

SCREENING OF PHYTOCHEMICALS AND ANTIDIABETIC ACTIVITY OF *Vitex negundo* leaves

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ABSTRACT

Diabetes mellitus is Major carbohydrate metabolic disorders. Insulin and oral hypoglycemic agents are still the major players in the management of the disease but many adverse effects, observed and they did not reduce diabetic associated complications. Currently available treatment is far from satisfactory and is expensive. Many indigenous Indian medicinal plants have been found to be successfully used to manage diabetes. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones. The present study aimed to evaluate the phytochemical and in vitro anti diabetic activity of Vitex negundo leaves was analyzed. Based on the results of the present study it can be concluded that the methanolic and aqueous extract of Vitex negundo leaves contain rich source of phytochemical. Vitex negundo leaves has potential antidiabetic activity. Antidiabetic activity may due to active compounds present in the extract. Further pre-clinical studies should be needed to confirm antidiabetic activity and isolation of antidiabetic compound.

Keywords:

Vitex negundo, Phytochemicals and Antidiabetic activity.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, its insulin action and both. It includes a group of metabolic diseases characterized by hyperglycemia. As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration. Therefore a therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia. Diabetes is a metabolic disorder of carbohydrate, fat and protein, affecting a large number of population in the world (Pareek *et al.*, 2009).

Diabetes mellitus is not a single disorder but it is a group of metabolic disorder characterised by chronic hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. Increased thirst, increased urinary output, ketonemia and ketonuria are the common symptoms of diabetes mellitus, which occur due to the abnormalities in carbohydrate, fat, and protein metabolism. When ketones body is present in the blood or urine, it is called ketoacidosis, hence proper treatment should be taken immediately, else it can leads to other diabetic complications (Craig *et al.*, 2009).

Natural anti-diabetic drugs from medicinal plants, is the other available therapy for the treatment of diabetes mellitus due to their well-known biological activity. Substances extracted

from fruiting bodies, cultured mycelia, and culture media have exhibited promising in vitro and in vivo biological activity including anti-diabetes (Ding *et al.*, 2010). Aldose reductase as a key enzyme, catalyze the reduction of glucose to sorbitol and is associated in the chronic complications of diabetes such as peripheral neuropathy and retinopathy. Use of aldose reductase inhibitors and α -glucosidase inhibitors has been reported for the treatment of diabetic complications (Jung *et al.*, 2011).

Many indigenous Indian medicinal plants have been found to be successfully used to manage diabetes. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones. However, search for new anti-diabetic drugs continue. Keeping this view, the present study aimed to evaluate the phytochemical and *in vitro* anti diabetic activity of *Vitex negundo* leaves were analyzed.

MATERIALS AND METHODS

Plant materials:

The fully mature *Vitex negundo* leaves was collected in December 2017 from, Melaperumalai, Thiruvarur district, Tamil Nadu, India. The leaf of *Vitex negundo* leaves was first washed well and dust was removed from the leaves. The leaves was spread out in a plain paper and shade dried at room temperature for about 10 days and makes a fine powder using grinder mixture. The powder was extracted with methanol. The extract was stored in refrigerator until used.

Preparation of alcoholic extract

2 gram of the powder of *Vitex negundo* leaves were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (methanol and water). The conical flask containing *Vitex negundo* leaves were shake it well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using whatman filter paper no.1 and filtrate used for further analysis.

Phytochemical screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

In vitro antidiabetic activity

In vitro α -amylase inhibition assay was carried out by the method of Apostolidis (2007). The α -glucosidase inhibitory activity was determined according to the method described by Apostolidis *et al.*, (2007).

RESULTS AND DISCUSSION

In the present study was carried out the phytochemical analysis on the *Vitex negundo* leaves revealed the presence of medicinally active constituents. The phytochemical characters of the *Vitex negundo* leaves investigated and summarized in Table-1. The phytochemical screening *Vitex negundo* leaves both extracts showed that the presence of saponins, flavonoids, polyphenol, triterpenoids steroids, alkaloids, carbohydrate, protein, anthroquinonesm, tannin, terpenoids and glycosides whereas phlopatannins was absent in aqueous extract.

