

## *in vitro* Antidiabetic Properties of the Fruits and Leaves of *Terminalia bellirica*

Manila, T.N.<sup>1</sup> Mary Shoba Das, C.<sup>1</sup> and Gayathri Devi, S.<sup>1\*</sup>

Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043, Tamil Nadu, India.

\*Email of the Corresponding author: [gayathridevi.adu@gmail.com](mailto:gayathridevi.adu@gmail.com)

### Abstract

*Terminalia bellirica* is a traditionally used medicinal plant claimed to possess antioxidant, antidiabetic and anti-inflammatory activity. The present study was conducted to evaluate the *in vitro* antidiabetic activity of the fruits and leaves of *Terminalia bellirica*. The *in vitro* alpha amylase and alpha glucosidase activity of different extracts of *Terminalia bellirica* was also determined. From the results, it is clear that the acetone extract of both the fruits and leaves of *Terminalia bellirica* shows strong inhibitory activity against alpha amylase and the aqueous extract of fruits and leaves of *Terminalia bellirica* were found to exhibit highest alpha glucosidase activity. The results obtained in the present study indicated that the *Terminalia bellirica* could be used for treating diabetes mellitus.

**Key words:** *Terminalia bellirica*, antidiabetic, alpha amylase, alpha glucosidase.

### 1. Introduction

Diabetes is a debilitating disease affecting millions of people worldwide. Since the disease has no known modern allopathic cure, it requires lifelong health. In fact modern medicine merely attempts to control the symptoms of diabetes like increased blood sugar level and tries to mitigate the various other complicated problems that can arise out of diabetes like increased cardiovascular risks, diabetic retinopathy, diabetic neuropathy and kidney failure leading to more diabetes related complications and an untimely death ( Biswas *et al.* 2011).

One antidiabetic therapeutic approach is to reduce gastrointestinal glucose production and absorption through the inhibition of carbohydrate digesting enzymes such as  $\alpha$ - amylase and  $\alpha$ -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 (Ahamad *et al.* 2011).

The treatment for type 2 diabetes mellitus is presently achieved through conventional drug which acts mainly stimulating insulin absorption and its release from pancreas or by the inhibition of carbohydrate degrading enzyme such as alpha glucosidase. It belongs to alpha -1, 4 glucan, glucanohydrolases, one of the important target enzyme for the conventional treatment of diabetes. It catalyses the initial step in hydrolyses of starch to maltose and maltotriose which then acted upon by alpha glucosidase, broken down into glucose that get absorbed by brush border of epithelium of the intestine and then enter into the blood stream. The condition arises due to excessive breakdown of starch by this enzyme is called as post prandial hypoglycemia (Jyothi *et al.* 2011).

Pancreatic alpha amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of oligoglucans. These are then acted on by alpha glucosidase and further degraded to glucose which on absorption enters in to blood stream (Sabu and Kuttan 2009).

Treatment enhancing beta cell function and reducing insulin resistance are key to improve metabolic control and retarding the development of diabetic complications. Based on WHO

recommendation, antidiabetic agents of plant origin are important for use in traditional medicinal (Palanuvej *et al.* 2009).

The practices of traditional medicine are based on hundred years of belief and observations, which predate the development and spread of modern medicine. In developing countries, there is an increasing attempt to incorporate traditional medicines, especially herbal preparations in the local health care systems and a modernized people are increasingly turning to herbal medicine (Jeyaprakash *et al.* 2011).

Folk medicinal practitioners have been treating diabetes for centuries and claim to have effective treatments for the disease as well as treatment for mitigating other symptoms arising from diabetes. They use both simple and complex formulations of medicinal plants for treatment of the disease (Rani *et al.* 2011). Some of the medicinal plants used for antidiabetic treatment are *Amaranthus cruentus* and *Moringa oleifera* exhibited significantly higher phenolic content. The methanolic extract of the investigated samples showed promising levels of  $\alpha$ -amylase (10–45 per cent) and  $\alpha$ -glucosidase (13–80 per cent) inhibition activities (Catherine *et al.* 2010).

