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Phytochemical Screening and Pharmacological Study of Antidiabetic Potential and Bioactive Compounds Present in Allium Sativum

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KEYWORDS
Allium sativum, Phytochemical screening, Pharmacological study, Amylase Enzyme assay, DNSA method

ABSTRACT

Introduction: The present study deals with phytochemical screening and pharmacological study of anti-diabetic potential and bioactive compounds present in *allium sativum*.

Material and Methods: A study of physicochemical parameters, solubility analysis, and quantitative and qualitative analysis of phytochemical screening of *allium sativum* was done. Then the Anti-diabetic activity was studied by alpha-amylase enzyme assay (Starch iodine color assay Method) and *in-vitro* alpha-amylase inhibitory activity (DNSA method).

Results: Glycoside, Alkaloid, and tannins showed maximum amounts in phytochemical analysis. *In-vitro* alpha-amylase inhibitory activity (DNSA method) shows that the standard drug acarbose produces 77.4 % inhibitory activity, and the test sample shows 69.3 % inhibitory activity at a concentration of 1250 µg/ml. *In-vitro* Alpha-amylase inhibitory activity (Starch-Iodine color assay method) shows that the standard drug acarbose produces 71.73 % inhibitory activity and the test sample shows 81.86 % inhibitory activity at a concentration of 1250 µg/ml.

Conclusions: The present study indicates that *allium sativum* is effective against a wide range of type-2 Diabetes mellitus. In both studies, the percentage of inhibition is nearly similar compared with the standard anti-diabetic drug acarbose. So, from the above study, it is concluded that allium sativum is effective against a wide range of type-2 Diabetes mellitus.

INTRODUCTION

One of the most common non-communicable diseases and a dangerous, lifelong ailment that affects people all over the world is diabetes mellitus (DM). Diabetes is caused by a complex interplay of environmental and genetic variables. It is a diverse set of metabolic illnesses that are clinically characterized by hyperglycemia, decreased tolerance to glucose, and other manifestable problems. Physiologically, it is characterized by malfunction of the pancreatic beta cells and a lack of insulin production or activity. Diabetes is a chronic metabolic condition that may be fatal in humans and is brought on by an erroneous balance of glucose homeostasis. The first step in the digestive process involves the enzyme -amylase, which hydrolyses polysaccharides into oligosaccharides like sucrose [1]. The second step involves the involvement of other enzymes, including glucosidase, sucrase, and maltase which catalyse the end step in hydrolysis of carbohydrates to release monosaccharides like glucose. Prior studies have shown that amylase and glucosidase inhibitors are susceptible to diabetes mellitus. A study on

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the secondary metabolic content is prompted by the fact that several phenolic Components were also able to block glucosidase activity (in vitro) and protect the cellular system from this harm. The side effects of medications like acarbose, which include gas, stomach distention, meteorism, and maybe diarrhea, are the primary drawbacks [2]. The use of natural medicines with anti-postprandial hyperglycemia activity, which undoubtedly has less or no adverse reaction than oral insulin and hypoglycaemics, is becoming more popular as a result of this concern. Glycosides, Alkaloids, essential oils, saponins, tannins, steroids, resins, terpenoids, proteins, flavonoids, and other phytochemicals are among those present in plants. examination of the anti-diabetic properties and pharmacological effects of the bioactive compounds found in Allium sativum. Allium sativum will thus be subject to physicochemical parameter research, solubility analysis, and quantitative and qualitative phytochemical screening. Then, using the amylase enzyme assay and alpha-amylase enzyme assay (Starch iodine color assay) techniques, the hypoglycaemic activity will be investigated. Garlic, sometimes referred to as Allium sativum **(Fig. 1)**, is a species of onion in the Alliaceae plant family and a member of the Liliales plant order [3]. Garlic is the most significant species of Allium. The word for garlic is 'Tantanwa' in Hausa. Flavonoids, phenols, and terpenoids are chemical substances that are active in medicinal plants and suppress the production of free radicals. These three substances are allegedly also present in garlic. This is because garlic has been used as medicine, including to preserve stamina, clear the respiratory system of phlegm and coughing, maintain good hair and skin, and relieve nausea, for thousands of years in addition to being used as a spice and culinary flavoring. It has long been recognized that garlic has medicinal uses. The plants are widely used as antibiotics and have anti-cancer, anti-atherosclerosis, and anti-diabetic properties. Garlic has been shown to reduce blood pressure, blood cholesterol, and blood sugar levels. It also prevents platelet mass formation. Herbal products have long been utilized for therapeutic purposes in almost every culture in the world, and the search for anti-diabetic drugs will continue for many years to come. Because the cost of giving current anti-diabetic pharmaceuticals is out of reach for the majority of low-income and rural communities, the use of plants to treat common conditions such as diabetes is rather common [4]. The study's major goal was to evaluate the phytochemical profile and in-vitro anti-diabetic activity of ethanolic (hot and cold) extracts of Allium sativum bulb for possible use in the treatment of hyperglycemia and hyperlipidemia associated with diabetes mellitus.