Table.1: Qualitative Phytochemical analysis of *Vitex negundo* leaves extract

| S.No | Phytochemicals | Aqueous Extract | Methanol Extract |
|------|----------------|-----------------|------------------|
| 1 | Tannin | + | + |
| 2 | Phlobatannins | - | + |
| 3 | Saponins | ++ | + |
| 4 | Flavonoids | + | ++ |
| 5 | Steroids | ++ | ++ |
| 6 | Terpenoids | ++ | + |
| 7 | Triterpenoids | + | + |
| 8 | Alkaloids | + | + |
| 9 | Carbohydrate | + | ++ |
| 10 | Protein | + | + |
| 11 | Anthroquinone | + | ++ |
| 12 | Polyphenol | ++ | + |
| 13 | Glycoside | ++ | + |

+ indicates presences ++ indicates high concentration and - Indicates absence,

Hassain *et al.* (2011) screened phytochemical constituents from methanol leaf extract of *Bombax malabaricum*. Various organic 11 solvent extracts of *Pedaliium murex* were subjected to preliminary phytochemical screenings by Thamizh mozhi *et al.* (2011). Selected 53 traditionally used medicinal plants from western region of India for their qualitative phytochemical screenings, total phenol and flavonoids contents. Pascaline *et al.* (2011) screened phytochemical constituents of some medicinal plants used by the Nandis of South Nandi District, Kenya.

Reena Ganesan *et al.*, (2013) aimed to carry out preliminary phytochemical of six different solvents extracts from leaf and leaf derived callus of *Sebastiania chamaelea*. The preliminary phytochemical analysis reflects the presence of phenolic compounds, carbohydrate, alkaloids, phytosterols, fats and oils, terpenoids. The result highlights among two extracts, leaf extract show negligible activity than callus extracts

***In vitro* Antidiabetic activity**

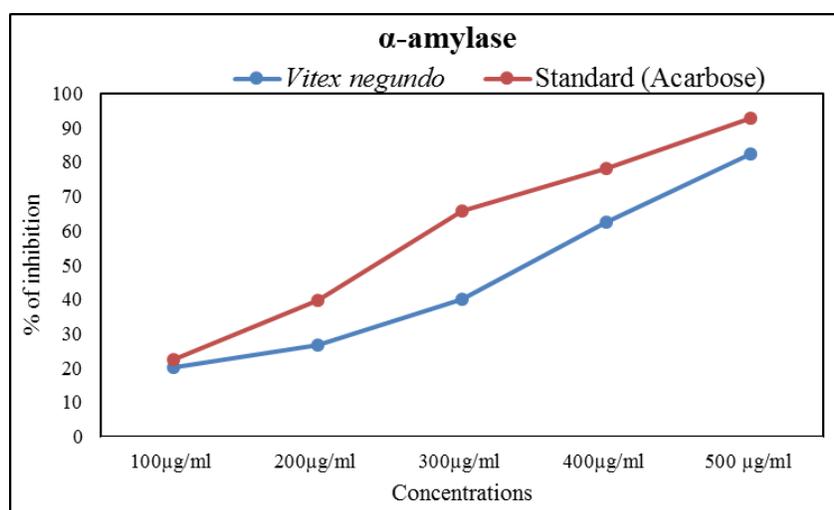
A study of ancient literature indicates that diabetes (Madhumeha/Prameha) was fairly well known and well-conceived as an entity in India. Regulation of glucose level in the blood of the diabetic patient can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species (Raghavendra *et al.*, 2010).

The intestinal digestive enzymes alpha-amylase plays a vital role in the carbohydrate digestion. One antidiabetic therapeutic approach reduces the post prandial glucose level in blood by the inhibition of alpha-amylase enzyme. These can be an important strategy in management of blood glucose. The in-vitro α -amylase inhibitory studies demonstrated that *Vitex negundo* well anti diabetic activity (Table 2). The percentage inhibition at 10, 20, 40, 60, 80 μ g/ml concentration of crude plant extracts shown concentration dependent reduction in percentage inhibition. At a concentration of 10 μ /ml of *Vitex negundo* has 82.23% and standard 92.84%.

