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# α-Amylase Inhibitory Activity of *Abrus precatorius* Linn. Leaves Ethanol Extract

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**Abstract :** The screening study of porcine pancreatic α-amylase inhibition was done on local area collected medicinal plants; a potent inhibitory activity was detected in the ethanol extract of *Abrus p recatorius* L. leaves. The enzyme assay reported fractionation of the extract led to the inhibition activity and has potent α-amylase inhibitor content. The mode of inhibition of extract against porcine pancreatic α-amylase was a mixed inhibition. This is the reported study that describes the potent α-amylase inhibitory activity of the ethanol extract of *A.precatorius* L.leaves.
**Keywords :** Porcine pancreatic α-amylase, *Abrus p recatorius* L., α-amylase inhibitor.

## Introduction

Diabetes mellitus is a metabolic disease characterized by hyperglycaemia resulting from irregular secretory action of insulin. There are many existing therapeutics for the treatment of diabetes, plant based drugs are generally considered much effective and safe. However, medicinal plants traditional usage for the treatment of diabetes lacks scientific validation.

Enzyme inhibitors have plays important role in many areas of disease control and treatment. The reviews of literature found that the plants which possess outstanding anti-diabetic property which could be possibly investigated further for the presence of α-amylase inhibitor[1].

Synthetic hypoglycaemic agents can produce serious side effects and are not suitable for use during pregnancy[2]. Therefore, the search of new more effective and safer hypoglycaemic agents has been important area of active research, and after the recommendations made by WHO on diabetes mellitus[3] research on hypoglycaemic agents from medicinal plants has become an important aspect of this study.

The antidiabetic effect of chloroform-methanol extract of *A. p recatorious L.*seed was studied in alloxan diabetic rabbits[4].Several compounds are identified in the leaves of *A. PrecatoriusL.*[5].

No more information reported from literature on the in vitro α-amylase inhibition activity of the *A.precatorius L.* leaves ethanol extract.

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Therefore, we consider vast potentiality of *A. precatorius L* .plant leaves as a source of α-amylase inhibitor study for characterizing their biological activities and chemical constituents.

## Material and Methods

### 2.1 Plant material

The leaves of *A.precatorius L.*plant were collected from local area identified and authenticate with the help of college Botany Department.

### 2.2 Extraction

The leaves of *A. precatorius L.*plant were dried under shade and then 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 collected extract was evaporated out to dryness. The obtained material was stored at $4^0$c in airtight bottles for experimental studies.

### 2.3 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[6-8].

### 2.4 Phytochemical Analysis

The extract was analysed for the active phyto-constituents such as phenols, tannins, flavonoids, alkaloids, saponins, terpenoids etc. according to the standard protocol[9].

### 2.5 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.

### 2.6 Statistical analysis

The experiments were performed out in triplicate and the results were expressed in mean ± SD.

## Results

The results showed that the *A.precatorius L.*leaves ethanol extract exhibited dose dependent α-amylase inhibitory activities by in vitroassay using potato starch as substrate.

### 3.1 In vitro alpha amylase inhibitory assay

The antidiabetic activity was investigated through the inhibition of α-amylase, an enzyme that made the digestion of starch and so reduced the glucose absorption. Acarbose is a standard drug at a concentration of (20-100μg/ml) showed α-amylase inhibitory activity from 40.94% to 57.12% with an $IC_{50}$ value 27.90μg/ml (Table 3.1).

**Table.3.1. α-Amylase inhibitory Activity of Acarbose.**

**Absorbance of the sample at 540nm Absorbance of Control = 0.513**

| Sr. No. | Concentration In (µg/ml ) | Absorbance | % Inhibition | $IC_{50}$ Value ( µg/ml) |
|---|---|---|---|---|
| 1 | 20 | 0.271 | 47.17 | 27.90 |
| 2 | 40 | 0.234 | 54.38 | |
| 3 | 60 | 0.208 | 59.45 | |
| 4 | 80 | 0.183 | 64.32 | |
| 5 | 100 | 0.160 | 68.81 | |

Ethanol extract (20-100µg/ml) of *A.precatorius L.*leaves exhibited potent α-amylase inhibitory activity in a dose dependent manner from 40.94% to 57.12% with an $IC_{50}$ value of 61.17µg/ml (Table 3.2).

