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**RESEARCH ARTICLE**

# "EXTRACTION OF VARIOUS EXTRACTS OF LEAVES OF *ALLAMANDA CATHARTICA* LINN. & ASSESSMENT OF THEIR HYPOGLYCEMIC POTENTIAL"

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## ABSTRACT

**Background:** Diabetes mellitus describes a metabolic disorder which results in increased blood glucose levels and disturbances of metabolism resulting from defects in insulin secretion, insulin action or both. Numbers of diabetic patients are increasing globally at large level. Ornamental plants of family Apocynaceae consists of several important medicinal plants with wide range of biological activities, interesting phytochemical constituents & have been traditionally used for the treatment of different ailments. **Objective:** Extraction & evaluation of the antidiabetic activity of extracts from leaves of *Allamanda cathartica* (Apocynaceae) Linn. **Methods:** Leaves of *A. cathartica* Linn. were collected, dried and extracted by using well established methods for alkaloids, flavonoids, steroids and crude extracts in polar and non-polar solvents. Evaluation of their antidiabetic activity was done with salivary alpha amylase and starch as substrate using chromogenic DNSA (2,4- Di nitro Salicylic Acid) method & Starch iodine method. All experiments were performed in 3 different sets each in triplicates. The data are expressed as

mean ± SEM (standard error of the mean). **Results:** The highest inhibition (26.29±0.44%) was obtained at concentration of 1.5 mg/ml of methanolic extract of leaves with an $IC_{50}$ value of 5.098 g/ml. Steroid extracts were also found to have high inhibitory potential with low $IC_{50}$ value. All other tested extracts (not alkaloids) were also showed good alpha amylase inhibition activity. **Conclusion:** Thus, all the tested leaf extracts might be effective in lowering the postprandial hyperglycemia by inhibiting salivary alpha amylase enzyme.

## INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. [1] Selecting the right scientific and systematic approach to biological evaluation of plant products, based on their use in traditional medicine is the key to ideal development of new drugs from plants. [2] The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential for systematic evaluation of plants used in traditional medicine for various ailments. Hence, there is need to screen medicinal plants for promising biological activity. [3] Over 50% of all modern drugs are of natural product origin and they play an important role in drug development programs of the pharmaceutical industry. [4] Many modern synthetic drugs may harm more than they help in curing diseases by its serious effects. On contrary, traditional medicines which make use of plants are much more esteemed being more safe without harmful effects and comparatively less expensive than many allopathic medicines. [5] Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value which have yet to be discovered. [6] In recent years, Diabetes mellitus has become a serious global health problem affecting about 10% population of the world. The most prevalent form both in the global and Indian scenario is the non-insulin dependent diabetes mellitus (NIDDM 2) which is associated with elevated postprandial hyperglycemia. [7] There is a list which include over 50 plants that are present in arid zone of Rajasthan have anti-diabetic potentials. [8] WHO has recommended the evaluation and mechanistic properties of the plants effective in such systems. [9] Alpha amylase inhibitor plays major role in the management of postprandial hyperglycemia. [10] The search for new pharmacologically active agents obtained by screening natural

sources such as medicinal plants or their extracts can lead to potent and specific inhibitors for α-amylase. [11] Inhibition of alpha amylase enzyme in the digestive tract of human is being considered to be effective in controlling diabetes by decreasing the absorption of glucose from starch. [12] Long-term day-to-day management of diabetes, with acarbose is well tolerated and can improve glycaemic control as monotherapy, as well as in combination therapy. [13] In Type 2 DM, hyperglycemia is a condition characterized by an abnormal post-prandial increase in blood glucose level. Many plant extracts and natural products have been investigated with respect to suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine. [14] Anti-diabetic property of extracts of higher plants in India has been reported. [15] Plants extracts inhibit the activity of alpha amylase enzyme at different concentrations have also been reported. [16] Hydro alcoholic extracts of *Azadirachta indica* (Neem) plant showed anti-hyprglycemic activity in streptozotocin treated rats and this effect is because of increase in glucose uptake and glycogen deposition in isolated rat hemi diaphragm. [17] No literature related to antidiabetic activity of *Allamanda cathartica* Linn. was found. Evaluation of antidiabetic potential of various extracts of leaves of *A. cathartica* Linn. has been carried out for the very first time in the present study.

