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**Journal of Chemical and Pharmaceutical Research, 2017, 9(12):25-29**

**Research Article**

**ISSN : 0975-7384**
**CODEN(USA) : JCPRC5**

# Alpha-Amylase Inhibitory Assay of *Argemone mexicana L.* Leaves

**Bhimraj Gawade[1*] and Mazahar Farooqui[2]**

[1]*Department of Chemistry, AD College, Kada, Maharashtra, India*
[2]*Dr Rafiq Zakaria College for Women, Aurangabad, Maharashtra, India*

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**ABSTRACT**

*In the present investigation, the study results revealed that the ethanol extract fraction of A. mexicana L. efficiently inhibits the enzyme, α-amylase in a similar manner like that of acarbose, a standard drug. The IC50 values of both are 27.83 µg/ml and 36.93 µg/ml respectively. Inhibition of α-amylase assay of ethanol fraction suggest that one of the mechanisms by which A. mexicana L. might be exhibiting their antidiabetic properties probably by inhibition of α-amylase activity leading to decrease of the rate of starch digestion, thus preventing sudden hyperglycaemia after intake of a starch in turn reduction of formation of glucose and consequently decrease in absorption of glucose. This investigation reported that the ethanol extract of A. mexicana L. leaves shows effective α-amylase inhibitory activity.*

**Keywords:** *A. mexicana L.*; α-amylase; Antidiabetic; Hyperglycaemia

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## INTRODUCTION

The alpha amylase enzyme inhibitors prevent dietary starches from being digested and absorbed by the body. They are useful for treating diabetes mellitus type-II. The α-amylase inhibitors act as an ant nutrient that control rate of digestion and absorption of carbohydrates. Also potentially become useful in control of obesity and diabetes.

*A. mexicana L.* is reported to possess medicinal benefits in traditional system of medicine [1]. Pharmacological activities of this plant were reported as Antimicrobial, Antidiabetic, Antioxidant, Hepatoprotective, Larvicidal, Wound healing, Cancer, Anthelmintic and Neuropharmacological studies. From these medicinal properties, this plant can be represented as a valuable source of medicinal compounds [2]. Synthetically prepared hypoglycaemic agents could produce serious side effects [3]. Therefore, WHO recommended that diabetes mellitus [4] research on hypoglycaemic agents getting from medicinal plants has been suggesting new area of active research.

Hypoglycaemic potentiality of aerial parts of *A. mexicana L.* ethanol and aqueous extracts was reported in Alloxan induced diabetic rats [5]. Water-alcohol mixture resulted fasting in reducing blood glucose levels in Streptozotocin induced hyperglycaemic wistar albino rats [6].

The crude plant extracts and their isolated chemical constituents show various biological activities and were identified in the leaves of *A. mexicana L.* [7]. The literature afforded no more information on the *in vitro* α-amylase inhibition assay of the *A. mexicana L.* leaves. Therefore, the present study has been designed to determine vast potentiality and effectiveness of *A. mexicana L.* leaves ethanol extract *in vitro* antidiabetic activity by using α-amylase enzyme assay for characterizing their biological activities and chemical constituents.

## MATERIALS AND METHODS

### Plant Material

*A. mexicana L.* plant leaves were collected from local area, identified and authenticate with the help of Botanist from our institute. The plant leaves rinsed with distilled water and dried in shade at room temperature.

### Extraction of Leaves

The dried leaves of *A. mexicana L.* plant were powdered. 10 gm of powdered plant material was dissolved in 100 ml of ethanol and kept on a magnetic stirrer for 2 hrs. Thereafter, it was extracted using a soxhlet apparatus sequentially with ethanol. The extract was collected and the solvent was evaporated out to dryness. The obtained material was stored at 4°C in airtight bottles for further studies.

### *In Vitro* α-Amylase Inhibitory Assay

A modified 3,5-dinitrosalicylic acid (DNS) method was adopted to estimate α-amylase inhibition activity, by quantifying the reducing sugar (maltose) liberated under the assay conditions. The enzyme inhibitory activity was expressed as a decrease in units of maltose liberated [8,9].