*Terminalia bellirica* is a native plant of India belonging *Combretaceae* family. It is a large deciduous buttressed tree with thick brownish grey bark having shallow longitudinal fissures. The bark is useful in the treatment of anemia and leucoderma. The fruits are thermogenic, anti-inflammatory, styptic, narcotic, digestive, expectorant, ophthalmic, antipyretic, antiemetic and rejuvenating, cough, bronchitis, strangury, skin diseases, leprosy, fever, ulcers, antidiabetic and general debility. The mature and dry fruits are useful in diarrhoea and dysentery. The oil obtained from the seed is trichogenous and is useful in dyspepsia (Warrier 1996).

With this background of information, the present study “*in vitro* antidiabetic of properties of the fruits and leaves of *Terminalia bellirica*” was undertaken with the objective to determine *in vitro* antidiabetic activity of *Terminalia bellirica*

## 2. Materials and methods

### 2.1 Collection of the plant samples

The plant *Terminalia bellirica* was freshly collected from in and around Kannur district, Kerala, India. The plant was duly authenticated by Botanical Survey of India, Tamil Nadu Agricultural University Coimbatore. A voucher specimen was deposited in the Department of Biochemistry, Avinashilingam University for Women, Coimbatore, Tamil Nadu, India (Plate 1).

### 2.2 Preparation of the extracts

The collected fruits and leaves of the plant were then washed and air dried in the shade at room temperature for complete drying. The dried sample was powdered. The powder obtained was extracted with suitable solvents using soxhlet apparatus until complete elution. The solvents used in sequential order were acetone, chloroform, ethanol and aqueous. The extract obtained was concentrated under vacuum on a rotary evaporator at 40°C and all of the dried extracts were stored at –20°C prior to assays of *in vitro* antidiabetic activity.

### 2.3 Quantification Assays

#### 2.3.1 *in vitro* alpha amylase inhibitory activity (Apostolidis *et al.* 2008)

Twenty five  $\mu$ l of 20 per cent (v/v) plant sample extract and 25  $\mu$ l of 20Mm phosphate buffer pH 6.9, containing porcine alpha amylase at a concentration of 0.5 mg/ml were incubated at 25°C for 10 min. After pre incubation, 25 $\mu$ l of 0.5% of starch solution in 20Mm phosphate buffer, pH 6.9, was added. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 50  $\mu$ l of 96mM 3, 5 dinitro salicylic acid (DNS) colour reagent. The

microplate was then incubated in a boiling water bath for 5 minutes and cooled to room temperature. Absorbance (A) was measured at 540nm.

Per cent inhibition was calculated as follows

$$\% \text{ of inhibition} = \frac{A_{540 \text{ Control}} - A_{540 \text{ Exp}}}{A_{540 \text{ Control}}} \times 100$$

### 2.3.2 *in vitro* alpha glucosidase inhibition assay (Kim *et al.* 2000)

Yeast alpha glucosidase was dissolved at a concentration of 0.1 U/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 upto equal volumes of dimethylsulfoxide and distilled water. Ten microliters of plant extract dilutions was incubated for 5 min with 50µl enzyme source. After the incubation, 50µl 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.

$$\text{Per cent alpha glucosidase inhibition} = 1 - B/A \times 100$$

A-The absorbance of control

B-The absorbance of sample containing extracts.

Acarbose used as positive control.

## 2.4 Statistical analysis

Statistical analysis was performed according to student t-test. The values ( $p < 0.05$ ) were considered to be significant.

## 3. Results

### 3.1 Alpha amylase activity of *Terminalia bellirica*

Figure 1 shows the alpha amylase inhibitory activity against different solvents of *Terminalia bellirica*. From this study, it is clear that the acetone extract of both the fruits (68.9 per cent) and leaves (89.6 per cent) of *Terminalia bellirica* shows strong inhibitory activity against alpha amylase.

The order of inhibitory activity against  $\alpha$ - amylase of fruits and leaves of *Terminalia bellirica* were acetone > ethanol > chloroform > aqueous. Both the aqueous extracts of fruits (6.89 per cent) and leaves (7.5 per cent) of *Terminalia bellirica* shows moderate inhibitory activity compared to other extracts. Acarbose is a known drug for alpha amylase inhibitor which shows 100 per cent alpha amylase inhibitory activity.

### 3.2 Alpha glucosidase inhibitory activity in fruits and leaves of *Terminalia bellirica*

The Figure 2 and 3 shows the inhibitory activity in fruits and leaves of *Terminalia bellirica*. From the figures, it is clear that the aqueous extract of fruits and leaves of *Terminalia bellirica* were found to exhibit highest alpha glucosidase activity. The other extracts such as acetone, chloroform and ethanol extracts of both the fruits and leaves also found to show maximum inhibitory activity.

