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Research Article

IN VITRO GLUCOSE BINDING ACTIVITY OF *TERMINALIA BELLIRICA*

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ABSTRACT

Herbal medicine has become an integral part of standard healthcares based on a combination of time honored traditional usage and ongoing scientific scrutiny of their therapeutic potential and safety. The petroleum ether, chloroform, ethanol, and aqueous extracts of leaves, fruits, and bark of *Terminalia bellirica* were evaluated for their effects on glucose adsorption, diffusion, and glucose transport across yeast cell. All the extracts could bind the glucose effectively, and the glucose binding capacity was directly proportional to the molar concentration of glucose. The rate of glucose diffusion across the membrane was found to increase from 30 to 180 minutes. The rate of uptake of glucose into the yeast cells was linear in all the five glucose concentration used in the study. Among the four extracts, ethanolic extract of leaves, fruits, and bark of *T. bellirica* show higher hypoglycemic activity while compared to other extracts. The hypoglycemic effect exhibited by the ethanol extracts of leaves, fruits, and bark is mediated by increasing glucose adsorption, glucose diffusion and glucose transport across the cell membrane as revealed by simple *in vitro* model of yeast cells.

Keywords: Glucose adsorption, Glucose diffusion, *Terminalia bellirica,* Yeast cells.

INTRODUCTION

Diabetes mellitus is the name given to a group of disorders characterized by absent or deficient insulin secretion or peripheral insulin resistance resulting in hyperglycemia. Currently, the presence of abnormally high glucose levels in the blood is the only criterion on which diagnosis of diabetes mellitus is based. There is increasing demand to use the natural products with antihypoglycemic activity [1].

Controlled hyperglycemia will decrease the risk of developing microvascular and macrovascular complications and provide better diabetes management hence preventing morbidity and mortality. The side effects associated with the prolonged use of insulin and other hypoglycemic drugs have necessitated the demand for safe and effective drugs especially of herbal origin. Different traditional medical systems are using the crude plant extracts or their active constituents for management of diabetes. Herbal drugs are considered free from side effects than the synthetic one. They are less toxic, relatively cheap, and popular [2].

A limited number of medicinal plant species have been studied and validated for their hypoglycemic properties using laboratory diabetic animal models and in clinical studies using human subjects. Several medicinal plants and their products (active, natural principles, and crude extracts) have been reported in the literature as having been used to control diabetes in the African traditional system of medicine [3].

Terminalia bellirica is a well-known traditional plant belongs to the family combretaceae, and it is locally known as dhandrika. It acts as laxative, regenerative, beneficial for hair, throat, eyes, skin disease, cough, cold, asthma, to arrest the bleeding, and induce deep sleep [4]. The present study deals with the *in vitro* hypoglycemic activity of leaves, fruits, and bark of *T. bellirica*.

METHODS

Collection of plant materials

The bark of *T. bellirica* 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.

Preparation of the extracts

The collected leaves, fruits, and bark of *T. bellirica* were washed and air-dried in the shade at room temperature for complete drying. The dried sample was powered. 10 g of powder was packed in a thimble, and 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 and the yields of the extracts were calculated. They were stored at −20°C until use. Apart from the solvent extracts, a fresh aqueous extract was also prepared.

Determination of glucose adsorption capacity

Glucose adsorption capacity of the samples was determined by the method of Ou *et al*. (2001) [5]. Briefly, the samples of plant extracts (1%) were added to 25 ml of glucose solution of increasing concentration (5, 10, 20, 50, and 100 mmol/L). The mixture was stirred well, incubated in a shaker water bath at 37°C for 6 hrs, centrifuged at 4800 r/minutes for 20 minutes and the glucose content in the supernatant was determined. Metronidazole was used as control. The concentration of bound glucose was calculated using the following formula:

$$\text{Glucose bound} = \frac{G1 - G6}{\text{Weight of the sample}} \times \text{Volume of solution}$$

G1 is the glucose concentration of the original solution.
G6 is the glucose concentration after 6 h.

