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Evaluation of *In Vitro* Alpha Amylase and alpha glucosidase inhibitory activities of bark of *Terminalia bellirica*

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Abstract

Diabetes mellitus is a chronic disease and known to be associated with obesity, hypertension, hyper-lipidemia, neuropathy and cardiovascular diseases. In the present study the petroleum ether, chloroform, ethanol and aqueous extracts of bark of *Terminalia bellirica* were subjected to α-amylase and α-glucosidase inhibitory assay. The extracts of *Terminalia bellirica* elicited a dose dependent inhibition against alpha amylase and glucosidase activity. The ethanol extract shows higher inhibitory activity than other extracts. From the present study, it is clear that the bark of *Terminalia bellirica* contain potential α-amylase and α-glucosidase inhibitors which can be used in the treatment of diabetes mellitus.

Keywords: *In vitro* antidiabetic activity, α-amylase inhibition, α-glucosidase inhibition, *Terminalia bellirica*

1. Introduction

Diabetes mellitus (DM) is the world's largest growing metabolic disorder of the endocrine system, presently affecting about 5-10 per cent people around the globe [1]. One antidiabetic therapeutic approach is to reduce gastrointestinal glucose production and absorption through the inhibition of carbohydrate digesting enzymes such as α- amylase and α-glucosidase. Inhibition of amylase and glucosidase enzymes involved in digestion of carbohydrates can significantly decrease the post prandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in management of blood glucose [2].

Management of diabetes without any side effect is still a challenge to the medical community. The use of the drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects. Thus searching for a new class of compounds is essential to overcome diabetic problems and the search for alternative drugs is continuous [3].

Herbal drugs are prescribed widely even their biological active compounds are unknown because of their effectiveness, less side effect and relatively low cost . One such plant expected to have *in vitro* antidiabetic activity is *Terminalia bellirica*, which is a well known traditional plant and it is locally known as dhandrika. It acts as laxative, regenerative, beneficial for hair, throat, eyes, skin disease, cough, and cold, asthma, to arrest the bleeding and induce deep sleep [4]. With this background of information, the present study aim to evaluate *in vitro* alpha amylase and alpha glucosidase inhibitory activities of bark of *Terminalia bellirica*".

2. Materials and Methods

2.1 Collection of plant materials

The bark of *Terminalia bellerica* was collected from Poondi area of Coimbatore, Tamil Nadu, India. The sample was identified and authenticated by Botanical Survey of India, TNAU, Coimbatore. The authentication number is BSI/SRC/5/23/2014-15/Tech 510.

2.2 Preparation of the extracts

Ten gram of the bark of *Terminalia bellerica* was dried, powered and packed in a thimble. Then it was serially extracted into solvents of increasing polarity petroleum ether, chloroform and ethanol using a Soxhlet apparatus. After extraction the solvents were evaporated to dryness in rotary evaporator and the yields of the extracts were calculated. They were stored at -20ºC until use. Apart from the solvent extracts, a fresh aqueous extract was also prepared.

2.3 Quantification assay

2.3.1 *In vitro* alpha amylase inhibitory activity

Diabetes is characterized by deranged metabolism, resulting from defect in the secretion and cellular action of insulin. *in vitro* alpha amylase inhibitory effect was estimated by the method explained by Apostolidis [5]. Twenty five µl of 20 percentage (v/v) plant sample extract and 25 µl of 20mM phosphate buffer pH 6.9, containing porcine alpha amylase at a concentration of 0.5 mg/ml were incubated at 25^0C for 10 min. After pre incubation, 25µl of 0.5% of starch solution in 20Mm phosphate buffer, pH 6.9, was added. The reaction mixture was then incubated at 25^0C for 10 min. The reaction was stopped with 50 µl of 96mM 3, 5dinitro salicylic acid (DNS) colour reagent. The microplate was then incubated in a boiling water bath for 5 mins and cooled to room temp. Absorbance (A) was measured at 540nm.

2.3.2 *In vitro* alpha glucosidase inhibitory activity

The alpha glucosidase inhibitory activity was measured by the method of Kim *et al*. [6]. Yeast alpha glucosidase was dissolved at a concentration of 0.1U/ml in 100mM phosphate buffer, pH 7.0, containing bovine serum albumin 2000 mg/l. and sodium azide 200 mg/ml which was used as enzyme source. Para nitro phenyl –alpha-D-glucopyranoside was used as substrate.

The plant extract (5%) was weighed and serial dilutions of 62.5, 31.25, 15.6, 7.8, 3.9 and 1.95 mg/ml, were made up equal volumes of dimethylsulfoxide and distilled water. Ten microliters of plant extract dilutions was incubated for 5 min with 50 microliter enzyme source. After the incubation, 50 microliter of substrate was added and further incubated for 5 min at room temperature. It was measured at the absorbance of 405 nm on a microtitre reader.

3. Results and Discussion

Diabetes is prone to many complications due to the nature of the disease. Long-standing diabetes can lead to heart, kidney and circulation problems including stroke. In traditional world, nutrition and health care have connectivity for which many plants are consumed as food in order to benefit health [7].