**Table.3.2 α-Amylase inhibitory effects of *A.precatorius L.*leaves ethanol extract.**

**Absorbance of the sample at 540nm Absorbance of Control = 0.513**

| Sr. No. | Concentration In (µg/ml ) | Absorbance | % Inhibition | $IC_{50}$ Value ( µg/ml) |
|---|---|---|---|---|
| 1 | 20 | 0.303 | 40.94 | 61.17 |
| 2 | 40 | 0.265 | 48.34 | |
| 3 | 60 | 0.257 | 49.90 | |
| 4 | 80 | 0.243 | 52.63 | |
| 5 | 100 | 0.220 | 57.12 | |

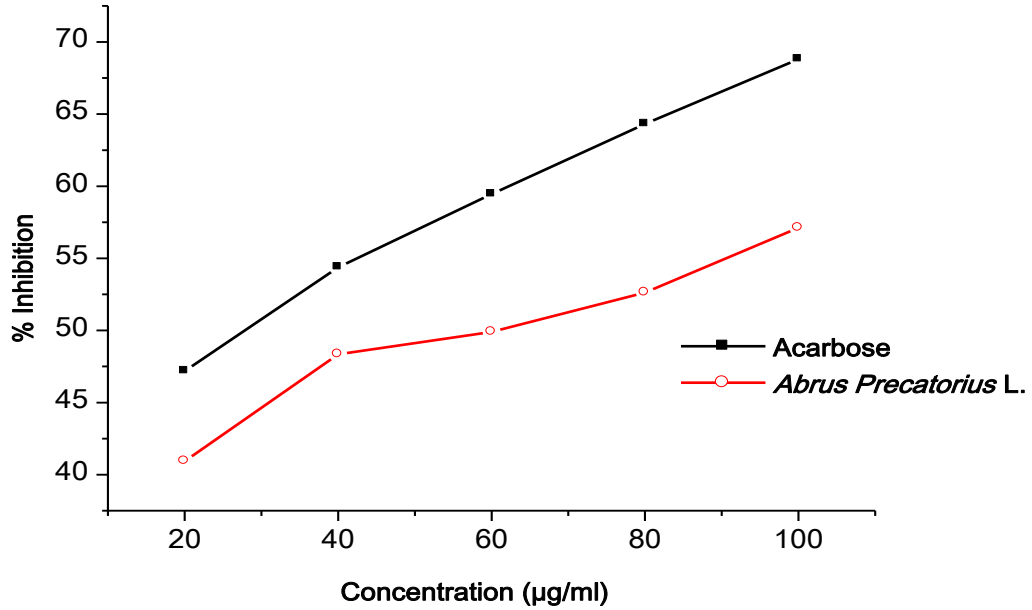



Fig. 3.1. A-Amylase inhibitory effect of *Abrus Precatorius L.* leaves ethanol extract

**3.2 Phytochemical Analysis**

Plant derived compounds are well known for their therapeutic values since ancient times. The qualitative analysis of the ethanol extract confirms the presence of alkaloids,Glycoside, tannins, flavonoids and phenols as shown in Table 3.3.