Effect of plant extracts on *in vitro* glucose diffusion

It was performed according to the method stated by Ahmed *et al*. (2011) [6]. A total of 25 ml of glucose solution (20 mmol/L) and the samples of plant extracts (1%) were dialyzed in dialysis bags against 200 mL of distilled water at 37°C in a shaker water bath. The glucose content in the dialysate was determined at 30, 60, 120, and 180 minutes using glucose oxidase peroxidase diagnostic kit. A control (metronidazole) test was carried out without sample. Glucose dialysis retardation index (GDRI) was calculated using the following formula:

$$\text{GDRI\%} = \frac{\text{Glucose content with addition of sample } (\text{mg / dL})}{\text{Glucose content of the control } (\text{mg / dL})} \times 100$$

Glucose uptake by yeast cells

Yeast cells were prepared according to the method of Cirillo [7]. Commercial baker's yeast was washed by repeated centrifugation (4200 r/minutes, 5 minutes) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1-5 mg) were added to 1 mL of glucose solution (5-25 mmol/L) and incubated together for 10 minutes at 37°C. The reaction was started by adding 100 µL of yeast suspension, vortexed, and further incubated at 37°C for 60 minutes. After 60 minutes, the tubes were centrifuged (3800 r/minutes, 5 minutes) and glucose was estimated in the supernatant. Metronidazole was used as control. The percent increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{Increase in glucose uptake } (\%) = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample.

RESULTS AND DISCUSSION

In vitro* glucose adsorption capacity of leaves, fruits, and bark of *T. bellirica

The glucose adsorption capacity of different extracts of leaves, fruits and bark of *T. bellirica* at different glucose concentration was investigated in this study, and the results were presented in Figs. 1-3.

All the extracts could bind glucose effectively, and the glucose binding capacity was directly proportional to the molar concentration of glucose. The samples were effective in adsorbing glucose at both lower and higher concentrations (5 and 100 mmol/L). Among all the extracts assessed, ethanolic extracts of fruits, leaves, and bark exhibited higher activity, this may be due to their both insoluble and soluble constituents and fibers from different sources are reported to adsorb glucose. The higher amounts of glucose were bound with increased glucose concentration. The adsorption of glucose also occurs even at lower concentration, it will reduce the amount of glucose available for transport across the intestinal lumen, consequently blunting the postprandial hyperglycemia.

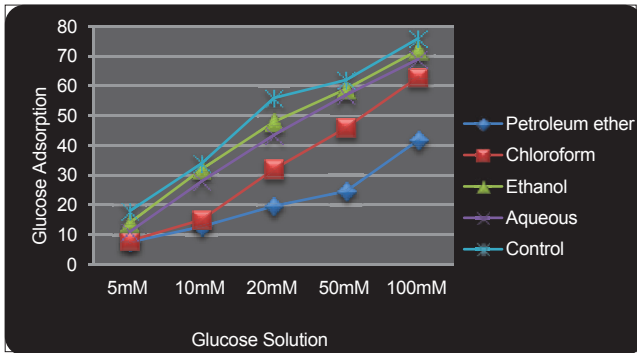

Fig. 1: Glucose binding capacity of leaves of *T. bellirica*

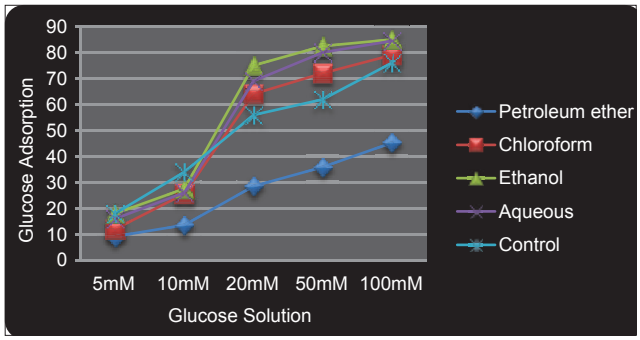

Fig. 2: Glucose binding capacity of fruits of *Terminalia bellirica*

Similar observation was reported by Bhutkar and Bhise [8] that the adsorption capacity of the extracts of *Albizzia lebbeck* and *Mucuna pruriens* was found to be directly proportional to the molar concentration of glucose.

***In vitro* glucose diffusion inhibitory assay**

The leaves, fruits, and bark extracts of different solvents (petroleum ether, chloroform, ethanol, and aqueous) were subjected to find out their glucose diffusion and GDRI across the dialysis membrane and the results obtained are shown in Tables 1-3.

The movement of glucose was monitored once in 30 minutes till 180 minutes. The rate of glucose diffusion across the membrane was found to increase from 30 to 180 minutes. Among the four extracts the ethanolic extract of fruits, leaves, and bark of *T. bellirica* exhibit higher inhibitory effects on movement of glucose into external solution across the dialysis membrane compared to other extracts and control.