## MATERIALS AND METHODLOGY

Leaves of *Allamanda cathartica* Linn. were collected from different localities of Jaipur. Shade dried, weighed and stored in different containers. Extraction of plant part in different polar, non polar solvents (Water, methanol and petroleum ether) and for their secondary metabolites (alkaloids, flavonoids and steroids) was carried out by well established methods.

### Extraction of Alkaloids

Alkaloids were extracted from leaves of the selected plant by well established method. [18] Finely powered sample (100g) of leaves was extracted in 20ml methanol after shaking for 15 min. After filtration, the filtrate was kept for drying. Thereafter residual mass was treated with 1% $H_2SO_4$ (5ml. 2 times).Extraction was then done in 10ml chloroform ($CHCl_3$) by using separating funnel. Organic layer of chloroform was rejected and aqueous layer was basified with 30% $NH_4OH$ ($P^H$=9-10). Again, extraction was done in 10ml chloroform & organic layer of chloroform (lower layer) was collected in a flask,

extraction was repeated with fresh chloroform and was dried in vacuo.

**Extraction of Flavonoids**

Leaves of selected plant were subjected to the flavonoid extraction following the method of Subramanian and Nagarjan. [19] One hundred gram of finely powdered sample was soxhlet extracted with 80% hot methanol (500ml) on a water bath for 24 h and filtered. Filtrate was re- extracted successively with petroleum ether (fraction I), diethyl ether (fraction II), and ethyl acetate (fraction III) using separating funnel. Petroleum ether fraction was discarded as being rich in fatty substances whereas diethyl ether and ethyl acetate fraction were analyzed for free and bound flavonoids respectively. The ethyl acetate fraction of sample was hydrolyzed by refluxing with 7% $H_2SO_4$ for 2 h (for removal of bound sugars) and the filtrate was extracted with ethyl acetate in separating funnel. Ethyl acetate extract obtained was washed with distilled water to neutrality. Diethyl ether (free flavonoids) and ethyl acetate fractions (bound flavonoids) were dried in vacuo and weighed.

**Extraction of Steroids**

Steroids were extracted from leaves of the selected plant by well established method, [20] after preliminary detection of steroids. Finely powdered sample (100g) of leaves was extracted in petroleum ether for 24hrs.After filtration; residual mass was treated with 15% ethanolic HCl for 4hr. Extraction was then done in ethyl acetate followed by washing in dis. water to neutralize the extract. Neutral extract was then passed over Sodium sulphate to remove moisture contents and was dried in vacuo. Chloroform was used for reconstitution of extract, filtered, dried and stored.

**Extraction of crude extracts in polar and non polar solvents**

Dry plant material (20 gm each) was taken separately in round bottomed flask in different polar & non polar solvents (water, methanol and petroleum ether) in the ratio of 1:10. Soxhlet extraction was carried out for 24 hours and filtered. Each filtrate was subjected to evaporation to obtain crude dried extract which was weighed and calculated for each gram plant material.

**In vitro salivary α amylase inhibitory assay:**

**1. Starch – Iodine color assay:**

Screening of plant extracts for α-amylase inhibitory activity was carried out in test tubes following the method of Xiao Z, [21] with slight modifications based on the starch

iodine test. Total assay mixture composed of 120 µl 0.02M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 1.5 ml of salivary amylase and plant extracts of concentration range 0.5-1.5 $mgml^{-1}$ (w/v) were incubated at 37°C for 10 min. Soluble starch (1% w/v) was then added to each reaction mixture and were incubated at 37°C for 15 min. Thereafter 1 M HCl (60 µl) was added to stop the enzymatic reaction, followed by the addition of 300 µl of iodine reagent (5 mM $I_2$ and 5 mM KI). Colour change was observed and the absorbance was recorded at 620 nm. Reaction tubes of control representing 100% enzyme activity which did not contain any plant extract. To eliminate the absorbance produced by plant extract, appropriate extract controls without the enzyme were also examined. Appearance of dark-blue colour indicates the presence of starch; a yellow colour indicates the absence of starch while a brownish colour indicates partially degraded starch in the reaction mixture. In the presence of inhibitors from the extracts the starch added to the enzyme assay mixture was not degraded and gave dark-blue colour complex whereas no coloured complex was developed in the absence of the inhibitor, indicating that starch was completely hydrolysed by α-amylase.