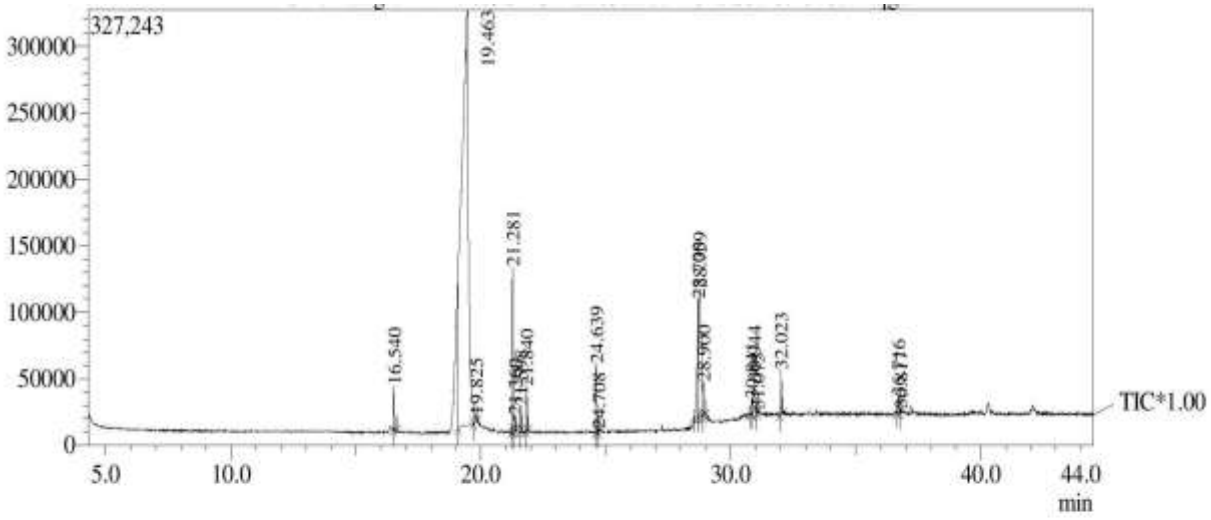
**Table 3.3 Phytochemical Tests Performed for Ethanol extract of *A. precatorius*leaves.**

| 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

**3.3 GC-MS analysis :**

The GC-MS study reveals the presence of many phytocompounds which contribute to α-amylase inhibitory activity (Figure 3.2).




Peak Report TIC

| Peak# | R.Time | Area | Area% | Height | Height% | Name | Base m/z |
|---|---|---|---|---|---|---|---|
| 1 | 16.540 | 61064 | 0.77 | 33745 | 3.82 | Phenol, 3,5-bis(1,1-dimethylethyl)- | 191.05 |
| 2 | 19.463 | 5968721 | 75.58 | 312122 | 35.30 | MOME INOSITOL | 87.00 |
| 3 | 19.825 | 53639 | 0.68 | 5664 | 0.64 | Pentanethioic acid, S-propyl ester | 57.00 |
| 4 | 21.281 | 254726 | 3.23 | 123648 | 13.98 | Neophytadiene | 68.05 |
| 5 | 21.360 | 27575 | 0.35 | 12026 | 1.36 | 2-METHYLBUTYL METHACRYLATE | 70.00 |
| 6 | 21.598 | 28602 | 0.36 | 18581 | 2.10 | 6-OCTEN-1-OL, 3,7-DIMETHYL-, PROPANOATE | 82.05 |
| 7 | 21.840 | 56889 | 0.72 | 33547 | 3.79 | 16-Heptadecenal | 81.05 |
| 8 | 24.639 | 103075 | 1.31 | 50977 | 5.77 | TETRADECANAL | 71.00 |
| 9 | 24.708 | 1480 | 0.02 | 2029 | 0.23 | Propane, 2-nitro- | 42.95 |
| 10 | 28.700 | 363797 | 4.61 | 87873 | 9.94 | BICYCLO[4.1.0]HEPTANE, 4,4-DIMETHYL-3-(3-METHYL-3-BUTENYLIDENE)-2-METHYLENE- | 77.00 |
| 11 | 28.759 | 653591 | 8.28 | 95708 | 10.82 | 3-Oxatricyclo[20.8.0.0(7,16)]triaconta-1(22),7(16),9,13,23,29-hexaene | 189.05 |
| 12 | 28.900 | 57676 | 0.73 | 25510 | 2.89 | 2-TETRADECYNAL, 4-HYDROXY- | 71.00 |
| 13 | 30.841 | 30199 | 0.38 | 9979 | 1.13 | 2-Cyclohexyl-1,3-dioxolane-4,5-dicarboxylic acid, dimethyl ester | 145.05 |
| 14 | 30.944 | 110935 | 1.40 | 23195 | 2.62 | Tricyclo[4.3.0.0(7,9)]non-3-ene, 2,2,5,5,8,8-hexamethyl-, (1.alpha.,6.beta.,7.alpha.,9.alpha.)- | 189.10 |
| 15 | 31.075 | 2849 | 0.04 | 2287 | 0.26 | 1-HEXEN-3-OL | 57.00 |
| 16 | 32.023 | 77088 | 0.98 | 33224 | 3.76 | BICYCLO(10.3.0)PENTADEC-1(12)-EN-13-ONE TOSYLHYDRAZONE | 69.05 |
| 17 | 36.716 | 39326 | 0.50 | 11358 | 1.28 | Carbonic acid, neopentyl cyclohexylmethyl ester | 83.10 |
| 18 | 36.817 | 6399 | 0.08 | 2697 | 0.31 | 3,4-PENTADIEN-2-ONE, 1,1,1-TRIFLUORO- | 69.00 |
| | | 7897631 | 100.00 | 884170 | 100.00 | | |