## 4. Discussion

Medicinal plants play a key role in the human health care. The traditional medicine refers to a broad range of ancient natural health care practices including folk/tribal practices as well as Ayurveda, Siddha, Amchi and Unani. These medical practices originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences without significant references to modern scientific principles. These practices incorporated ancient beliefs (Kumar *et al.* 2011).

Diabetes and its different types is an age old disease for clinicians since centuries. Many aspects of diabetes needs to be explored with respect to physiological actions of insulin and the various clinical features of this disease such as tissue complication, hence proper treatment in relation to diet and antidiabetic agents is emphasized. Plants and plant extracts were used to combat the disease as early as 1550 B.C., with as many as 400 "prescribed" effective medications to control diabetes (Patel *et al.* 2010).

*Terminila bellirica* also referred to as, Beleric Myrobalan in English, Bibhitaki in Sanskrit, Locally known as Bahera in India, has been used for centuries in the Ayurveda, a holistic system of medicine originating from India. The dried fruit used for medicinal purposes. It is a large deciduous tree with a buttressed trunk, a thick brownish gray bark with shallow longitudinal fissures, attaining a height of between 20 and 30 meters. The leaves are crowded around the ends of the branches, alternately arranged, margins entire, elliptic to elliptic-ovate, rounded tip or sub-acute, midrib prominent, pubescent when young and becoming glabrous with maturity. The flowers are pale greenish yellow with an offensive odor, borne in axillary spikes longer than the petioles but shorter than leaves. The fruits are ovoid grey drupes, obscurely 5- angled, narrowed into a very short stalk (Amrithpal 2011).

Pancreatic alpha amylase is a key enzyme in the digestive system and catalyses the initial step in the hydrolysis of starch to a mixture of smaller oligosaccharides consisting of maltose, maltotriose and number of  $\alpha$  (1-6) and  $\alpha$  (1-4) oligoglucans. These are then acted on by  $\alpha$  -glucosidases and further degraded in to glucose which on absorption enters the blood stream. Degradation of this dietary starch proceeds rapidly and leads to elevated post prandial hyperglycemia. Hence starch digestion retardation by inhibition of alpha amylase plays a key role in the control of diabetes. Inhibitor of pancreatic alpha amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the post prandial serum glucose levels (Tarling *et al.* 2008).

Our results are corroborative with that of Dinesh kumar *et al.* 2011 who have reported that the ethanol extracts of *Mangifera indica*, *Azadirachta indica* and also petroleum ether extract of *Murraya koenjii* (at a concentrations 10-100 $\mu$ g/ml) showed maximum alpha amylase inhibitory activity from 35.79 $\pm$  0.33 to 62.49 $\pm$ 0.34%, 16.50 $\pm$ 1.23 to 66.66  $\pm$  0.93 per cent and 21.57  $\pm$  1.46 to 60.78  $\pm$  0.55% with an IC<sub>50</sub> value of 37.86  $\pm$  0.32  $\mu$ g/ml, 62.99  $\pm$  1.20  $\mu$ g/ml and 59.0  $\pm$  0.51  $\mu$ g/ml respectively.

Chloroform extract of *Cocculus hirsutus* at 60  $\mu$ g/ml was found to show an inhibition of 83.33 per cent (IC<sub>50</sub> value 70.48 $\pm$ 18.39) and the benzene extract of *Basella rubra* exhibited 82.83 per cent inhibition at 100  $\mu$ g/ml (IC<sub>50</sub> value 80.97 $\pm$ 8.12) against  $\alpha$ -amylase. The acetone extract of *Cocculus* at 100  $\mu$ g/ml showed an inhibition of 79.10 per cent and the methanol extract of *Cocculus* showed an inhibition of 77.2 per cent at a concentration of 100  $\mu$ g/ml respectively (Jyothi *et al.* 2011).

