20 mg/ml) of callus and plant exhibited potent *α*-amylase inhibitory activity and it was concentration dependent. The bulb extract exhibited lower % inhibitory activity from 11.32±0.04 to 49.57±0.02 and for callus it was remarkably significant ranging from 73.03±0.05 to 80.25±0.08. Acarbose as a standard drug showed maximum *α*-amylase inhibitory activity.

***In vitro* *α*-glucosidase inhibitory assay**

Tabulated and graphical data **(Table 2, Graph 2)** of *α*-glucosidase inhibition assay plant extract showed % inhibitory activity from 87.52±0.09 to 98.06±0.11 while callus extract and standard drug activity is ranging from 70.10±0.10 to 83.72±0.04 and 75.65±0.14 to 98.08±0.04 respectively. The values of callus extract lying closer to standard drug indicate high potential of callus to serve as raw material in formulation of standard anti-diabetic drug.

Table 1. *In vitro* anti-diabetic activity of methanol extract of *Allium sativum* using α-amylase inhibitory method.

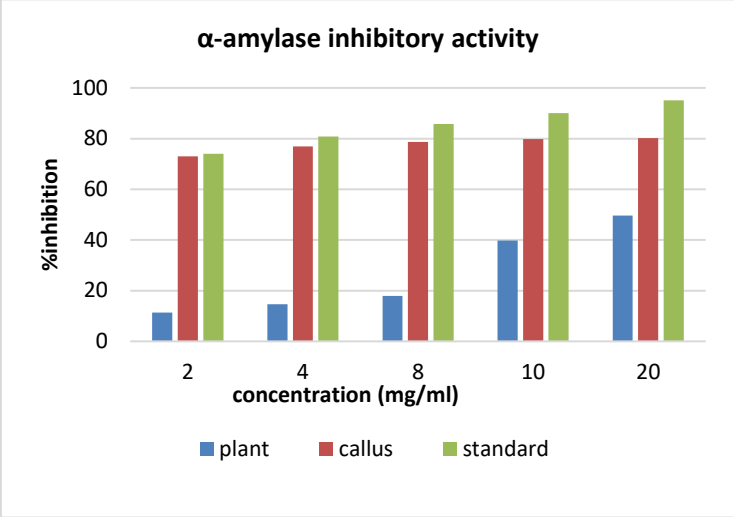
	Concentration(mg/ml)				
	2	4	8	10	20
Callus extract	73.03±0.05	76.85±0.07	78.71±0.02	79.77±0.06	80.25±0.08
Plant extract	11.31±0.04	11.40±0.06	17.96±0.03	83±0.05	49.57±0.02
Acarbose drug	74.05±0.03	80.83±0.05	85.76±0.03	90.08±0.04	95.06±0.02

Values are expressed as Mean±SD, n=3

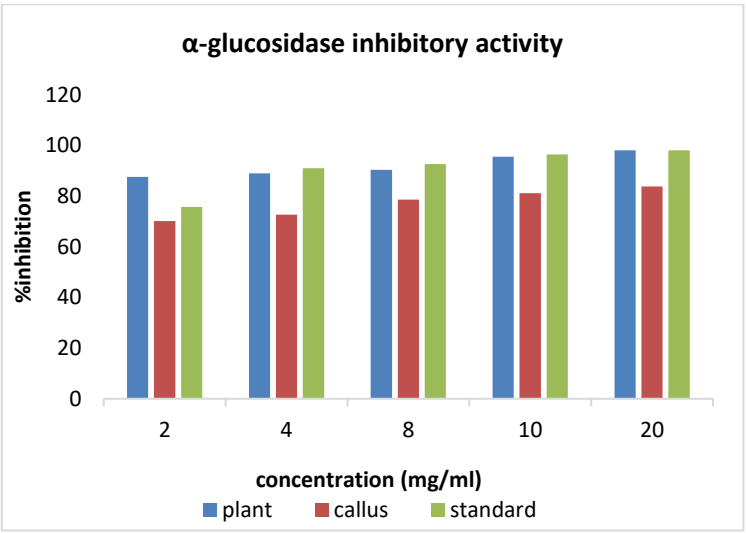
Table 2: *In vitro* anti-diabetic activity of methanol extract of *Allium sativum* using α-glucosidase inhibitory method and comparison with standard drug.

	Concentration(mg/ml)				
	2	4	8	10	20
Callus extract	70.10±0.10	72.51±0.09	78.61±0.06	81.07±0.04	83.72±0.04
Plant extract	87.52±0.09	88.93±0.07	90.30±0.12	95.45±0.09	98.06±0.11
Acarbose drug	75.65±0.14	90.94±0.08	92.65±0.07	96.42±0.04	98.08±0.02

Values are expressed as Mean±SD, n=3



Graph 1. *In vitro* anti- diabetic activity of methanol extract of *Allium sativum* using *α*-amylase inhibitory method and comparison with standard drug.



Graph 2. *In vitro* anti-diabetic activity of methanol extract of *Allium sativum* using *α*-glucosidase inhibitory method and comparison with standard drug.

DISCUSSION

In this study, callus extract is showing significant hypoglycemic activity by *in vitro* assay. Alpha amylase and alpha glucosidase are enzymes involved in breakdown of oligosaccharides and disaccharides into monosaccharides [18]. The inhibition of these enzymes increases the digestion time of carbohydrates and reduces the glucose absorption in blood [19]. Various synthetic drugs such as Acarbose, Viglibose etc. are available to inhibit the activity of alpha amylase and alpha glucosidase enzymes but it causes various side effects such as diarrhoea, bloating etc. [20]. Active principles in the callus extract which are attributing to high carbohydrates catabolizing enzyme inhibitory activity could be isolated and identified using HPTLC and GC-MS techniques. Synthesis of anti- diabetic biomolecules could be scaled up by biotic or abiotic elicitors. Callus could be a continued source of uncontaminated raw material under controlled condition irrespective of season and weather on year round basis. Thus *In vitro* study clearly indicates that the active anti-diabetic constituent in the callus extract if explored appropriately could be helpful in presenting the diabetic patients a novel, plant-based drug for glucose absorption in the gastrointestinal tract.

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