In the present study, the inhibitory activity of different extracts of increasing polarity (petroleum ether, chloroform and ethanol) and aqueous extract of bark of *Terminalia bellirica* was investigated and the results are given below. The *in vitro* alpha amylase inhibitory activity of different extracts of *Terminalia bellirica* exhibited good antidiabetic activity.

The per cent inhibition ranging from 20µg/ml to 100 µg/ml concentration of extracts of bark of *Terminalia bellirica* shows concentration dependent (Figure I). The results revealed that the ethanolic extract at a concentration of 100µg/ml showed a maximum per cent inhibition (67.7), followed by the aqueous extract (57.1), chloroform (65.3) and petroleum ether (32.8). The IC_{50} value of standard drug acarbose was found to be 54µg/ml (Table I).

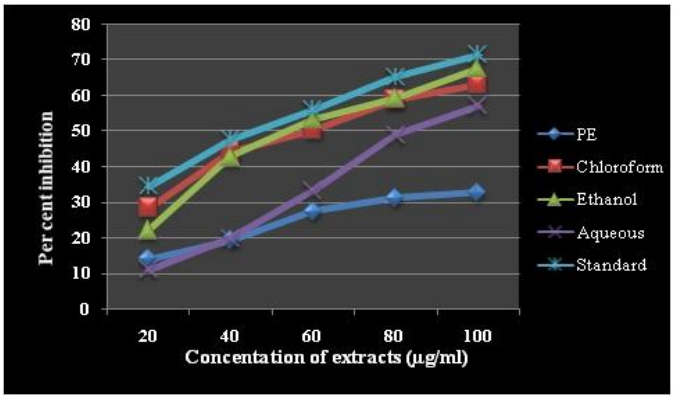

Figure 1: Alpha amylase activity of bark of *Terminalia bellirica*

Table 1: IC_{50} Value of alpha amylase activity of bark of *Terminalia bellirica*

Solvents	IC_{50} Value
Petroleum Ether	130
Chloroform	58
Ethanol	58
Aqueous	88
Standard	54

The extracts of bark of *Terminalia bellirica* elicited a dose dependent inhibition against alpha glucosidase activity (Figure II). The ethanol extract revealed a significant inhibitory activity of α-glucosidase. The per cent inhibition varied from 28.3%- 81.7% for lowest to highest concentration (20µg/ml to 100µg/ml). The IC_{50} value of standard drug acarbose was found to be 44 µg/ml (Table II).

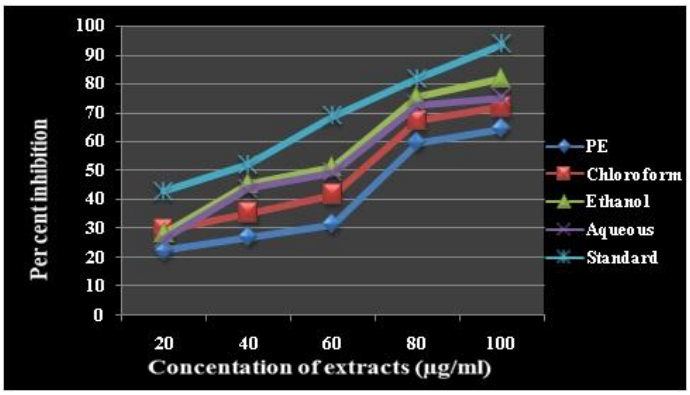

Figure 2: Alpha glucosidase activity of bark of *Terminalia bellirica*

Table 2: IC_{50} value of alpha glucosidase activity of bark of *Terminalia bellirica*

Solvents	IC_{50} Value
Petroleum Ether	72
Chloroform	74
Ethanol	46
Aqueous	62
Standard	44

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch yield glucose and maltose. Alpha amylase inhibitors bind to alpha-bond of polysaccharide and prevent breakdown of polysaccharide into mono and disaccharide [8]. The pancreatic and intestinal glucosidases are the key enzymes of dietary carbohydrate digestion and inhibitors of these enzymes may be effective in retarding glucose adsorption [9]. This is because only monosaccharides are readily taken up from the intestine and all other carbohydrates have to be broken-down enzymatically before they can be absorbed [10].

Bachhawat *et al*. [11] found that the bark of *Terminalia arjuna* was the most potential inhibitor of the enzyme α-glucosidase. The study carried out by Riris [12] revealed that the ethanol extract of bark of *Vatica pauciflora* Blume showed higher α-glucosidase inhibitory activity (91%) than the hexane (29%), ethyl acetate (60.8) and aqueous (78.3%). Gayathri and Jeyanthi [13] demonstrated that the aqueous extract of bark of *Polyalthia longifolia* exhibited very good inhibition of α-amylase and α-glucosidase with an inhibition of 94.4% and 94.2% at a volume of 200µl. Since *Terminalia bellirica* also exhibited a very strong inhibition against α-amylase and α-glucosidase, it can be concluded it has a great antidiabetic potentials.

4. Conclusion

In vitro studies observed that the ethanol extract of bark of *Terminalia bellirica* posses an appreciable α-amylase and α-glucosidase inhibitory activity which may be due to the presence of secondary metabolites. Hence it could be used to treat the metabolic disorders like diabetes mellitus. However, these results should be confirmed by *in vivo* models and clinical trials for their effective utilization as therapeutic agents.

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