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# A Comparative Study between Aqueous and Ethanolic Extracts of *Allium Odorum* Linn with Reference to its Antioxidant and Alpha-Amylase Inhibition Activities

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**ABSTRACT:** *Allium odorum* Linn belongs to the family Liliaceae which is established as a potent medicinal plant in folkware medicinal system. The plant is native to China as well as Japan and also cultivated in North-east part of India as an essential part of culinary. In this study, leaves of this plant were collected from Imphal East district of Manipur and extracted in water and ethanol. The extracts were screened for the presence of various bioactive compounds and its inhibition activity against α-amylase. Both aqueous and ethanol extracts showed presence of bioactive compounds like sterol, tannin, saponin, di-terpines and sugars whereas bioactive compounds like flavonoid, glycoside, and amino acids were present only in ethanol extracts. Major bioactive compound like alkaloid test showed negative result during the study. The sample was also found to exhibit a maximum of 89.27% and 69.79% of α-amylase inhibition activity in ethanol and water extract respectively. When sample was subjected to its antioxidant activity it exhibited 84.85% and 65.88% by DPPH method and a maximum of 67.73% and 57.97% by $H_2O_2$ method in ethanol and water extract respectively. Hence, from this study the sample can be used as a potential source for anti-oxidant and anti-diabetic agent which will help us to substitute some commercially available synthetic drugs near future. 

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## INTRODUCTION

Medicinal plants play an important role in human health. Almost 80% of the world population fully depends on medicinal plants for meeting their health care needs [1]. Bioactive compounds extracted from medicinal plants contain many organic compounds which provide definite physiological action in our body. For an example, phenols and flavonoids isolated from medicinal plants are reported to have positive impact on health and cancer prevention [2].Antioxidants are the chemicals that can prevent or slower cell damage. They have important preventive roles in changing the flavour and nutritional quality of food. They also prevent illness such as different types of cancers, cardiovascular and neurological diseases etc. Polyphenols are the most significant compounds for antioxidant properties of the plant raw material [3-5]. Plant mainly uses alpha amylase inhibition property to protect themselves from insect. They follow the mechanism of changing digestive property of alpha amylases and proteinases in the gut of insects resulting in abnormal feeding behaviour [3,6]. Diabetes is a clinical condition recognised by high level of glucose accumulated in the blood as cell does not respond to the insulin that is secreted by pancreas. Compounds like metformin, gliclazides, glitazones/vitagliptin/saxagliptin etc are predominantly supplied to patient to control the blood glucose level. Hence, plants with alpha amylase inhibition property are always very crucial as there are always needs for new source of antidiabetic molecule [7-8]. *Allium* species belongs to the family Liliaceae. It is the plant which is the native of China and Japan. Many natural products have been produced from this genus as they are used to cure tumor, cardiovascular

disease, thrombosis, cholesterolmia and hyperglycemia [9-12]. *Allium odorum* is a small annual plant with leaves that are small, green, grass-like, narrowly linear, and flattish. Seeds are black, depressed, globose or uniform. Fruits are capsule that open longitudinally along the capsule wall between the partitions of the locule [11,13].

# MATERIALS AND METHODS

**Sample collection and sample preparation**

Leaves of *A. odorum* were collected from Imphal East District of Manipur in the month of March 2017 and was identified based on its vernacular name. The leaves were rinsed with tap water followed by distilled water and dried in the shade. The dried samples were grinded into the fine powder with the help of a grinder and kept in an air tight container for further use.

**Sample preparation**

An amount of 200 grams of dried powder was placed in Soxhlet apparatus for extraction using ethanol as solvent. The solvent was then evaporated and crude extract were collected for further use [3]. In another setup, an amount of 200 grams of fine powder f the sample were mixed in cold water an kept in deep freezer for 72hr and centrifuge at 3000rpm. Solvent free crude were collected for further investigation [14].

**Screening for Phytochemicals [15-20]**

The crude extract was screened for the presence of different bioactive compounds. 20mg/mL solvent concentration of the crude extract was prepared and further used for the tests

**Test for sterol**

**Salkowski test-** The crude extract (100mg) was shaken with chloroform (2mL) followed by addition of concentrated sulphuric acid (2mL) along the side of the test tube. Reddish brown colour development indicates the presence of sterol.

**Test for alkaloid**

Few mg about (15mg) of extract was stirred with1% HCl (6mL) on water bath for 5min and filtered.