Fig 1: *Allium sativum bulb*

MATERIALS AND METHODS

The *Allium sativum* was purchased in May 2022 from the local market of Balasore. All the solvents were of analytical grade and purchased from Qualigens (Thermo Fisher Scientific). Distilled water was collected from Pharmaceutics Lab, Centurion University of Technology and Management, Balasore, Odisha.

Extraction of Allium sativum bulb

The Allium sativum bulb was allowed to dry in the shade before being ground into a fine powder and stored in an airtight container. Individual cold percolation procedures were used to extract 100gm of dry powder using different solvents with varied polarity for physicochemical analysis. For the solubility research, the solvent was evaporated to dryness and the dried crude extracts were kept in an airtight container at 40˚ C [5].

Qualitative Analysis of Phytochemicals

The extract of Allium Sativum was subjected to phytochemical analysis in terms of quality. The ethanolic extract was used for further phytochemical screening.

Detection of Alkaloids

A few drops of Mayer's reagent were taken with drug aqueous extract. The presence of alkaloids is indicated by a yellow/white/reddish precipitate [6].

Detection of Amino acid

Ninhydrin reagent at 0.1/0.25% w/v was added to the extract and heated for a short while. Blue/violet color formation shows the presence of amino acids [7].

Detection of Anthraquinone

The ethanol extract was mixed with isopropyl alcohol and a drop of concentrated ammonium hydroxide

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solution. The presence of anthraquinone is indicated by the production of red shade [8].

Detection of Pholorotannins

Take 2 mL ethanolic extract and then add 2mL of 1% HCl and the alcoholic acidified mixture boil. A red precipitate was formed and it confirmed the presence of phlorotannin [8].

Detection of Flavonoids

To 2 ml of aqueous extract, add a few drops of 10% sodium hydroxide solution. The presence of saponins is indicated by the presence of yellow shade [8].

Detection of Cardiac Glycosides (Keller Killian's Test)

The alcoholic extract was mixed with 0.5 ml of a strong $Pb(C_2H_3O_2)_2$ solution and an equal volume of water. After that, it was cut and filtered. The same amount of chloroform was used to extract it from the filtrate. Evaporation was used to dry the chloroform extract, and the residual material was dissolved in 3 ml of CH_3COOH with a few drops of $FeCl_3$ solutions. The resultant mixture was transferred to a test tube containing 2 ml of concentrated H_2SO_4. If a reddish-brown layer appears, the presence of digitoxoside is shown by the layer turning bluish-green after standing [9].

Detection of Saponins (Froth Test)

Distilled water was mixed with the plant extract. If the foam formed lasts for 10 minutes, saponins are present [10].

Detection of Tannins

An aqueous extract of 1 ml is added with a few drops of alcoholic $Fecl_2$ solution (0.1%). Then greenish-black, dark blue soluble compounds sign of the presence of tannins [11].

QUANTITATIVE ANALYSIS OF PHYTOCONSTITUENT

Quantitative evaluation for the detection of alkaloids, flavonoids, and saponin was evaluated after the qualitative evaluation was done by the different standard methods.