The treatment for type2 diabetes mellitus is presently achieved through conventional drug which acts mainly stimulating insulin absorption and its release from pancreas or by the inhibition of carbohydrate degrading enzyme such as alpha glucosidase. It belongs to alpha -1, 4 glucan, glucanohydrolases, one of the important target enzyme for the conventional treatment of diabetes. It catalyses the initial step in hydrolyses of starch to maltose and maltotriose which then acted upon by alpha glucosidase, broken down into glucose that get absorbed by brush border of epithelium of the intestine and then enter into the blood stream. The condition arises due to excessive

breakdown of starch by this enzyme is called as post prandial hypoglycemic (Jyothi *et al.* 2011).

The hypoglycemic potential of *Carpesium abrotanoides* was evaluated by the glucosidase inhibition assay. The optimal concentration of *Carpesium abrotanoides* required for the 50 per cent inhibition (IC<sub>50</sub>) against alpha-glucosidase was 44.22 µg/ml. Acarbose was used as positive control with IC<sub>50</sub> value of 2.5 µg/ml (Mayur *et al.* 2010).

*Scaphium scaphigerum* G. Don, *Litsea glutinosa* L, *Hibiscus esculentus* L, *Ocimum canum* Sims, *Trigonella foenum-graecum* L., *Plantago ovata* seeds and *Basella alba* Linn showed the inhibitory percentage of 82.6, 41.0, 37.6, 32.8, 30.6, 27.0, 25.0 and 19.7 per cent respectively whereas, *S.scaphigerum* was further investigated and found that the concentration for 50 per cent inhibition of α-glucosidase activity (IC<sub>50</sub>) was 0.17 per cent (Palanuvej *et al.* 2009).

Both the fruits and leaves were found to show the highest inhibitory activity against alpha amylase. The aqueous extract of leaves and fruits of *Terminalia bellirica* were also found to show the highest inhibitory activity against alpha glucosidase and thereby proves the antidiabetic activity of the plant.

In conclusion, there is an evidence to support both the fruits and leaves of *Terminalia bellirica* have an antidiabetic activity. Hence, they may be useful as therapeutic agents for radical related pathological damage.

## 5. Findings

This research received no specific grant from any funding agency in the public, commercial or not – for – profit sectors.

## References

- Ahamad, J., Naquvi, J.K., Showkat, R., Ali, M.M. and Shuaib, M. (2011), “Review on role of natural alpha-glucosidase inhibitors for management of diabetes mellitus”, *IJBR.*, 2(6):374-380.
- Amrithpal, S.S. (2011), “Phytochemistry and Ethnopharmacology”, *Science Publishers*, 357-361.
- Apostolidis, E., Jayaprakasha, G.K. and Girenavar, B.S. (2008), “Antidiabetic activities of rio red grape fruits and sour orange fruit extracts in different *in vitro* model systems”, *Bioresour. Technol.*, 99(10).
- Biswas, R.K., Ishika, T., Rahman, M., Swarna, A., Khan, T., Monalisa, N.M. and Rahmatullah, M. (2011), “Antidiabetic plants and formulations used by folk medicinal practitioners of two villages in Narail and Chuadanga Districts, Bangladesh”, *American-Eurasian Journal of Sustainable Agriculture*, 5(2): 158-167.
- Catherine, K.H., Pandey, P., Arya, C.M. and Ahmed, Z. (2010), “Evaluation of antidiabetic activity of two important memory enhancing medicinal plants *Baccopa monnieri* and *Centella asiatica*”, *Indian. J. Pharmacol.*, 44(1): 114–117.
- Jeyaprakash, K., Ayyanar, M., Geetha, K.N. and Sekar, T. (2011), “Traditional uses of medicinal plants among the tribal people in Theni District (Western Ghats), Southern India”, *Asian Pacific Journal of Tropical Biomedicine*, S20-S25.
- Jyothi, K.S.N., Hemalatha, P. and Challa, S. (2011), “Evaluation of α-amylase inhibitory potential of three medicinally important traditional wild food plants of India”, 5(2): 95-99.
- Kim, J.S., Kwon, C.S. and Son, K.H. (2000), “Inhibition of α-glucosidase and amylase by Luteolin, a flavonoid”, *Biosci Biotech Biochem.* 64:2458-2461.

Kumar, H.C., Ramesh, A., Kumar, S.N.J. and Ishaq, M.B. (2011), "A review on hepatoprotective activity of medicinal plants", *IJPSR.*, 2(3): 501-51.