**a. Mayer`s test-** To a portion of filtrate, Mayer`s reagent (potassium mercuric iodide solution) ( 1mL ) was added. Cream colour precipitation indicates the presence of alkaloid.

**b. Wagner`s test**- Potassium iodide ( 2g ) and iodine ( 1.27g ) were dissolved in distilled water ( 5mL ) and the solution was diluted to 100 mL distilled water. Few drops of this solution were added to the filtrate. Brown colour precipitation indicates the presence of alkaloid.

**Test for tannins**

The extract was stirred with distilled water (10mL) and filter. A few drops of 5% ferric chloride were then added. Green colour development indicates the presence of tannins.

**Test for flavonoid**

**a. Alkaline reagent test-** The crude extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow, which become colourless on addition dilute acid indicates the presence of flavonoids.

**b. Sulphuric acid test-** A fraction of extract was treated with concentrated sulphuric acid. Appearance of orange colour indicates the presence of flavonoid.

**c. Lead acetate test-** A small amount of extract was treated with lead acetate.

**Test for saponin**

A few mg of the test residue was diluted with distilled water in a test tube and shaken vigorously and heated in a water bath for 5 min. Development of frothing indicates the presence of saponin.

**Test for glycoside**

Anthraquinone glycoside (Borntrager`s test )- The extract solution is boiled with dilute sulphuric acid, filtered and to the filtrate chloroform was added and shaken well. The organic layer is separated to which ammonia is added.

Development of red colour of the ammonical layer indicates the presence of glycoside.

**Cardiac glycoside (Keller-Killiani test)-** Extract (0.5g) was shaken with distilled water (5mL). To this glacial acetic acid (2mL) containing a few drops of ferric chloride was added followed by sulphuric acid (1mL) along the side of the test tube. Formation of ring at the interface gives positive result.

**Test for diterpenes**

Extract was dissolved in water and treated with 3-4 drops of copper acetate solution.

Appearance of ceramal green colour indicates the presence of diterpenes.

**Test for protein**

**a. Biuret test-** A few mg of the residue was taken in water and 1mL of 4% copper sulphate was added to it. Violet ring development indicates the presence of protein.

**b. Xanthoproteic test-** A little residue was taken with 2mL of water and 0.5mL of concentrated nitric acid was added to it. Yellow colour development indicates the presence of protein.

**Test for amino acid**

**Ninhydrin test-** The ninhydrin reagent is 0.1% w/v solution of ninhydrin in n-butanol. A little of this reagent was added to the test extract. Development of violet colour indicates the presence of amino acid.

**Test for sugar**

**a. Molisch test**- A few mg of the test extract was placed in the test tube containing .5mL of water and it was mixed with 2mL of concentrated sulphuric acid added from the side of the inclined test tube so that the acid formed a layer beneath the aqueous solution without mixing. Development of violet ring indicates the presence of sugar.

**b. Barfoed test-** The reagent was prepared by dissolving 13.3g of crystalline neutral copper acetate in 200mL of 1% acetic acid solution The test residue was dissolved in water and heated with a little of the reagent. Green colour development indicates the presence of sugar.

**Test for phenol**

Extract was treated with 3-4 drops of 10% ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

**Alpha-amylase Inhibition Activity [21,22]**

A quantity of 0.1g of starch was dissolved in 100mL of acetate buffer (16mM) to prepare 0.1% starch solution. Similarly, 27.5 mg of alpha amylase was dissolved in 100 mL of distilled water to prepare the enzyme solution. Sodium potassium tartarate solution and 3, 5 dinitro salicylic acid solution at 96 mM were mixed to prepare the colorimetric reagent. Different concentration (200, 400, 600, 800, 1000µg/mL) of extract were placed in different test tubes. To each test tube starch (1mL), amylase (1mL) was added and incubated for 15mins. After incubation DNS (1mL) was added and heated at 100°C and observed the colour change. The absorbance was taken at 540nm against a blank. The percentage of α-amylase inhibition was calculated using

$$\%Inhibition = \frac{A_{Control} - A_{sample}}{A_{Control}} \times 100$$

**Antioxidant Assay (23, 24, 25)**

**DPPH Method**

The antioxidant activity of ethanol and water extracts of the plant extract were assayed according to the standard method with slight modification. DPPH (2,2-diphenyl picryl hydrazyl) is composed of stable free radical molecules and is purple in colour. The antioxidant molecules (present in the test sample) react with DPPH and after the incubation time, the purple colour gets converted to yellow colour. Stock of the plant sample was made by dissolving 1mg of sample in 1 mL of distilled water. The absorbance was taken at 540nm against suitable blank using Ascorbic acid as a standard.