Estimation for alkaloids

The Harborn method was used to calculate the concentrations of alkaloids. 10 mg of drug extract was mixed along with 40 ml of 10% acetic acid in methanol, sealed in the beaker, and placed at room temperature for 4 hours. The mixture was concentrated to one-third of its original volume by adding ammonium hydroxide solution drop by drop until complete precipitation occurred and then filtered. A UV spectrophotometer was used to measure absorbance at 415nm [12].

Estimation for flavonoids

The flavonoid content was determined spectrophotometrically, in which flavonoids in the plant extract react with aluminum chloride and potassium acetate in the reagent to produce a colored product that can be detected at 415nm using a UV spectrophotometer. In brief, one ml of 2% aluminum chloride in methanol was combined with one ml of extract at a concentration of one mg per ml. A UV spectrophotometer was used to detect the absorbance at 415nm after one hour of incubation at room temperature [13].

Estimation for saponin

Saponins were extracted into ethanol/water solutions. Following this, evaporation was used to remove the alcohol content, and the saponins were extracted into n-butanol from the aqueous phase. The plant sample's dry powder was added to a 20% ethanol solution. A bath of hot water was used to heat the suspension for 3–4 hours while stirring continuously at a temperature of 55–60 °C. The mixture was exuded, and the solid drug powder residue was extracted once again using a solution of 20% ethanol in the same volume. To decrease the volume to around 8 ml, the two mixed solutions were evaporated over a water bath at 80–90° C. To eliminate contaminants from the initial solution, the concentrated solution was put into

a 100 ml separating funnel along with 5 ml of diethyl ether. The ether layer with impurities was discarded, and the aqueous layer was recovered for further extraction. The purification procedure was then done once more, followed by the addition of 12 ml of n-butanol, followed by two washes with 4 ml of aqueous sodium chloride (5%), yielding a total of 24 ml of mixed n-butanol solutions. The residual aqueous solution was transferred to a porcelain crucible that had been dried, pre-weighed and weighed at a consistent weight while being dried at 60° C. The saponin product is the leftover residue [14].

$$Total\ Saponin\ Content\ (\%) = \frac{Weight\ of\ the\ Saponin\ Residue}{Weight\ of\ the\ Plant\ Material} \times 100$$

PHARMACOLOGICAL ACTIVITY OF THE EXTRACT

In vitro anti-diabetic activity by amylase enzyme assay (DNSA METHOD*)*

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A 0.1% w/v starch solution and 15 ml of sodium acetate buffer were added. 27.5 mg of alpha-amylase was mixed with 100 ml of distilled water to generate the enzyme solution. The colorimetric reagent was prepared, and the sodium-potassium tartrate solution and di-nitro salicylic acid solution were mixed. The control tube contains only the reagent and a test sample ranging from 100 to 500 g/ml. As a consequence, 500 µl of the sample was mixed with 500 µl of starch solution and 500 µl of alpha-amylase solution, and incubated for 10 minutes at 37 ° C. The process was stopped after 1 mL of 3, 5, and di-nitro salicylic acids were added. The samples were then placed in a water bath for 3-5 minutes before being cooled at room temperature. The mixture was diluted with 10 cc of distilled water. The absorbance at 540 nm was measured after replacing the test sample with dimethyl sulfoxide. Acarbose is used as a standard [15].

$$Percentage\ of\ Inhibition = \frac{Abs\ of\ Control - Abs\ of\ Sample\ drug}{Abs\ of\ Control} \times 100$$

In vitro anti-diabetic activity Alpha amylase enzyme assay (starch-iodine color assay method)

The test uses the starch-iodine color assay method. Test samples of various concentrations are obtained. 500 µL of 0.02 M buffered sodium phosphate containing 0.04 units of - amylase solution was left to incubate for 10 minutes at 37 °C. After that, 500 µL of soluble starch was added to each reaction and incubated at 37 °C for 15 minutes. After adding 1 M HCl (20 µL), the color changed and absorbance at 620nm was measured. The control reaction with 100% enzyme activity was used [16]. The enzyme activity inhibition was calculated as follows:

$$Percentage\ of\ Inhibition = \frac{Abs\ of\ Control - Abs\ of\ Sample\ drug}{Abs\ of\ Control} \times 100$$