Mayur, B., Sandesh, S., Shruti, S. and Yum, S.S. (2010), "Antioxidant and  $\alpha$ -glucosidase inhibitory properties of *Carpesium abrotanoides* L", *Journal of Medicinal Plants Research*, 4(15): 1547-1553.

Palanuvej, C., Hokputsa, S., Tunsaringkarn, T. and Ruangrunsi, N. (2009), "*in vitro* glucose entrapment and alpha- glucosidase inhibition of mucilaginous substances from selected Thai medicinal plants", *Scientia Pharmaceutica*, 77: 837-849.

Patel, V.R., Patel, P.R. and Kajal, S.S. (2010), "Antioxidant activity of some selected medicinal plants in Western region of India", *Advances in Bio. Res.*, 4(1): 23-26.

Rani, B.A., Pugin, A. and Wendehenne, D. (2011), "New insights into nitric oxide signaling in plants", *Annual Review of Plant Biology*, 59: 21-39.

Sabu, M.C. and Kuttan, R. (2009), "Antidiabetic and antioxidant activity of *Terminalia bellerica*. Roxb", *Indian Journal of Experimental Biology*, 47: 270-275.

Tarling, S., Prasad, S.K., Kumar, R. and Hemalatha, S. (2008), "An overview on antidiabetic medicinal plants having insulin mimetic property", *Asian Pacific Journal of Tropical Biomedicine*, 320-330.

Warrier, S. (1996), "Indian medicinal plants", *Shanda Publication, Kerala*, 327-330.

## Biography

Manila, T.N. is a M.Sc. student of Department of Biochemistry, Avinashilingam University, Coimbatore. She is currently doing a research in the antioxidant and antidiabetic properties of *Terminalia bellirica*.

Mary Shoba Das, C. is currently pursuing her Ph.D in the Department of Biochemistry, Avinashilingam University, Coimbatore and working in the same plant.

Dr. S. Gayathri Devi is working as Assistant Professor (SS) in the Department of Biochemistry, Avinashilingam University, Coimbatore. Her field of interest in research is on the medicinal plants with antioxidant and antidiabetic property.