**2. Glucose-DNSA color assay:**

Inhibition assay was performed using chromogenic DNSA method. [22] Total assay mixture composed of 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 1ml of salivary amylase and 400 µl extracts of concentration ranging from 0.5-1.5 $mgml^{-1}$(w/v) was incubated at 37°C for 10 min. After pre-incubation, 580 µl of 1% (w/v) starch solution was added to each tube and were subjected to incubation at 37°C for 15 min. Reaction was then terminated by adding1.0 ml DNSA reagent and each tube was placed in boiling water bath for 5 min., cooled to room temperature and the absorbance was measured at 540 nm. Control containing no plant extracts showed 100% enzyme activity. To eliminate the absorbance produced by plant extract, appropriate extract controls with the extract in the reaction mixture except for the enzyme were also included (negative control). Percent inhibition of alpha amylase was calculated as follows:

Percent Relative enzyme activity= (enzyme activity in test sample with extract/enzyme activity in control)*100.

% Inhibition in the $\alpha$-amylase activity= (100−% Relative enzyme activity).

**Statistical Data Analysis**

All experiments were performed in 3 different sets, each in triplicate. The data are expressed as mean ± SEM. Statistical difference, ANOVA and linear regression analysis were performed using Graph pad prism 5 statistical software. The $IC_{50}$ values were determined from plots of percent inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values. The $IC_{50}$ values were defined as the concentration of the extract, containing the α-amylase inhibitor that inhibited 50% of the alpha amylase activity.

## RESULTS

Results revealed that various extracts of leaves of *Allamanda cathartica* L. exhibit alpha amylase inhibitory activity of different level (Table 1).

**Extracts with high inhibitory effect on alpha amylase activity:**

Methanolic extracts (at a concentration of 0.5- 1.5 mg/ml) showed the highest alpha amylase inhibition with high percent inhibition (9.20±0.50% to 26.29±0.44%) and low $IC_{50}$ value (5.09 g/ml). Steroid extracts (% inhibition= 24.72±0.40% to 35.62±0.66%, $IC_{50}$ value= 7.38 g/ml) were also exhibit good level of alpha amylase inhibition. (Table 2)

**Extracts with moderate inhibitory effect on alpha amylase activity:**

Free flavonoid (% inhibition= 13.40±0.63% to 21.3±0.60%, $IC_{50}$ value= 39.13 g/ml), bound flavonoid (% inhibition= 10.17±1.05% to 17.89±1.00%, $IC_{50}$ value= 24.03 g/ml), petroleum ether (% inhibition= 7.62±0.75% to 15.45±0.50%, $IC_{50}$ value= 52.44 g/ml) & water extract (% inhibition= 13.67±0.50% to 21.33±0.51%, $IC_{50}$ value= 42.13 g/ml) of leaves were recorded to have moderate alpha amylase inhibitory activity. (Table 3)

**Extracts with low inhibitory effect on alpha amylase activity:**

Alkaloids of leaves were found to have low (insufficient) inhibitory activity with low % inhibition (21.9±0.55% to 23.97±0.40%) and high IC50 value (945387.29 g/ml). (Table 4)

The alpha amylase inhibitory activity of different extracts of leaves of *Allamanda cathartica* Linn. has been shown in figure 1.