RESULT AND DISCUSSION

The herbal medicinal plants contain several active phytoconstituents [17]. *Allium sativum* indicates phytochemicals such as alkaloids, amino acids or primary and secondary amine, anthraquinones, flavonoids, glycosides, phlorotannins, tannins, saponins, and terpenoids present in the ethanolic extract [18] **(Table 1)**.

Table 1. The Qualitative Analysis of Phytochemical Screening Allium-sativum Extracts

Phytochemical Constituents	**Ethanolic Extract**	**Hot Extract**	**Cold Extract**	**Color**
Alkaloids	−	−	−	Turbidity
Amino Acids or Primary and Secondary Amine	+	+	+	Blue-Violet
Anthraquinones	−	−	−	−
Phlobatannins	−	−	−	−
Flavonoids	+ +	+	+	Yellow
Glycosides	+ + +	+ + +	+ + +	Violet
Saponins	+	+	+	Forth
Tannins	+ + +	+ + +	+ + +	Blue-black
Whereas: Present (+)**, Fairly Good Amount** (++) **, Good Amount-** (+ + +)**, Absent** (−)				

Therefore furthermore, analytical methods were adopted for qualitative and quantitative analysis of phytochemical screening. In this study, ethanol was employed as a solvent to extract secondary metabolites from samples of garlic. This led to the compounding and dissolution of all the elements that made up these secondary metabolites in the solvent. Due to their versatility, ethanol solvents are successfully utilized in the extraction process to dissolve both organic and inorganic compounds. Because of its universal properties, ethanol may bind every chemical element found in natural materials, including polar, semi-polar, and non-polar ones. Physical and chemical properties of Allium sativum oil, including drying loss, water-soluble and acid-insoluble, ash total ash value, acetone, petroleum ethe, and methanol yield values, were

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measured and displayed in **(Table 2)**. There were more water-soluble components present than alcohol-soluble components, as evidenced by the values of acid-insoluble, water-soluble, alcohol-soluble, and aqueous soluble extractive values being 1.93%, 3.51%, and 2.18% correspondingly. Solubility tests of *allium sativum* ethanolic extract were performed using different solvents **(Fig. 2)** in different polar and non-polar solvents. *Allium sativum* plant extract was dissolved in a polar solvent (water) gradually depending upon polarity. All the extracts showed maximum solubility in water followed by hexane, chloroform, toluene, ethyl acetate, acetone, dimethyl sulphoxide, diethyl formamide, and water respectively.

Table 2. Physicochemical parameters of *Allium Sativum*

Sl.no	Parameters	Values
1	PH	12.24± 0.23
2	Water soluble ash	6.95± 0.29
3	Total ash	7.8± 0.35
4	Acetone soluble extractive value	8.54± 0.86
6	Acid insoluble ash	7.79± 0.27
7	Alcoholic soluble extractive value	12.4± 0.72
8	Aqueous soluble extractive value	11.5± 0.23
9	Loss on drying	7.24 ± 0.16