Table 2: *In vitro* α -amylase inhibition of *Vitex negundo*

| Concentrations | <i>Vitex negundo</i> | Standard (Acarbose) |
|----------------|----------------------|---------------------|
| | % of inhibition | |
| 100 μ g/ml | 20.12 \pm 1.40 | 22.45 \pm 1.57 |
| 200 μ g/ml | 26.66 \pm 1.86 | 39.61 \pm 2.74 |
| 300 μ g/ml | 40.12 \pm 2.80 | 65.74 \pm 4.67 |
| 400 μ g/ml | 62.66 \pm 3.28 | 78.31 \pm 5.48 |
| 500 μ g/ml | 82.23 \pm 5.75 | 92.84 \pm 6.49 |

Values are expressed as Mean \pm SD for triplicates

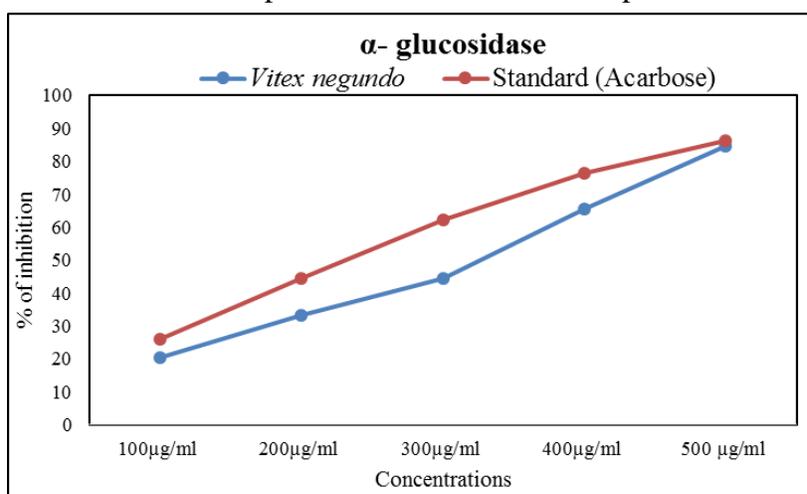
**Figure 1: *In vitro* α -amylase inhibition of *Vitex negundo***

α -glucosidase catalyzes the final step in carbohydrate digestion which leads to postprandial hyperglycemia. Inhibitors of α -glucosidase are useful in the control of hyperglycemia as they delay carbohydrate digestion and causing reduced glucose absorption rate which consequently reduce the postprandial plasma glucose rise (Tarling *et al.*, 2008). These inhibitors have been found useful in the control of diabetes mellitus over many years (Layer *et al.*, 1986 Tundis *et al.*, 2010) Many scientists have investigated the plants containing various phytochemicals that exhibit additive and synergistic interaction in antidiabetic properties which exert positive health-promoting effects (Samad *et al.*, 2009). In this present study, *in vitro* α -glucosidase inhibitor activity of methanolic extract of *Vitex negundo* was evaluated. The retardation and delay of carbohydrate absorption with a plant-based α -glucosidase inhibitor offers a prospective therapeutic approach for the management of type 2 diabetes mellitus. The values show that *Vitex negundo* has 84.65% and standard 86.14% (Table 3).

Table 3: *In vitro* α -glucosidase inhibition of *Vitex negundo*

| Concentrations | <i>Vitex negundo</i> | Standard (Acarbose) |
|----------------|----------------------|---------------------|
| | % of inhibition | |
| 100 μ g/ml | 20.45 \pm 1.43 | 25.92 \pm 1.81 |
| 200 μ g/ml | 33.10 \pm 2.31 | 44.45 \pm 3.11 |
| 300 μ g/ml | 44.48 \pm 3.11 | 62.35 \pm 4.36 |
| 400 μ g/ml | 65.59 \pm 4.59 | 76.45 \pm 5.35 |
| 500 μ g/ml | 84.65 \pm 5.92 | 86.14 \pm 6.02 |

Values are expressed as Mean \pm SD for triplicates

**Figure 2: *In vitro* α -glucosidase inhibition of *Vitex negundo***

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide in to mono and disaccharide. In our experimental study it was observed that methanolic extract of *Vitex negundo* demonstrated significant Alpha amylase inhibition activity as compared to standard drug acarbose and thus confirmed the antidiabetic activity.

CONCLUSION

In India the prevalence rate of diabetes estimated to be 1-5% complication are the major cause of morbidity and mortality in diabetic mellitus. There is an increasing demand by the use of animal products of natural products due to the side effects associated with used of insulin and oral hypoglycemic agents. The first scientific report that provides convincing phytochemicals and antidiabetic activity evidence for the relevance of *Vitex negundo* leaves thus providing scientific validity to its traditional consumption by the local populace of south India. *Vitex negundo* leaves extract had a good potential for therapeutic use against diabetic.

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