

## EVALUATION OF *IN VITRO* ANTIDIABETIC ACTIVITY OF METHANOLIC EXTRACT OF SEAGRASS *HALOPHILA BECCARII*

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Received: 09 March 2018, Revised and Accepted: 23 April 2018

### ABSTRACT

**Objectives:** The objectives of this study were to analyze the *in vitro* antidiabetic activity of methanolic extract of seagrass.

**Methods:** The methanolic extract of *Halophila beccarii* was tested for its *in vitro* antidiabetic potential through inhibition of carbohydrate digestive enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase, glucose diffusion assay, glucose adsorption capacity, and enhancement of glucose uptake activity in yeast cells.

**Results:** The crude extract of *H. beccarii* showed strong inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase as on comparison with the standard enzyme inhibitor acarbose. The antidiabetic activity was found to be concentration dependent, and the antihyperglycemic activity was demonstrated through the inhibition of the glucose movement and facilitated diffusion of glucose. The rate of glucose diffusion across the membrane was found to be increased from 30 to 180 min.

**Conclusion:** The seagrass collected from Pulicat Lake was identified as *H. beccarii* based on the morphology of the plant, shape of the leaves, and brown pigmentation on the leaves. According to the results, *H. beccarii* showed antidiabetic activity by the inhibition of carbohydrate digestive enzymes and glucose movement; hence, it is significantly reduced the post-postprandial hyperglycemia.

**Keywords:** Seagrass, *Halophila beccarii*, Facilitated diffusion,  $\alpha$ -Amylase,  $\alpha$ -Glucosidase, Antidiabetic, Carbohydrates.

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### INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder due to the deficiency of insulin or resistance to the insulin, and further, it leads to macrovascular and microvascular diseases [1]. The strategic treatment for diabetes mainly focuses on stimulation of endogenous insulin secretion and enhancement of insulin action on target tissue and also to inhibit the degradation of oligo- and disaccharides [2,3]. The majority of the synthetic drugs are targeted toward the inhibition of carbohydrate digestive enzymes such as  $\alpha$ -glucosidase and  $\alpha$ -amylase. The  $\alpha$ -glucosidase catalyzes the breakdown of oligo- and/or disaccharides into monosaccharides, and the inhibition of these enzymes reduces blood glucose levels by interfering the absorption of monosaccharides through the mucosal border in the small intestine. Similarly, the inhibition of  $\alpha$ -amylases delays the absorption of glucose and thereby controls the serum glucose levels [4].

Currently, herbal medicines are getting more importance in the treatment of diabetes [5,6]. The seagrass plants are a group of marine hydrophilus angiosperms live in an estuarine or in the marine environment. The seagrass is reported to be widely used in the folklore medicine as sedatives and for the treatment of wounds and fever [7]. Thus, in this study, the seagrass was chosen to investigate its effect on  $\alpha$ -amylase and  $\alpha$ -glycosidase inhibitory activities and glucose homeostasis. The novelty of this work is, *in vitro* studies have not been studied earlier. And also, this complete research study is a new work done on seagrass, i.e., *Halophila beccarii* species as no one had prior studies.

### METHODS

#### Collection of seagrass sample

The seagrass plants were collected from sea coast near Pulicat Lake at Nellore district, AP, India. The seagrass plant was botanically identified

as *H. beccarii* belongs to *Hydrocharitaceae* family. The collected plants were brought to laboratory under aseptic conditions and washed with tap water and distilled water. The washed material was shade dried, and the dried samples were then ground to fine powder using mixer grinder.

#### Preparation of extract

About 20 g dry powder of *H. beccarii* was soaked in 200 ml of methanol for about 1 week. The solution was then filtered using a Whatman No. 1 filter paper, and the extract was evaporated to dryness with Rota evaporator model under reduced pressure at 40°C. The dried methanolic extract was dissolved in 0.1% dimethyl sulfoxide (DMSO) and stored at 4°C for further use.