## DISCUSSION

A number of medicinal plants and their formulations are used for treating diabetes in the traditional Indian Ayurvedic system as well as in ethno medicinal practices. Many plant extracts and natural products have

been investigated with respect to suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine. Retardation of starch digestion by inhibition of enzyme such as α-amylase would play a key role in the control of diabetes. Till now, studies for alpha amylase inhibitory activity was done using extracts of multiple plants, but this is the first time *Allamanda cathartica* leaf extracts have been tested for this particular action. We found the methanolic and steroid extract to have most hypoglycemic potential. Further studies are required to find out the mode of action of these plant extracts as alpha amylase enzyme inhibitors and to qualify the action of different constituents in the extract.

## CONCLUSION

The results of the present study indicate that leaves of this plant exhibits potent inhibitory activity on salivary alpha amylase enzyme. $IC_{50}$ value of methanolic and steroid extracts of leaves are lowest than other extracts of leaves, indicating high inhibitory potential of these extracts. Thus, these extracts might be helpful in identification of new potent lead molecule for natural amylase inhibitors.

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**Table 1: Level of alpha amylase inhibitory activity of different extracts of leaves of *A. Cathartica* Linn.**

| Name of plant part | Name of extract | Level of inhibitory activity |
|---|---|---|
| **Leaf** | Alkaloid | **-** |
| | Steroid | ++ |
| | Free flavonoid | + |
| | Bound flavonoid | + |
| | Pet ether | + |
| | Methanol | ++ |
| | Water | + |

***Note:*** *Indication of inhibitory potential of extract: (++) extracts with high inhibitory effects on alpha amylase, (+) extracts with moderate inhibitory effects on alpha amylase activity, (-) extracts with insignificant (low) inhibitory effects on alpha amylase activity.*

**Table 2: Extracts with maximum inhibitory effects on the alpha amylase activity**

| Sr. No. | Name of extracts | Concentration (mg/ml) | % inhibition | Regression equation | $IC_{50}$ Value |
|---|---|---|---|---|---|
| **1.** | Methanolic extract | 0.5 | 9.20±0.50 | Y=4.021+1.384X | 5.098 |
| | | 1.0 | 13.59±1.00 | | |
| | | 1.5 | 26.29±0.44 | | |
| **2.** | Steroid | 0.5 | 24.72±0.40 | Y=4.478+0.601X | 7.388 |
| | | 1.0 | 27.45±0.76 | | |
| | | 1.5 | 35.62±0.66 | | |

***NOTE****: Values are given as mean ± SD (n=3). One way analysis of variance was used which show significant difference with respect to control (P≤0.05)*

**Table 3: Extracts with moderate inhibitory effects on the alpha amylase activity**

| Sr. No. | Name of extracts | Concentration (mg/ml) | % inhibition | Regression equation | $IC_{50}$ Value |
|---|---|---|---|---|---|
| **1.** | Bound flavonoid | 0.5 | 10.17±1.05 | Y=3.952+0.759X | 24.030 |
| | | 1.0 | 15.24±0.58 | | |
| | | 1.5 | 17.89±1.00 | | |
| **2.** | Free flavonoid | 0.5 | 13.40±0.63 | Y=4.019+0.616X | 39.132 |
| | | 1.0 | 14.34±0.40 | | |
| | | 1.5 | 21.3±0.60 | | |
| **3.** | Water extract | 0.5 | 13.67±0.50 | Y=4.087+0.562X | 42.126 |
| | | 1.0 | 18.13±0.32 | | |
| | | 1.5 | 21.33±0.51 | | |
| **4.** | Petroleum ether extract | 0.5 | 7.62±0.75 | Y=3.767+0.717X | 52.440 |
| | | 1.0 | 8.64±0.71 | | |
| | | 1.5 | 15.45±0.50 | | |

***NOTE**: Values are given as mean ± SD (n=3). One way analysis of variance was used which show significant difference with respect to control (P≤0.05)*

**Table 4: Extracts with low inhibitory effects on the alpha amylase activity**

| Sr. No. | Name of extracts | Concentration (mg/ml) | % inhibition | Regression equation | $IC_{50}$ Value |
|---|---|---|---|---|---|
| **1.** | Alkaloid | 0.5 | 21.90±0.55% | Y=4.265+0.123X | 945387.29 |
| | | 1.0 | 22.63±0.87% | | |
| | | 1.5 | 23.97±0.40% | | |

