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# Phytochemical Analysis and Inhibitiory Activity of Petroleum Ether Extract of T. Divaricata Leaves Against Carbohydrate Hydrolysing Enzyme α–Amylase

KEYWORDS: T. Divaricata, Alpha Amylase inhibition assay, phytochemical Screening.


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**ABSTRACT** *T.divaricata a common garden plant in tropical countries has been used as a traditional medicine. Phytochemical analysis of T.divaricata revealed the presence of Steroids, Reducing Sugar, Proteins, Terpenoids, Gums, Tannins, and Saponins. In this study significant inhibition effect of carbohydrate hydrolysing enzyme alpha amylase in the leaves extract of T. divaricata have been observed. As result the concentration of carbohydrate i.e. glucose will be controlled and in turns it will controlling type 2 Diabetes mellitus. So, further comprehensive pharmacological and phytochemical investigations are needed to elucidate the specific chemical compounds responsible for alpha amylase inhibition and their mode of actions. It can be further purified and used as an alternative for synthetic drugs.*

## INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder that affects the metabolism ofcarbohydrates, proteins, fat, electrolytes and water. It includes a group of metabolic diseases characterized by hyperglycemia, in which blood glucose levels are elevated either because the pancreas do not produce enough insulin or cells do not respond to the produced insulin or both (West IC,2000; WHO, 2006). Epidemiological studies and clinical trials strongly support that hyperglycemia is the main cause of complications related with coronary artery disease, cerebrovascular disease, renal failure, blindness, limb amputation, neurological complications and pre-mature death(Lopez CA ,2001).

Currently, 347 million people worldwide have diabetes (Danaei G,et al., 2011). The frequency of this disorder is on the rise globally, is likely to hit 300 million by 2025 with India projected to have the largest number of diabetic cases (Gupta OP, Phatak S 2003). In 2004, an estimated 3.4 million people died from consequences of high fasting blood sugar (Global Health Risks, 2009). More than 80% of diabetes deaths occur in low- and middle-income countries (Mathers CD, Loncar D. 2006). WHO projects that diabetes will be the 7th leading cause of death in 2030(WHO, 2011).

The most abundant enzyme in human saliva, α-amylase salivary, possess biological functions, its hydrolytic activity is responsible for the initial break down of starch to oligosaccharides. Because of its central role in digestion of food, Ethnopharmacological approach and bioassay-guided isolation have provided a lead in identifying potential α-amylase inhibitors from plant sources. Currently, methods to determine the levels of α-amylase inhibitor are based on the measurement of α-amylase activity using the chromogenic DNSA method staining power in the presence or absence of an inhibitor during the action of the enzyme on soluble starch or by using an alkaline reactive whose reduction products are determined photometrically as reported by Bernfeld (Bernfeld P. 1955).

Plants have long been used for the treatment of diabetes. Naturally available α - amylase inhibitor from medicinally important plants are shown to be effective in managing postprandial hyperglycemia which is a major concern in type-2 diabetes.

Present study was on plant T. Divaricata leaves which has many roles in daily life. The most common medicinal use of crude T. divaricata extract involves its antimicrobial action against infectious diseases such as syphilis, leprosy, and gonorrhoea, as well as its antiparasitic action against worms, dysentery, diarrhoea, and malaria (Van Beek et al., 1984).

T.Divaricata belongs to the family Apocynaceae is an ornamental, flowering, evergreen shrub that generally grows to a height of 6ft and comes under the genus Tabernaemontana which consists of 100-110 species of flowering plants (Rahman M D et al., 2011). It is a common garden plant found in tropical countries incuding Brazil, Egypt, India, Sri Lanka, Vietnam, Malaysia and Thailand (Wasanapratchayasakulet al., 2008).The plant have medicinal value and have been used traditionally for the treatment of ulcers and rheumatism(Orient Longman 1996), other medicinal properties of the plant include Anticancer (Akhila Sravya Dantu et al., 2012), Anixolytic (Basavaraj Pet al., 2011), Antidiabetic (Masudur rahman M D et al., 2011) and Anticonvulsant (Basavaraj Pet al., 2011) activities. The main aim of the present work was to evaluate the α - amylase inhibitory activity and phytochemical analysis of Petroleum Ether extract of dried leaves of T.divaricata.

## MATERIALS AND METHODS

### 1. Materials

**Plant materials:**

The leaves of T.Divaricata were collected in the month of November 2013 from the fields around the area of Bhaurad in Akola district, Maharashtra, India.

**Preparation of extracts:**

The leaves were washed thoroughly 2-3 times with running tap water, leaf material was then air dried under shade, after complete shade drying the plant material was grinded in mixer, and the powder was kept in small plastic bags with paper labelling. The powdered plant material was successfully extracted using Whatman No.1 filter paper and Soxhlet extractor by the solvent Petroleum Ether

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($60^0$-$80^0$) according to their increasing polarity respectively. The extract obtained was filtered and evaporated to dryness at 37 °C under Incubator.

**Chemicals:**
All the chemicals were purchased from Hi Media Laboratories Pvt. Limited, Mumbai, India, Quligens Fine Chemicals, Glaxo Smith Kline Pharmaceuticals Limited, Mumbai India unless otherwise stated.

**Methods:**
**I) α–Amylase Enzyme Inhibition by 3, 5-Dinitrosalicylic Acid Assay Method**
α-Amylase inhibition assay was performed using chromogenic method adopted from Sigma – Aldrichwhich was adopted from Bernfeld (1955).