#### Inhibition of $\alpha$ -amylase activity

The effect of extract on the inhibition of  $\alpha$ -amylase activity was measured by 3,5-dinitro salicylic acid (DNS) method using starch as a substrate [8]. Briefly, 10 mg of pancreatic  $\alpha$ -amylase was dissolved to 10 ml of distilled water and substrate solution was prepared by mixing starch (1%) with 20 mmol phosphate buffer (pH 6.9) and  $\alpha$ -amylase activity was calculated based on the decreased levels of maltose. The reaction was initiated with the addition of different concentrations (100–500  $\mu$ g) of extract of *H. beccarii* and 100  $\mu$ l of  $\alpha$ -amylase to 400  $\mu$ l of starch solution. The reaction mixture was incubated at 37°C for about 10 min. After incubation, the reaction was terminated by adding 100  $\mu$ l of DNS reagent (96 mmol DNS and 30 g sodium potassium tartrate in 0.5 M NaOH) and placed in water bath at 60°C. After 15 min, this mixture was diluted with 900  $\mu$ l distilled water, and  $\alpha$ -amylase inhibition was determined by measuring absorbance at 540 nm. Control was maintained by replacing the extract with DMSO (0.1%). The percentage of  $\alpha$ -amylase inhibition was calculated.

#### Inhibition of $\alpha$ -glucosidase activity

The  $\alpha$ -glucosidase inhibitory activity of extract of *H. beccarii* was studied as per the method of Suthindhiran *et al.* [9]. The reaction mixture containing

different concentrations of extract ranging from 100 µg to 500 µg, 50 µl of phosphate buffer (100 mmol), pH 6.8, and 100 µl α-glucosidase (1 U/ml) was preincubated at 37°C for about 15 min. After preincubation, the reaction mixture was treated with 50 µl of P-nitrophenyl α-D glucopyranoside in 5 mmol in phosphate buffer pH 6.8 and incubated at 37°C for 20 min. Finally, the reaction was terminated with 50 µl of Na<sub>2</sub>CO<sub>3</sub>. The absorbance of yellow color para-nitrophenol released from PNPG was measured at 405 nm. Control was maintained without methanolic extract. The percentage inhibition of α-glucosidase activity was calculated.

#### Determination of glucose adsorption capacity

Glucose adsorption capacity of the extract was calculated as per the standard method of Ou *et al.* [10]. About 1 ml of the extract of *H. beccarii* was added to 25 ml of glucose solution in different concentrations ranging from 5 to 100 mmol and incubated in shaker water bath at 37°C for about 6 h. After incubation, the reaction mixture was centrifuged at 4800 rpm for 20 min, and the residual glucose of the supernatant was measured. Metronidazole used as a control standard. The concentration of bound glucose was calculated using the following formula.

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

G1 - Glucose concentration of original solution

G6 - Glucose concentration after 6 h.

#### Glucose diffusion assay

The diffusion of glucose, in the presence of extract of *H. beccarii*, was assessed using dialysis model by Edwards *et al.* [11], Ahmed *et al.* [12]. The experimental setup was made by loading 1 ml of extract (500 µg/ml) and 1 ml of glucose solution (20 mm in 0.15 M NaCl) into the dialysis bag (12000 MW, Hi-Media laboratory). The dialysis bag was sealed at both ends and placed in a 200 ml of beaker containing 45 ml of 0.15 M NaCl. The control bag was loaded with 1 ml of 0.15 M NaCl, 20 mm glucose, and 1 ml distilled water. The quantity of glucose in the external solution was measured. The beaker was placed in orbital shaker at room temperature (20±2°C). All tests were carried out in triplicate, and the concentrations of glucose were estimated using the O-Toluidine method.