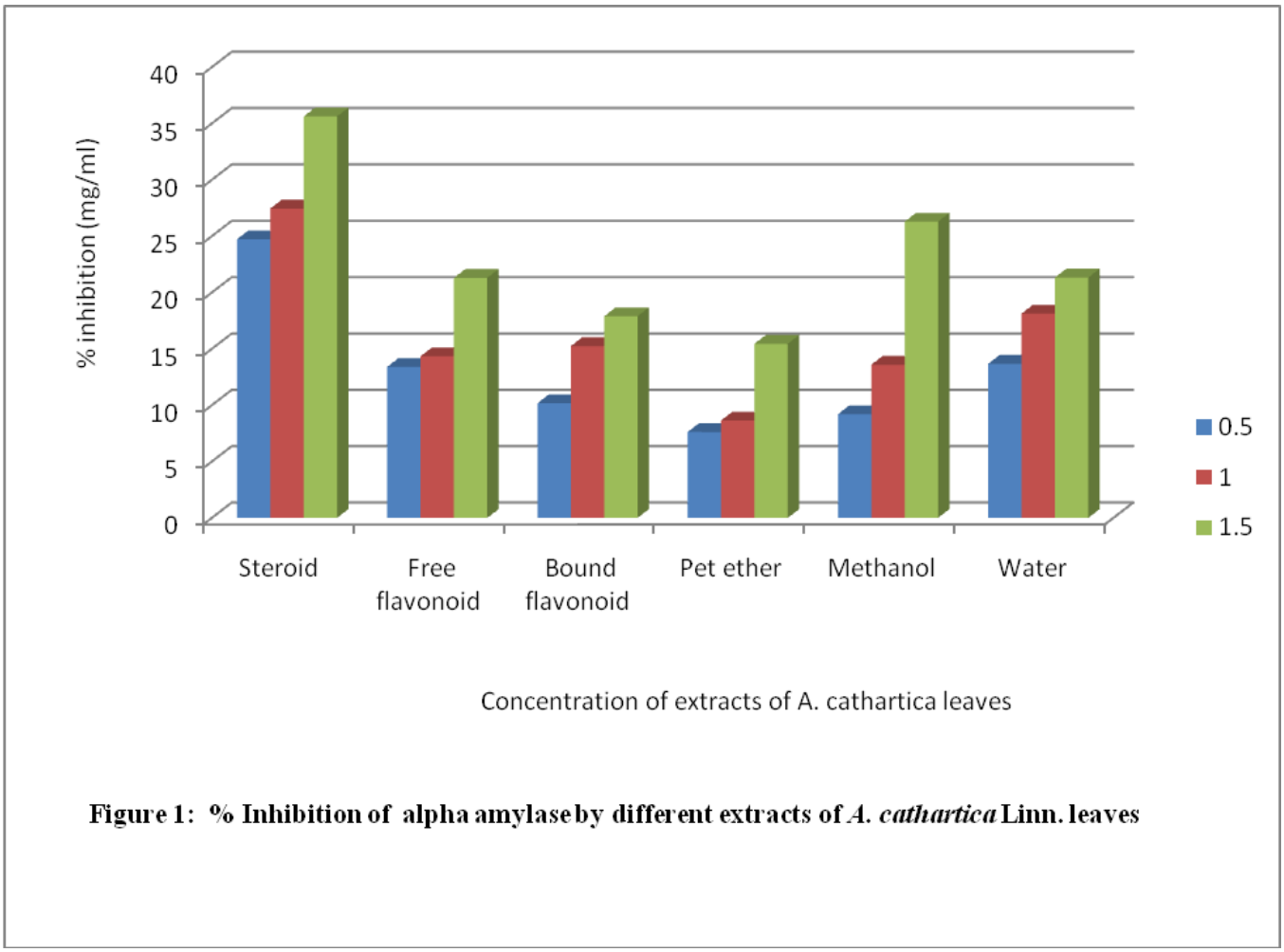


**Figure 1: % Inhibition of alpha amylase by different extracts of *A. cathartica* Linn. leaves**