Three sets of experiments were conducted: Three test tubes were taken & labelled as blank, standard and test. Primarily 0.5 ml of starch (1%) solution was added in all tubes,followed addition of inhibitor i.e. 0.5 ml of plant extract (10 mg/ml)was done in only Test. Incubate at 37 ° c for 5 to 10 min.Then add of 0.5 ml of Amylase (0.01g /100 ml of D.w.) solution in std. and test. After that 0.5 ml of distilled water was added in all the test tube. Finally, 0.5 ml of the DNSA reagent was added in all the tubes whichwere then placed in boiling water bath for 15 min. The tubes were cooled and the reaction mixture was diluted with 5 ml of distilled water. Now absorbance was recorded at 540 nm against blank.

**II) Phytochemical Screening:**
Qualitative phytochemical screening was carried out with thefollowing methods.

**1. Test for Steroids:**
1 ml extract was dissolved in 10 ml chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids(Gibbs, R.D., 1974).

**2. Test for alkaloids:**
2 ml extract and 0.2 ml dilute hydrochloric acid was taken in a test tube. Then 1 ml of Mayer's reagent was added. Yellow colourprecipitate indicates the presence of alkaloids (Shazid Md. Sharker et al., 2011).

**3. Tests for reducing sugar:**
Benedict's test: 0.5 ml of aqueous extract and 5 ml of Benedict's solution was taken in test tube, boiled for 5 min and allowed to cool spontaneously. A red colour precipitate of cuprous oxide indicating the presence of a reducing sugar (Shazid Md. Sharker et al., 2011).

**4. Test for Terpenoids:**
2 ml extract was added to 2 ml of acetic anhydride and concentration of $H_2So_4$.Formation of blue, green rings indicate the presence of terpenoids.(Ayoola, G.A.et al., 2008).

**5. Test for Fatty Acids :**
0.5 ml extract was mixed with 5 ml of ether. The extract was allow for evaporation on filter paper and dried the filter paper. The appearance of transparencyon filter paper indicates the presence of fatty acids (Ayoola, G.A.et al. 2008).

**6. Test for Flavonoids :**
A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract. Immediate development of a red colour indicates the presence of Flavonoids (Shazid Md. Sharker et al., 2011).

**7. Test for Gums :**
0.5 ml extract was taken and then Molish reagent and sulphuric acid were added. Red violet ring produced at the junction of two liquids indicates the presence of gums (Shazid Md. Sharker et al., 2011).

**8. Test for Tannins :**
To the 2 ml of extract few drops of 1% lead acetate was added. A yellowish precipitate indicated the presence of tannins (Treare G.E. and Evans W.C., 1985).

**9. Test for Saponins :**
5 ml extract was mixed with 20 ml of distilled water and agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins (Kumar A. et al.,2009).

**10.Test for Anthocyanins :**
2 ml extract is added to 2 ml of 2N Hcl and ammonia. The appearance of pink-red turns blue-violet indicates the presence of anthocyanins (Paris, R. and H. Moyse, 1969).

**Result and Discussion:**

**Figure no. I: α-Amylase Inhibition**

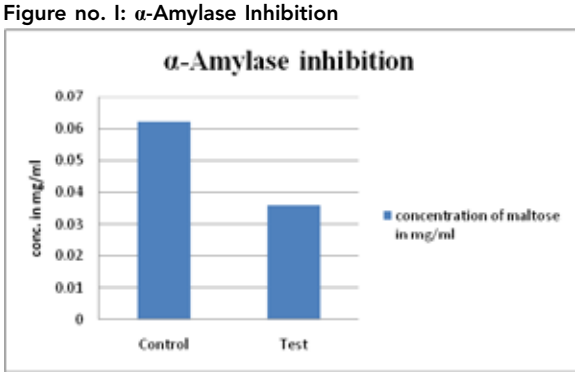


**Table no. 1: Phytochemical analysis of Tabernaemontana Divaricata**

| No. | Phytochemicals | P. Ether Extract of T.Divaricata |
|---|---|---|
| 1. | Steroids | + |
| 2. | Alkoloids | - |
| 3. | Reducing Sugar | + |
| 4. | Terpenoids | + |
| 5. | Fatty Acids | - |
| 6. | Flavonoids | - |
| 7. | Gums | + |
| 8. | Tannins | + |
| 9. | Saponins | + |
| 10. | Anthocynins | - |

**(+) = Positive (-) = Negative**

In the present study Petroleum Ether Extract of leaves of T. divaricata of concentration 10mg/ml used in the alpha amylase inhibition activity. The alpha amylase inhibition activity was studied with the help of 3, 5 – Dinitro Salicylic Acid Assay method. Thus from the figure no. I the concentration of maltose in control and Test was found to be 0.062 and 0.036 mg/ml/min respectively which clearly showed the inhibitory activity of T. divaricata on alpha amylase activity.

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Besides this Phytochemical analysis revealed the presence of Steroids, Reducing Sugar, Proteins, Terpenoids, Gums, Tannins, and Saponins. In this study significant inhibition effect of carbohydrate hydrolysing enzyme alpha amylase in the leaves extract of T. divaricata have been observed. As result the concentration of carbohydrate i.e. glucose will be controlled and in turns it will controlling type 2 Diabetes mellitus. So, further comprehensive pharmacological and phytochemical investigations are needed to elucidate the specific chemical compounds responsible for alpha amylase inhibition and their mode of actions. It can be further purified and used as an alternative for synthetic drugs.

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