**$H_2O_2$ Method**

A concentration of 40mM hydrogen peroxide was prepared in phosphate buffer of pH 7.4. Different concentration of samples and distilled water were added to $H_20_2$ (0.6 mL, 40mM). The absorbance was taken at 230nm against a blank using Ascorbic acid as a standard. In both cases percentage of scavenging activity was calculated using the following equation:

$$\%Inhibition = \frac{A_{Control} - A_{sample}}{A_{Control}} \times 100$$

**Determination of Total Phenolic Content (25, 26)**

Phenolic content is determined by Folin Ciocalteu method. An aliquot of 0.5mL of extract (1mg/mL) was mixed with 2.5mL Fc reagent (previously diluted with distilled water 1:10) and 2mL (75%) of $Na_2CO_3$. The tubes were vortex for 15 sec and allowed to stand for 30min at 40°C for colour development. Absorbance was then measured at 765nm. Results were expressed as mg gallic acid equivalents (GAE)/g sample.

**Statistical analysis**

Bioactive activity namely antioxidant and α-amylase inhibition activity for both the extracts were expressed as mean ± standard deviation (SD) of three replicates with the help of software.

# RESULT AND DISCUSSION

**Phytochemical Screening**

The plant medicinal value relies in the active phytochemicals that generates certain physiological impact on humans. A study on *Allium sativum* showed the rich presence of alkaloids, reducing sugar, flavonoids, glycosides, cardiac glycosides, tannin and phenolic compounds, saponins, amino acid & triterpenoids in aqueous and methanolic extract of garlic leaves [27]. In the present study, the phytochemical screening of crude extract of *Allium odorum* also revealed the presence of various secondary metabolites. Water extracts showed the presence of sterol, tannin, saponin, diterpenes, sugars, flavonoids, whereas ethanol extract showed positive results for sterol, tannin, saponin, glycoside, diterpenes, amino acids, sugars and phenol. Alkaloid was absent in both ethanol and water extract **(Table 1).** Therefore, the presence or absence of such compounds depend largely on the extent of accumulation, the amount of plant material used and the analytical method employed

**TABLE 1: Result of Phytochemical screening for both the extracts.**

| SL. NO. | PHYTOCHEMICALS | RESULTS | |
|---|---|---|---|
| | | ETHANOL | WATER |
| 1 | Sterol | +ve | +ve |
| 2 | Alkaloid | -ve | -ve |
| 3 | Tannin | +ve | +ve |
| 4 | Flavonoid | +ve | +ve |
| 5 | Saponin | +ve | +ve |
| 6 | Glycoside | +ve | -ve |
| 7 | Diterpenes | +ve | +ve |
| 8 | Amino acid | +ve | -ve |
| 9 | Sugar | +ve | +ve |
| 10 | Phenol | +ve | - ve |

+=present; -ve=absent

***In vitro* alpha amylase inhibition activity**

Although there are reports of alpha amylase inhibitory activity for six *Allium* species [28], very few reports are available for *A.odorum* in Manipur. In our present study, both the extracts showed an increase in dose dependent concentration with inhibition activity of 89.27% at 1000 µg/mL and 69.79% at 1000 µg/mL for ethanol and water extracts respectively, which is quite strong as compared to the results of [28]. A positive control was maintained and percentage of inhibition for both the extracts were tabulated **(Table 2).** The $IC_{50}$ values for

ethanol and water extract was found to be 253.63 µg/mL and 575.10 µg/mL respectively.

**Antioxidant activity**

**Antioxidant activity by DPPH Method**

The extracts showed noticeable antioxidant activity through DPPH methods. Antioxidant value was found to be maximum (84.85%) at 200 µg/mL which is quite effective as against the inhibition of 82.2% for 80% ethanolic garlic extract studied by [29]. $IC_{50}$ value for ethanol and water extract was found to be 56.12 µg/mL and 143.86 µg/mL respectively. Ascorbic acid was used as a standard while a separate positive control was also maintained.

**H2O2 Method**

$H_2O_2$ assay of *T. zeylanicum* extract exhibited a dose-dependent hydrogen peroxide inhibition where methanolic extract possessed good ability of scavenging with $IC_{50}$ values 0.122 mg/ml [30]. But our study with *A.odorum* showed good scavenging ability with ethanolic extract. $IC_{50}$ value for ethanol and water extract was found to be 73.08 µg/mL and 115.95 µg/mL respectively. Ascorbic acid was used as a standard while a separate positive control was also maintained.