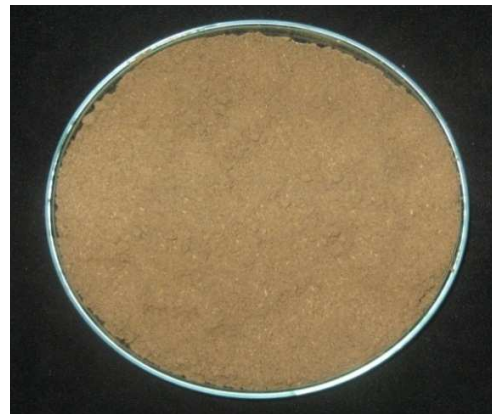
**PLATE I**



*Terminalia bellirica*

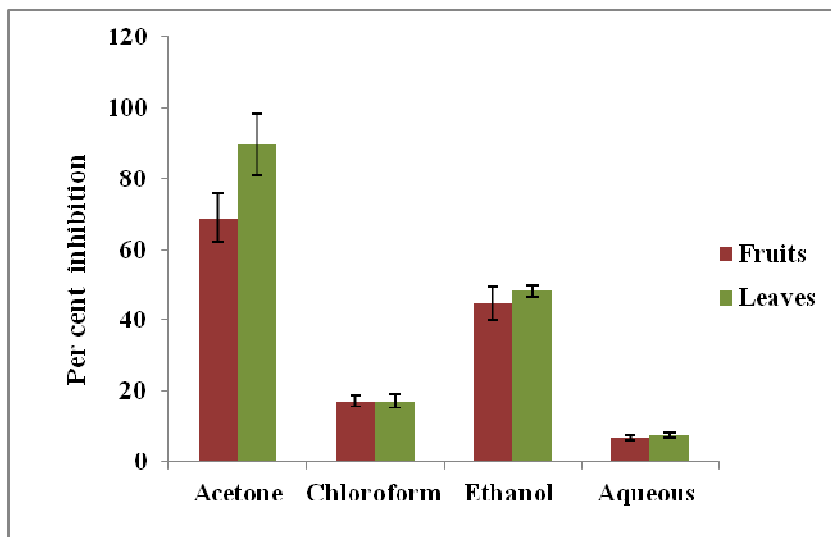


**FRUIT POWDER**

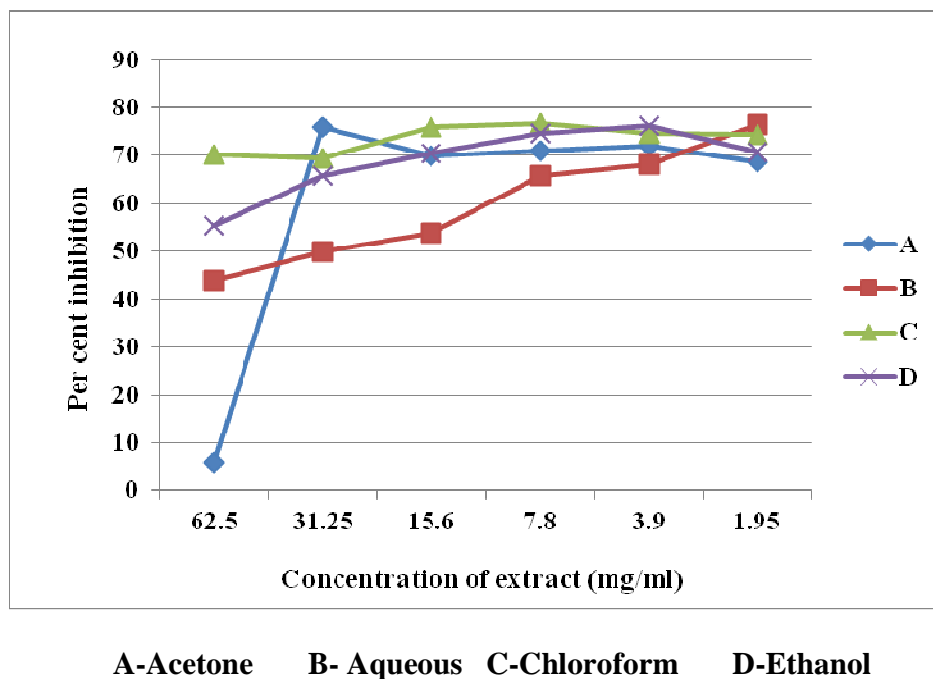


**LEAF POWDER**

**Figure 1 Alpha amylase inhibitory activity in fruits and leaves of *Terminalia bellirica***

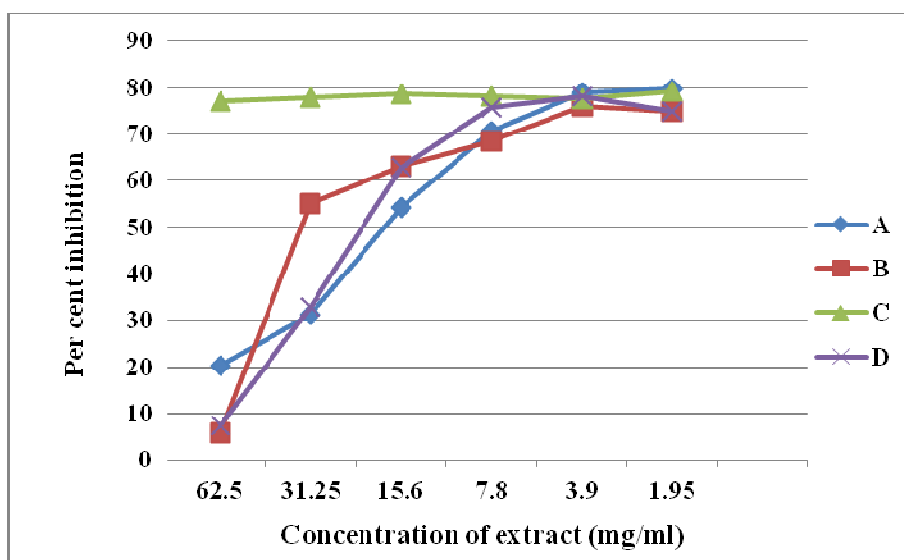


**Figure 2 Alpha glucosidase inhibitory activity of fruits of *Terminalia bellirica***





**Figure 3 Alpha glucosidase inhibitory activity of the leaves of *Terminalia bellirica***



**A-Acetone    B- Aqueous    C-Chloroform    D-Ethanol**