### Phytochemical Analysis

The ethanol extract of *A. mexicana L.* was analysed for the active phyto-constituents such as phenols, tannins, flavonoids, alkaloids, saponins, terpenoids etc. according to the standard protocol [10].

### GC-MS Analysis

GC-MS analysis was carried out on Shimadzu GC-MS model number QP 2010S. The column Rxi-5Sil MS, 30 meter length, 0.25 mm ID, 0.25 μm thickness was used. The organic compounds were identified by comparison of mass spectra with the inbuilt libraries NIST–11 and WILEY-8.

### Statistical Analysis

The experimental tests were performed in triplicate and the results expressed in mean ± SD.

## RESULTS AND DISCUSSION

The ethanol extract of *A. mexicana L.* leaves results showed that it exhibited dose dependent porcine pancreatic α-amylase inhibitory activities by *in vitro* assay using potato starch as substrate.

### *In Vitro* Alpha Amylase Inhibitory Assay

The antidiabetic activity was investigated through the inhibition of porcine pancreatic α-amylase enzyme that made the digestion of starch and so reduced the glucose absorption. Acarbose is a standard drug at a concentration of (20-100 μg/ml) showed α-amylase inhibitory activity from 47.17% to 68.81% with an $IC_{50}$ value 27.83 μg/ml, whereas Ethanol extract (20-100 μg/ml) of *A. mexicana L.* leaves exhibited potent α-amylase inhibitory activity in a dose dependent manner from 46.39% to 57.90% with an $IC_{50}$ value of 36.95 μg/ml (Figure 1 and Table 1).

**Table 1: % Inhibition α-amylase assay of standard and *A. mexicana L.* leaves extract**

| Sr. No. | Concentration In (μg/ml ) | % Inhibition of Standard | % Inhibition of extract |
|---|---|---|---|
| 1 | 20 | 47.17 | 46.39 |
| 2 | 40 | 54.38 | 50.68 |
| 3 | 60 | 59.45 | 53.02 |
| 4 | 80 | 64.32 | 52.24 |
| 5 | 100 | 68.81 | 57.9 |
| $IC_{50}$ Value ( μg/ml) | | 27.83 | 36.95 |

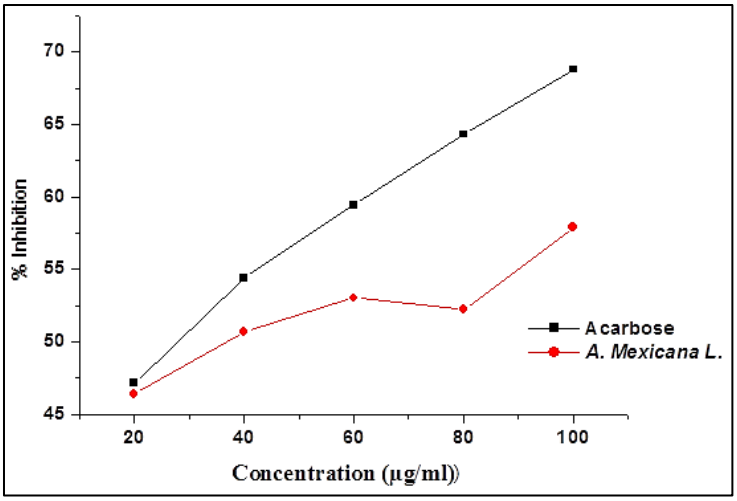


**Figure 1: α -Amylase inhibitory assay *A. mexicana* L. leaves ethanol extract**

## Phytochemical Analysis

The qualitative phytochemical analysis of the ethanol extract confirms the presence of alkaloids, protein, glycoside, tannins, flavonoids, steroids and phenols as shown in Table 2.