GDRI is a useful *in vitro* index to predict the effect of a fiber on the delay in glucose absorption in the gastrointestinal tract [9]. The GDRI was found to be a decrease in an increase with time. The higher GDRI value indicates higher retardation index of glucose by the sample. The higher GDRI is found in the ethanolic extracts of fruits (72.9%), leaves (72.4%), and bark (42.9%) respectively at 30 minutes. The decrease in glucose diffusion could be attributed to the inhibition of the enzyme α-amylase, which delays/prolongs the glucose release from starch. This inhibition could be possibly due to several factors centering fiber in terms of concentration, encapsulation of enzyme and starch to reduce the accessibility. In general, carbohydrate digestion is delayed in the presence of inhibitors of carbohydrate hydrolyzing enzymes. Thus, it could be suggested that the medicinal plants inhibit α-amylase activity thereby exerting a hypoglycemic effect [6].

Glucose uptake by yeast cells

The rate of glucose transport across the cell membrane was studied in an *in vitro* system comprising of yeast cells suspended in different glucose concentration (5-25 mmol/L). The results were depicted in Figs. 4-6. The amount of glucose remaining in the medium after a specific time interval serves as an indicator of glucose uptake by the yeast cells. The rate of uptake of glucose into the yeast cells was linear in all the 5 glucose concentration. The ethanolic extracts of leaves, fruits, and bark of *T. bellirica* exhibited significantly higher activity than the other extracts such as petroleum ether, chloroform, and aqueous. However, the percent increase in the glucose uptake by the yeast cells was observed to be inversely proportional to the glucose concentration and was found to be decreased with increase in molar concentration of glucose.

The mechanism of glucose transport across the yeast cell membrane has been receiving attention as *in vitro* screening method for hypoglycemic

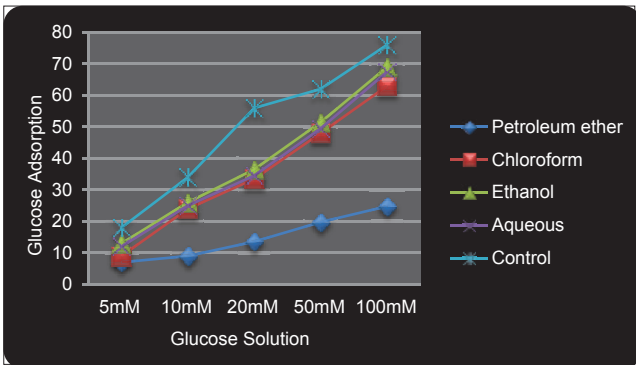

Fig. 3: Glucose binding capacity of bark of *Terminalia bellirica*

Table 1: Effect of leaf extracts of *T. bellirica* on glucose diffusion and GDRI

Extracts	Glucose content in dialysate (mMol/L)			
	30 minutes	60 minutes	120 minutes	180 minutes
Petroleum ether	2.34±0.29 (57.9)	2.63±0.20 (52.6)	3.75±0.35 (32.5)	4.20±0.34 (24.3)
Chloroform	2.01±0.46 (63.9)	2.86±0.22 (48.4)	4.26±0.28 (23.3)	4.73±0.25 (14.7)
Ethanol	2.67±0.26 (72.4)	2.41±0.23 (56.5)	3.23±0.30 (41.7)	3.76±0.27 (32.3)
Aqueous	4.01±0.25 (25.8)	4.49±0.40 (23.4)	4.61±0.23 (21.6)	4.72±0.24 (14.7)
Control	2.50±0.36 (54.9)	2.84±0.32 (48.8)	4.07±0.38 (26.4)	4.41±0.22 (20.3)

Values in parenthesis indicate GDRI. Values are expressed by mean±SD of six samples in each group. SD: Standard deviation, GDRI: Glucose dialysis retardation index, *T. bellirica: Terminalia bellirica*

Table 2: Effect of fruits extracts of *T. bellirica* on glucose diffusion and GDRI

Extracts	Glucose content in dialysate (mMol/L)			
	30 minutes	60 minutes	120 minutes	180 minutes
Petroleum ether	1.67±0.21 (69.9)	2.30±0.28 (58.5)	3.23±0.15 (41.7)	3.76±0.16 (32.3)
Chloroform	1.88±0.27 (66.2)	2.67±0.19 (52.0)	3.64±0.11 (34.4)	4.99±0.26 (26.2)
Ethanol	1.50±0.14 (72.9)	2.19±0.32 (60.5)	2.89±0.27 (47.9)	3.32±0.29 (40.2)
Aqueous	2.71±0.11 (51.2)	2.94±0.16 (47.1)	3.82±0.21 (31.2)	4.42±0.27 (24.5)
Control	2.50±0.36 (54.9)	2.84±0.32 (48.8)	4.07±0.38 (26.4)	4.41±0.22 (20.3)

Values in parenthesis indicate GDRI. Values are expressed by mean±SD of six samples in each group. SD: Standard deviation, GDRI: Glucose dialysis retardation index, *T. bellirica: Terminalia bellirica*