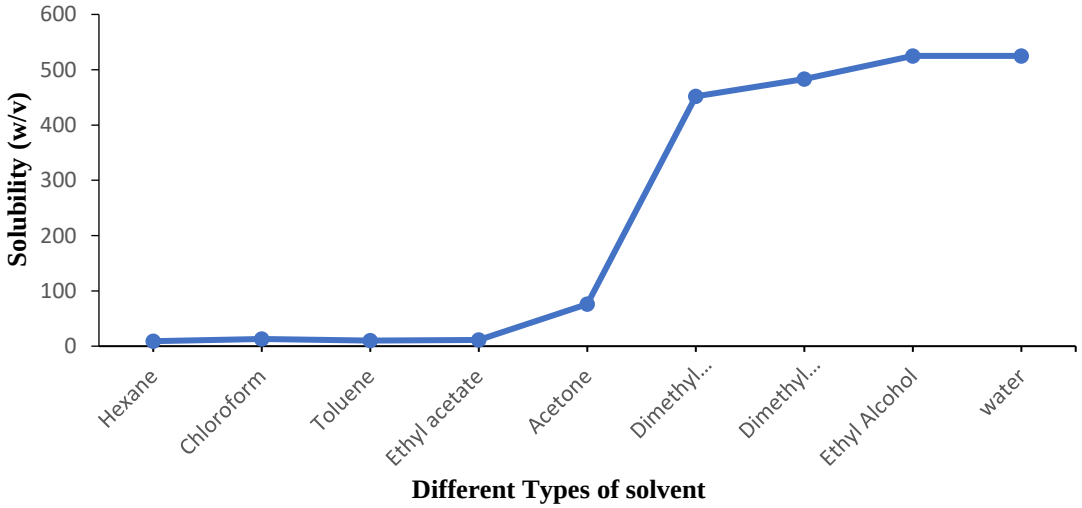

Fig 2: Solubility of Allium sativum methanol extract in different solvents

Among all the tested solvents, the least solubility was observed in hexane. The quantitative phytochemical screening of all three extracts of *Allium sativum* was tabulated in **(Table 3)**. Previously very few research studies were available for *Allium sativum* in Odisha. Alkaloids were found to be a sufficient percentage to make about 6.34%, 7.16%, and 6.14% respectively in all three different extractive mediums. Evaluating the plot of % α-amylase inhibition by the DNSA method as a function of ethanolic extract concentrations **(Fig. 3)**, the IC50 values were calculated.

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Table 3. Phytochemical Screening of Allium sativum Extracts: Quantitative Analysis (%)

Phytochemical Constituents	Water Extract		Ethanolic Extract
	Hot	Cold (Room Temperature)	
Alkaloids	6.34± 0.05	6.14± 0.05	7.16± 0.05
Flavonoids	2.1± 0.03	2.4± 0.03	3.4± 0.05
Saponins	2.23± 0.03	3.24± 0.02	3.45± 0.02

In our present observation, the ethanolic extract presents an increase in dose-dependent concentration with the inhibiting activity of 69.3% at 1250 µg/ml by alpha-amylase inhibitory activity. The standard positive control acarbose showed an inhibiting activity of 77.4 %. Which was quite a good result compared with the standard. Positive control was maintained and inhibition was calculated, plotted in (**Table-4**).

Table-4. DNSA method (Alpha-amylase inhibitory activity) for both standard and test sample

Standard (Acarbose)		Ethanolic extract of Allium sativum		
Concentration (µg/ml)	**Percentage of Inhibition**	**IC 50 values**	**Percentage of Inhibition**	**IC 50 values**
250	65.5	677.468	56.6	864.622
500	66.5		59.2	
750	73.4		61.3	
1000	75.6		67.6	
1250	77.4		69.3	

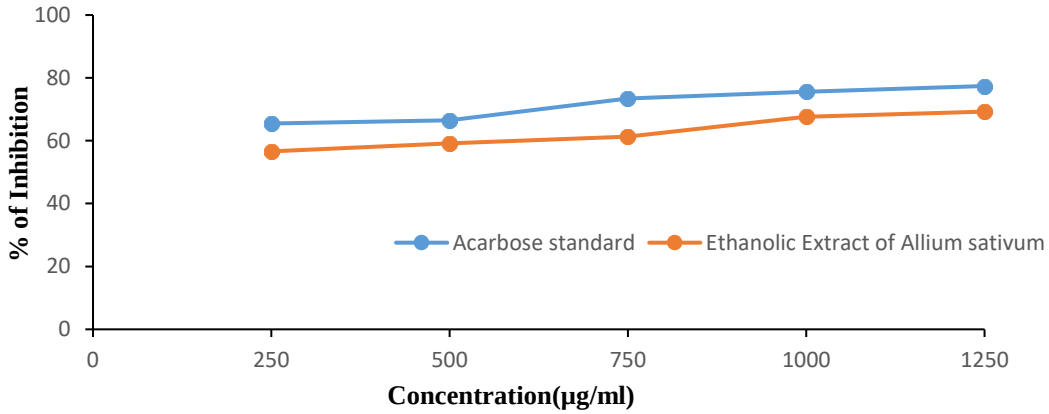

Fig 3. DNSA method (Alpha-amylase inhibitory activity) for both standard and test sample

Evaluating % α-amylase inhibition by starch-iodine color assay method of Ethanolic extract of plant and acarbose as standard **(Fig. 4)**.