#### Uptake of glucose by yeast cells

Different concentrations of extract of *H. beccarii* (1–5 mg) were added to 1 ml of glucose solution (5–25 mmol/L) and preincubated for 10 min at 37°C. The reaction was initiated by the addition of 100 µl of yeast cell suspension according to the method of yeast cells [13], mixed well and incubated at 37°C for 60 min. After incubation, the tubes were centrifuged at 3800 rpm for 5 min and the supernatant was collected and the absorbance was measured at 620 nm. Metformin was used as a standard. The percent of glucose uptake by yeast cells was calculated on comparison with control sample [14].

## RESULTS

The seagrass collected from Pulicat Lake was identified as *H. beccarii* based on the morphology of the plant and shape of the leaves Fig. 1. The present study focused on detecting the ability of *H. beccarii* to serve as an effective antidiabetic agent. The whole plant of *H. beccarii* was extracted with methanol and extract was dissolved in 0.1% DMSO and analyzed for *in vitro* antidiabetic activity.

The extract showed a percentage inhibition of 30.2±0.25 and 75.1±0.07 at 100 µg/ml and 500 µg/ml, respectively. The IC<sub>50</sub> value of extract was found to be at 270 µg/ml, whereas IC<sub>50</sub> value of acarbose was observed at 183 µg/ml; the result was given in Table 1. Acarbose is a standard antidiabetic drug and it is competitively and reversibly inhibiting the pancreatic α-amylase.

α-Glucosidase activity is the hallmark to indicate the state of diabetes. The extract of *H. beccarii* exhibited prominent inhibitory action on α-glucosidase. The concentration-based inhibition was noticed with a

percentage inhibition of 50% and 90% at 100 µg/ml and 500 µg/ml of extract, respectively, and IC<sub>50</sub> was found at 100 µg/ml. On the other hand, the standard acarbose exhibited 50% inhibition of α-glucosidase at 75 µg/ml, and the obtained results were represented in Table 2.

The glucose adsorption capacity of extract was investigated, and the results were presented in Fig. 2. The adsorption of glucose was noticed at all concentrations of extract, and the binding capacity was found to be proportional to the molar concentration of glucose.

A simple dialysis model was used to determine the glucose diffusion inhibition by *H. beccarii*. After 3.0 h of dialysis, the movement of glucose from the control sample reached a peak level, and the concentration in the external solution was 18.3±0.3 mmol/l (5.3%) Fig. 3. *H. beccarii* extract 500 µg/ml was used to analyze time-dependent impact on glucose diffusion. The glucose diffusion was significantly decreased after 60 min and external glucose concentration was 1.4±0.3 mmol/L. After 3 h of dialysis, the overall 71% decrease in total glucose diffusion compared to control.

Glucose concentrations inside the dialysis tubing were reciprocally coupled to the glucose concentrations in the external solution (control, 1.7±1.21 mmol/L and 18.6 mmol/L in extract). The proportion of glucose transport across the cell membrane of yeast cells was studied in an *in vitro* system consisting of different concentrations of extract of *H. beccarii* (1–5 mg/ml) and glucose ranging from 5 to 25 mmol/L.

The percentage of glucose uptake by yeast cells increases with increase in the concentration of extract of *H. beccarii* Fig. 4. The amount of residual glucose in the sample after a specific time serves as an indicator of the glucose uptake by the yeast cells. At lower concentration, i.e., 1 mg/ml, the percentage of glucose uptake was 30±0.20 initiated but as concentration increases the percentage of uptake reached maximum 75±0.32.