Table 3: Effect of bark extracts of *T. bellirica* on glucose diffusion and GDRI

Extracts	Glucose content in dialysate (mMol/L)			
	30 minutes	60 minutes	120 minutes	180 minutes
Petroleum ether	3.34±0.17 (39.9)	3.73±0.37 (32.8)	3.40±0.26 (38.7)	4.20±0.31 (24.3)
Chloroform	3.17±0.60 (42.9)	3.51±0.23 (36.8)	3.58±0.05 (35.6)	3.76±0.26 (32.3)
Ethanol	3.75±0.14 (32.5)	3.04±0.36 (45.3)	4.26±0.32 (23.3)	3.67±0.17 (34.0)
Aqueous	4.51±0.31 (18.7)	4.17±0.35 (24.9)	3.58±0.34 (35.5)	4.73±0.19 (14.7)
Control	2.50±0.36 (54.9)	2.84±0.32 (48.8)	4.07±0.38 (26.4)	4.41±0.22 (20.3)

Values in parenthesis indicate GDRI. Values are expressed by mean±SD of six samples in each group. SD: Standard deviation, GDRI: Glucose dialysis retardation index, *T. bellirica: Terminalia bellirica*

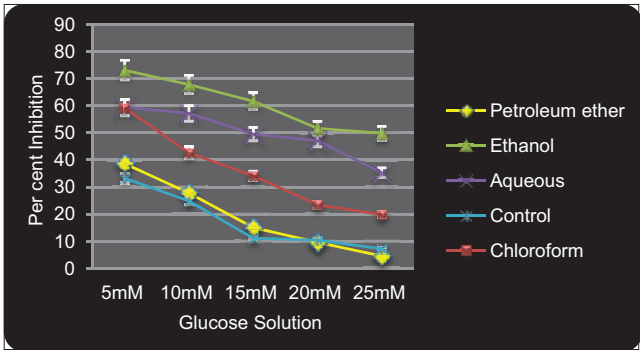

Fig. 4: Effect of leaf extracts on the uptake of glucose by yeast cells

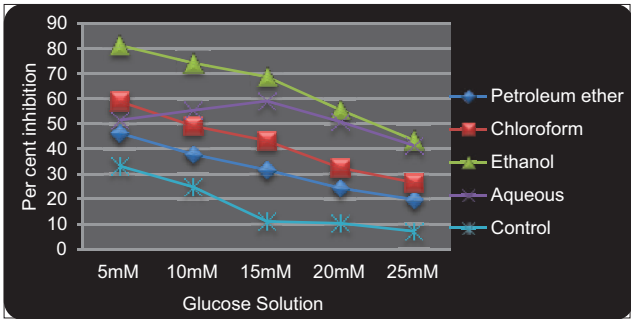

Fig. 6: Effect of bark extracts on the uptake of glucose by yeast cells

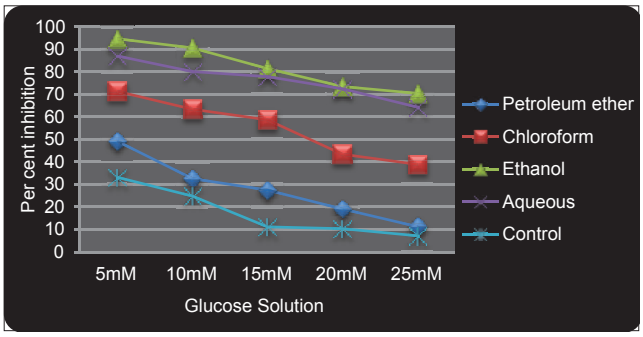

Fig. 5: Effect of fruit extracts on the uptake of glucose by yeast cells

effect of various compounds/medicinal plants. Recent studies on the transport of non metabolizable sugars and certain metabolizable glycosides suggest that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers. It is reported that in yeast cells (*Saccharomyces cerevisiae*) glucose transport is extremely complex, and it is generally agreed that glucose is transported in yeast is by a facilitated diffusion process. Facilitated carriers are specific carriers that transport solutes down the concentration gradient. This means that effective transport is only attained if there is the removal of intracellular glucose [10].

CONCLUSION

The results of the present study revealed that the ethanolic extracts of leaves, fruits, and bark of *T. bellirica* showed the maximum *in vitro*

hypoglycemic activity mediated by increasing glucose adsorption, decreasing glucose diffusion rate and by glucose transport across the cell membrane. However, these results should be confirmed by *in vivo* models and clinical trials for their effective utilization as therapeutic agents.

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