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Table-5. Starch-Iodine colour assay method (Alpha-amylase inhibitory activity) of both standard and test sample)

Standard (Acarbose)		**Ethanolic extract of Allium sativum**		
Concentration (µg/ml)	**Percentage of Inhibition**	**IC 50 values**	**Percentage of Inhibition**	**IC 50 values**
250	63.65	747.098	71.34	757.349
500	61.02		73.32	
750	67.09		77.67	
1000	69.34		79.53	
1250	71.73		81.86	

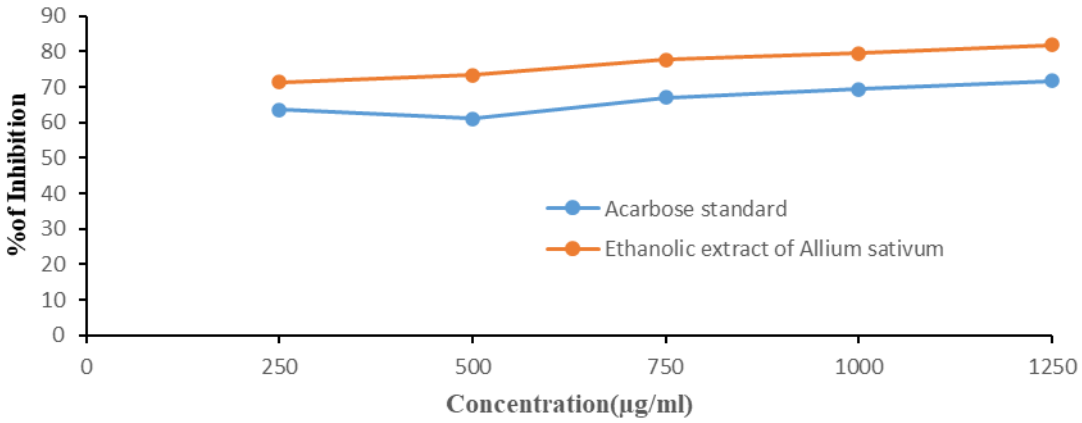

Fig 4. Comparative study of alpha-amylose inhibitory activity (Starch-Iodine color assay method.

In our present observation, the ethanolic extract presents an increase in dose-dependent concentration with the inhibiting activity of 81.86 % at 1250 µg/ml by alpha-amylase inhibitory activity. The standard positive control acarbose showed an inhibiting activity of 71.73%. It was not a satisfactory observation when it was compared with the standard. Positive control was maintained and inhibition was calculated, plotted in (**Table-5**).

CONCLUSION

The ethnopharmacological use of *Allium sativum for the treatment of diabetes mellitus is being developed as a possible first step in the development of alternative and less expensive treatments for the condition.* In this work, we performed phytochemical screening as well as a pharmacological assessment of the anti-diabetic potential and bioactive compounds found in Allium sativum. So, we conducted a physiochemical parameter study, solubility analysis, and quantitative and qualitative phytochemical screening of Allium Sativum. The anti-diabetic activity is then assessed using an DNSA method and starch iodine color assay method. According to the findings of this work, Salvia officinal is helpful against a wide variety of type 2 diabetes mellitus. In both investigations, the percentage of inhibition was found to be similar to that of the conventional anti-diabetic

medicine acarbose. So, from the above study, it is concluded that Allium sativum was effective against a wide range of type-2 Diabetes mellitus.

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CONFLICT OF INTEREST

There is no conflict interest.

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