**Table 2: Phytochemical tests performed for analysis of *A. mexicana L.* leaves extract**

| Phytochemicals | Test Performed | Result |
|---|---|---|
| 1. Alkaloid | a) Mayer's Reagent | + |
| | b) Dragendorff's Reagent | + |
| | c) Wagner's Reagent | - |
| | d) Hager's Reagent | - |
| 2. Carbohydrate | a) Molisch's Reagent | - |
| | b) Fehling's Solution | - |
| | c) Barfoed's Reagent | - |
| | d) Benedict's Solution | - |
| 3. Protein and amino acids | a) Biuret Reagent | + |
| | b) Xanthoproteic Test | + |
| | c) Ninhydrin Reagent | - |
| | d) Millon's Reagent | - |
| 4. Glycoside | a) Modified Brontrager's Test | + |
| | b) Legal's Test | - |
| | c) Keller Kiliani Test | - |
| 5. Tannin | a) Gelatin Test | - |
| | b) Ferric Chloride Test | + |
| | c) Lead Acetate Test | - |
| 6. Saponin | a) Froth Test | - |
| | b) Foam Test | - |
| 7. Flavonoids | a) Lead Acetate Test | + |
| | b) Shinoda Test | + |
| | c) Alkaline Reagent Test | + |
| | d) Ferric Chloride Test | + |
| 8. Steroids | a) Salkowaski's Test | + |
| | b) Libermann- Burchard's Test | - |
| 9. Triterpenoids | a) Salkowaski's Test | - |
| | b) Libermann- Burchard's Test | - |
| 10. Phenolic compounds | a) Ferric Chloride Test | ++ |

(+) for present, (++) more intense, (+++) highly intense and (-) for absent

## GC-MS Analysis

A GC-MS of the ethanol extract was carried out up to maximum 44.0 minutes, which shows eight different peaks. The first peak appears that 16.533 minutes retention time. The maximum peak area was found at retention time 36.745 minutes as in chromatogram (Figure 2). The library search shows maximum amount of 1-Eicosanol

compound (Table 3), it will be interesting to investigate further the bioactivity of this compounds when isolated in pure form.