## DISCUSSION

The digestion of starch begins in the mouth with the catalytic fusion of α-amylase secreted in saliva. The process of digestion is continued



Fig. 1: Seagrass *Halophila beccarii*

Table 1: Percentage of inhibition of α-amylase activity

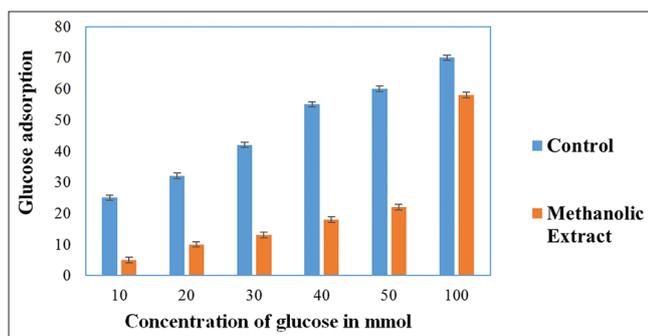
Sample	Concentration µg/ml	% of inhibition	IC <sub>50</sub> µg/ml
Methanolic extract of seagrass	100	50.30±0.03	270 µg/ml
	200	60.03±0.02	
	300	70.03±0.02	
	400	78.02±0.07	
	500	90.02±0.07	
Acarbose standard	100	45.30±0.02	183 µg/ml
	200	68.02±0.44	
	300	72.22±0.20	
	400	75.40±0.08	
	500	85.01±0.05	

Values are expressed as mean±SEM of three independent experiments, statistical significance 1% level (p<0.01). SEM: Standard error of the mean

**Table 2: Percentage of inhibition of  $\alpha$ -glucosidase activity**

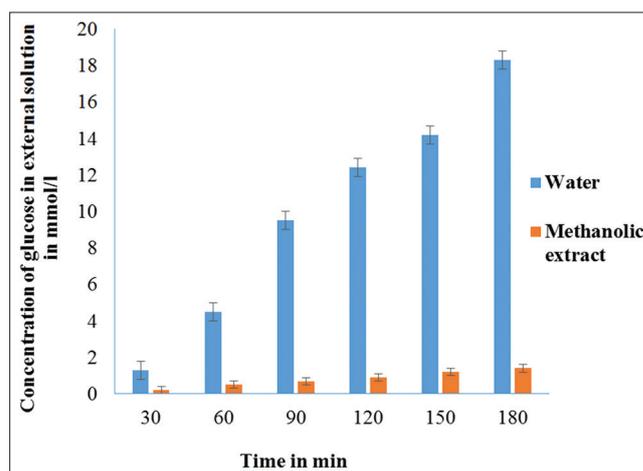
Sample	Concentration $\mu\text{g/ml}$	% of inhibition	$\text{IC}_{50}$ $\mu\text{g/ml}$
Methanolic extract of seagrass	100	30.02 $\pm$ 0.25	100 $\mu\text{g/ml}$
	200	40.07 $\pm$ 0.56	
	300	55.03 $\pm$ 0.12	
	400	68.02 $\pm$ 0.07	
	500	75.02 $\pm$ 0.08	
Acarbose standard	100	60.03 $\pm$ 0.02	75 $\mu\text{g/ml}$
	200	68.02 $\pm$ 0.04	
	300	75.05 $\pm$ 0.02	
	400	82.07 $\pm$ 0.08	
	500	95.01 $\pm$ 0.05	

Values are expressed as mean $\pm$ SEM of three independent experiments, statistical significance 1% level ( $p < 0.01$ ). SEM: Standard error of the mean

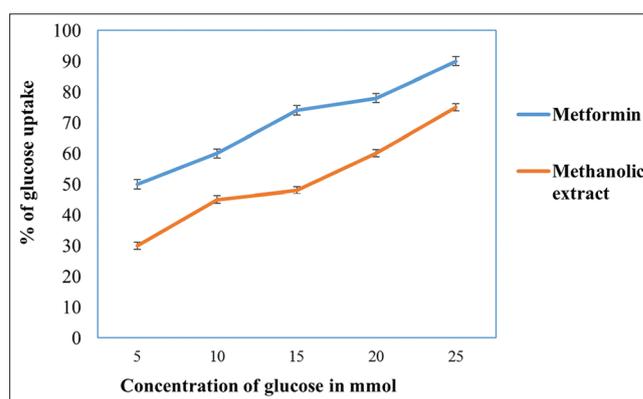


**Fig. 2: Effect of *Halophila beccarii* extract on adsorption of glucose. Values are expressed as mean $\pm$ standard error of the mean of three independent experiments, statistical significance 1% level ( $p < 0.01$ ).**