**Fig. 3.2: GC-MS Chromatogram of *A.precatoriusL.*leaves ethanol extract.**

## Discussion

Inhibition activity of α-amylase enzyme *A.precatorius L.* leaves ethanol extract was tested for antidiabetic activity by DNS method. The extract exhibited dose dependent α-amylase inhibitory activities by in vitro assay using potato starch as substrate. *A.precatoriusL* .leaf is reported to have a broad range of therapeutic effects, like antibacterial, antifungal, antitumor, analgesic, anti-inflammatory, antispasmodic, anti-diabetic, antiserotonergic, anti-migraine, including treatment of inflammation, ulcers, wounds, throat scratches and sores[10].

The GC-MS and preliminaryqualitative phytochemical analysis ofethanol leaf extract confirmed the presence of secondary metabolites[11].The largest proximate content of the leavesis carbohydrate and minerals. The leaves are rich in potassium and calcium than other minerals[12].Flavonoid glycoside was isolated and characterized by using thin layer chromatographyin the leaves[13].

Several bioactive constituents like abrine, trigonelline,abruslactone A, hemiphloin, abrusoside A, abrusoside B, abrusoside C, abrusoside D, arabinose, galactose, xylose, choline, hypaphorine, precatorine, glycyrrhizin, montanyl alcohol, inositol, D-monomethyl ether, pinitol,3,4-Dihydroxy Benzoic Acid[14]are identified in the leaves of A. Precatorius[15].All the chemical groups identified in the leaves of *A. precatorius L.*find themselves in the traditional preparation[16].

The enzyme assay study reported that the extracted triterpene ketone, lupenone, together with 24-methylenecycloartenone andluteolin as a potent α-amylase inhibitor[17]. The leaves of *A. precatorius L.*possess

hypoglycaemic property by assessing the hypoglycaemic effect of the aqueous extract on some biochemical parameters in normal and alloxan-induced diabetic albino rats[18].

Phytochemical screening of our study is also reported that *A. precatoriusL.*leaves ethanol extract presence of alkaloids, carbohydrates, protein, tannins, flavonoids,steroids, triterpenes and phenolic compounds etc.(Table 3.3).The review of literature shows that there is an ability to inhibit intestinal α-glycosidase and pancreatic α-amylase enzymes in the active phytochemicals.

Acarbose was used as the standard for positive control; it inhibited the α-amylase activity with an $IC_{50}$ value 27.90µg/ml, while the $IC_{50}$ value of the ethanol extract was found to be 61.17µg/ml. This indicates that the ethanol extract has potent α-amylase inhibitor in comparison with standard (Fig. 3.1). The specific bioactive compounds which are responsible for this inhibition were studied through GC-MS analysis (Fig. 3.2).

## Conclusion

*A. precatorius L.*leaves showed that Ethanol extract has significant potent α-amylase inhibitory activity. The overall activity depends on phytochemical contents were present in the extract. It could be a source of natural antidiabetic agent, that have more importance as therapeutic agent for prevent and management of type-II diabetes. Therefore, it was concluded that ethanol extract of *A. PrecatoriusL.*leavesshowed potent antidiabetic activity and more investigations are proposed to validate these claims by identifying bioactive components with potential therapeutic benefits.

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