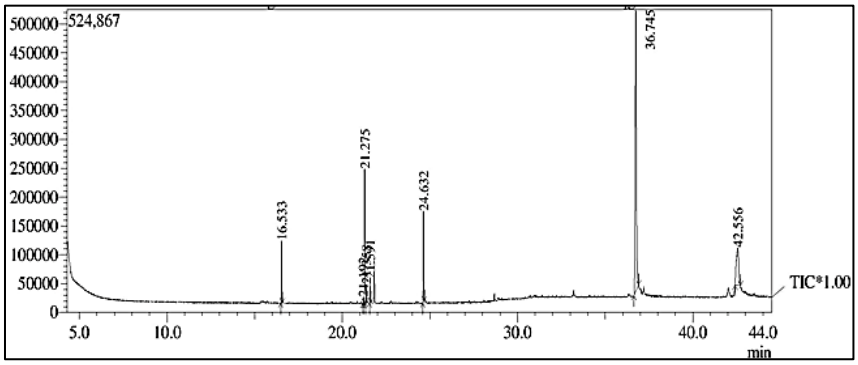


**Figure 2: GC-MS Chromatogram of *A. mexicana L.* leaves ethanol crude extract**

**Table 3: Phytochemicals detected in GC-MS analysis of *A. mexicana L.* leaves extract**

| Peak # | R. Time | Area | Area% | Height | Height % | Name | Base m/z |
|---|---|---|---|---|---|---|---|
| 1 | 16.533 | 178670 | 4.45 | 107727 | 9.49 | Phenol, 2,4-Bis(1,1-Dimethylethyl)- | 191.1 |
| 2 | 21.192 | 18168 | 0.45 | 10418 | 0.92 | Butanal, 3-Hydroxy- | 70.05 |
| 3 | 21.275 | 493338 | 12.29 | 232730 | 20.5 | Neophytadiene | 68.05 |
| 4 | 21.358 | 79370 | 1.98 | 32211 | 2.84 | 2-Undecene, 9-Methyl-, (E)- | 70.1 |
| 5 | 21.591 | 68998 | 1.72 | 40975 | 3.61 | 16-Heptadecenal | 82.05 |
| 6 | 24.632 | 322263 | 8.03 | 157666 | 13.89 | 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol | 71.05 |
| 7 | 36.745 | 2238028 | 55.78 | 488528 | 43.03 | 1-Eicosanol | 83.1 |
| 8 | 42.556 | 613751 | 15.3 | 65129 | 5.74 | Oxirane, Hexadecyl- | 43 |
| | | 4012586 | 100 | 1135384 | 100 | | |

The *in vitro* antidiabetic activity was studied by using α-amylase inhibitory assay modified 3,5-dinitrosalicylic acid (DNS) method. The ethanol extract of *A. Maxicana L.* leaves possessed significant antidiabetic activity [11] in comparison with standard drug acarbose. The ethanol extract showed inhibition of α-amylase at $IC_{50}$ value of 36.95µg/ml, whereas standard showed inhibition of α-amylase at $IC_{50}$ value 27.83 µg/ml (Table 3).

*A. mexicana L.* leaves pharmacological studies has been shown that various notable pharmacological activities [12]. Its leaves along with black pepper are used to cure diabetes. Phytochemical analysis of the extract revealed the presence of various bioactive components of which alkaloids, flavonoids, tannin, steroids [13]**,** saponins, anthraquinones and aqueous extracts evaluate relative contribution of different polyphenols [14] were the most predominant [15,16]. The flavonoids and tannin contents were present in maximum level in the aqueous extract of *A. mexicana L.* leaf followed by stem and root extracts [17]. Alkaloids, tannin, glycosides were reported to exhibit antidiabetic characteristics [18]. The administration of chloroform and aqueous fractions of hydroalcoholic extract of *A. mexicana L.* to alloxan induced hyperglycaemic rat's demonstrated prominent reduction in blood sugar level [19]. The salivary alpha-amylase inhibitory assay also reveals that chloroform fraction possess Salivary alpha-amylase inhibitory potential [20]. The specific bioactive compounds which are responsible for inhibition were studied through GC-MS analysis (Figure 2). The ethanol leaves extract were confirmed the presence of Phenol,2,4-Bis(1,1-Dimethylethyl)-, Butanal, 3-Hydroxy-, Neophytadiene, 2-Undecene,9-Methyl-,(E)-,16-Heptadecenal, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 1-Eicosanol, Oxirane,hexadecyl- (Table 3). Neophytadiene is an enzyme inhibitor [21]. 3,7,11,15-tetramethyl-2-hexadecen-l-ol (phytol) have antidiabetic, α-glucosidase inhibition activity [22]. Phytol has a potential role in the management of insulin resistance and metabolic disorders that accompany diabetes or obesity [23]. Thus due to existence of Neophytadiene, Phytol and other compounds in extract showed maximum α-amylase inhibitory activity [24].

## CONCLUSION

The potent α-amylase inhibitory activity showed by *A. mexicana L.* leaves ethanol extract has significant role in management of diabetes. These overall activities due to bioactive phytochemicals were present in the extract. The leaves of *A. mexicana L.* could be a source of natural antidiabetic agent, which has contribution of most significant therapeutic agents responsible for prevent and management of type-II diabetes. Thus, it was concluded that *A. mexicana L.* leaves ethanol extract showed potent *in vitro* antidiabetic activity. The more investigative study is

proposed to validate these claims by separation, isolation and identifying bioactive components with potential therapeutic value.

**ACKNOWLEDGMENT**

The authors are grateful to the Director, KFRI, Kerala for GC-MS facility, Research Institute, Maulana Azad College, Dr. Rafiq Zakaria College for Women, Aurangabad, HOD and Principal, A. D. College, Kada, for providing facilities.

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