**TABLE 2: α-Amylase inhibition activity for both the extracts.**

| Sl. No. | Conc. Of sample (µg/mL) | Ethanol | | $IC_{50}$ value (µg/mL) | Water | | $IC_{50}$ value (µg/mL) |
|---|---|---|---|---|---|---|---|
| | | Absorbance (Values represent mean±SD, n=3) | % inhibition | | Absorbance (Values represent mean±SD, n=3) | % inhibition | |
| 1 | 200 | 0.266± 0.57 | 45.15 | 253.63 | 0.517±0.56 | 33.54 | 575.10 |
| 2 | 400 | 0.201±0.55 | 58.55 | | 0.473±0.48 | 39.20 | |
| 3 | 600 | 0.136±0.55 | 72.16 | | 0.368±0.50 | 52.69 | |
| 4 | 800 | 0.094±0.51 | 80.61 | | 0.305±0.52 | 60.79 | |
| 5 | 1000 | 0.052±0.53 | 89.27 | | 0.235±0.52 | 69.79 | |

**TABLE 3: Antioxidant activity by DPPH method.**

| Sl. No. | Conc. Of sample (µg/mL) | Ethanol | | $IC_{50}$ value (µg/mL) | Water | | $IC_{50}$ value (µg/mL) |
|---|---|---|---|---|---|---|---|
| | | Absorbance (Values represent mean±SD, n=3) | % inhibition | | Absorbance (Values represent mean±SD, n=3) | % inhibition | |
| 1 | 50 | 0.211±0.80 | 49.27 | 56.12 | 0.324±0.72 | 24.29 | 143.86 |
| 2 | 100 | 0.154±0.86 | 62.98 | | 0.267±0.76 | 37.61 | |
| 3 | 150 | 0.100±0.93 | 75.96 | | 0.207±0.77 | 51.63 | |
| 4 | 200 | 0.063±0.87 | 84.85 | | 0.146±0.84 | 65.88 | |

**TABLE 4: Antioxidant activity by $H_2O_2$ Method.**

| Sl. No. | Conc. Of sample (µg/mL) | Ethanol | | $IC_{50}$ value (µg/mL) | Water | | $IC_{50}$ value (µg/mL) |
|---|---|---|---|---|---|---|---|
| | | Absorbance (Values represent mean±SD, n=3) | % inhibition | | Absorbance (Values represent mean±SD, n=3) | % inhibition | |
| 1 | 50 | 0.343±0.13 | 47.55 | 73.08 | 0.533±0.18 | 40.11 | 115.95 |
| 2 | 100 | 0.303±0.18 | 53.66 | | 0.427±0.23 | 52.02 | |
| 3 | 150 | 0.254±0.14 | 61.16 | | 0.410±0.20 | 53.93 | |
| 4 | 200 | 0.211±0.16 | 67.73 | | 0.375±0.24 | 57.97 | |

**Determination of phenolic content**

Total phenolic content was examined for the ethanol extract. Plant rich in phenolic compounds exhibit antioxidant property through redox properties. The ethanol extract showed 30.12 mg of GAE/g. Higher phenolic content showed better bioactivity. Therefore, the better antioxidant result may be due to this high phenolic content. Our results correlate with the study done by [31] that also revealed the ethanol extract to exhibit highest phenolic content compared to other extracts.

Medicinal plants are of great importance to the health care system. Plants such as vegetables, fruit, spices, etc have been used and have been continuously screened for new molecules with medicinal value. Even though many synthetic drugs are readily available, people prefer traditional folk medicine because of their lesser side effects.

# CONCLUSION

The present investigation revealed that this plant contain bioactive compound which could be used for several purpose. The sample was found to exhibit a maximum of 89.27% and 69.79% of α-amylase inhibition activity in ethanol and water respectively. The sample showed a maximum of 84.85% by DPPH method and 67.73% by $H_2O_2$ method of antioxidant property in ethanol extract. Comparing the results of ethanol extract with aqueous extract, ethanol extract possessed better bioactive activities. The results from the present study indicate that *Allium odorum* possessing antioxidant properties could serve as free radicals inhibitor or scavenger and can also be established as a potent anti-diabetic drug in future though animal modelling is required.

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# CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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NIL

# DATA AVAILABILITY

Not declared.

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