in the intestine due to the production of  $\alpha$ -amylase. The extract of *H. beccarii* showed tremendous effect on the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes. Therefore, it reduces the digestion of complex carbohydrates and reduces absorption of glucose which further results in the reduction of blood glucose concentration.  $\alpha$ -Glucosidase, located in the intestinal lumen and brush border membrane of intestine, is actively engaged in the digestion of polysaccharides and disaccharides before their absorption [15]. Acarbose, a complex oligosaccharide, is a structural analog of starch and competitively inhibits  $\alpha$ -glucosidase which delays the digestion of starch and disaccharides. Thus, the inhibition of  $\alpha$ -glucosidase is an ideal therapeutic approach to decrease the absorption of carbohydrates [16]. This study provides an alternative natural product from seagrass with hyperbolic potency and lesser side effects than present synthetic drugs. The inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase may lead to reduction in postprandial hyperglycemia which is an important risk factor for cardiovascular diseases [17]. Thus, the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase is important to control post-postprandial hyperglycemia in the treatment of diabetes. The extract was more effective in adsorbing glucose at both lower and higher concentrations which further reduces the amount of glucose transport across the intestinal lumen. The data clearly projects that *H. beccarii* is potential in arresting the glucose diffusion which further confirms that the *H. beccarii* is capable of regulating the movement of glucose out of the cells into bloodstream and thus control the post-postprandial glucose levels [18] reported the inhibitory activity of medicinal plants against glucose diffusion. Uptake of glucose by yeast is widely used screening method for evaluating the antidiabetic activity of natural products. Yeast cells take up glucose by facilitated diffusion. In this process effective transport of extracellular glucose into the cells is attained through carriers that transport solutes down the concentration gradient.



**Fig. 3: Effect of *Halophila beccarii* extract on diffusion of glucose. Values are expressed as mean $\pm$ standard error of the mean of three independent experiments, statistical significance 1% level ( $p < 0.01$ ).**



**Fig. 4: Effect of *Halophila beccarii* extract on uptake of glucose by yeast cells. Values are expressed as mean $\pm$ standard error of the mean of three independent experiments, statistical significance 1% level ( $p < 0.01$ ).**

For treating diabetes, in the traditional Indian Ayurvedic system, there are several medicinal plants and their formulations are available as well as in ethnomedicinal practices as their principal bioactive components showed good  $\alpha$ -amylase inhibitory activity [19].

*In vitro* analysis of the antidiabetic activity of seagrass is in accordance with the previous study of medicinal plants, wherein there is a positive relationship between the total polyphenol and flavonoid content and the ability to inhibit intestinal  $\alpha$ -glucosidase and pancreatic  $\alpha$ -amylase [20,21]. Up to now, no reports are available on *in vitro* antidiabetic of seagrass.

## CONCLUSION

The present study is the first report on validating the antidiabetic activity of *H. beccarii* using appropriate *in vitro* techniques. The study provides the possible mechanism of glucose lowering activity of *H. beccarii* which was explained through inhibition of  $\alpha$ -amylase,  $\alpha$ -glucosidase, and glucose movement, diffusion of glucose across cell membrane and by increasing glucose adsorption.

## ACKNOWLEDGMENT

The authors are much thankful to CURIE facilities, Sri Padmavati Mahila Visvavidyalayam for the experimental analysis.

**AUTHORS' CONTRIBUTIONS**

Concept and collection of data - Vani. M. Writing the article and critical review of the article - Vani. M and Vasavi. T. Final approval of the article - Prof P. Uma Maheswari Devi.

**CONFLICTS OF INTEREST**

Nil.

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