ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Inhibition of carbohydrate degrading enzymes by the root of *Borassus flabellifer* – an *in vitro* evaluation

Natarajan K, Sangeetha R*

Department of Biochemistry, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai – 600 117, Tamil Nadu, India

Article History:

Received on: 09.05.2019
Revised on: 03.08.2019
Accepted on: 10.08.2019

Keywords:

Aldose reductase, anti-hyperglycemic, amylase, diabetes mellitus, glucosidase, Borassus flabellifer root

ABSTRACT

Diabetes mellitus is a metabolic disorder which is characterized by hyperglycemia. Chronic elevation in blood glucose level causes micro and macrovascular complications. Therapeutic management of diabetes involves the control of hyperglycemia by inhibiting carbohydrate degrading enzymes like amylase and glucosidase. This study was aimed at investigating the inhibitory potential of the root of *Borassus flabellifer* against enzymes such as amylase and glucosidase. The hexane, chloroform, petroleum ether, ethanol, and hydroalcoholic extracts of the root of *Borassus flabellifer* were studied for their inhibitory potential against amylase and glucosidase. The inhibitory potential of the extracts were compared to the potential exhibited by the standard inhibitor, acarbose. The hydroalcoholic extract was found to be the most potent inhibitor of both the enzymes with an IC_{50} of 652.37 and 607.06 μg/ml, much comparable to that of acarbose (612.55 and 550.38 μg/ml). The extract could inhibit 50 % of aldose reductase activity at a concentration of 1 mg/ml. The observations of this study suggest that the root of *Borassus flabellifer* may be a promising source for developing effective oral anti-hyperglycemic drugs.

*Corresponding Author

Name: Sangeetha R
Phone: 9884204394
Email: sara_dna@yahoo.co.in

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i1.1797

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Diabetes is a metabolic disorder with increasing prevalence and affects millions of people around the world. Diabetes mellitus is characterized by disturbances in the metabolism of carbohydrates, proteins, and lipids. Glucose homeostasis is affected, and hyperglycemia ensues. This clinical condition is attributed to a deficiency of insulin secretion, insulin action, or both (Dutta and Mukhopadhyay, 2016). Individuals affected by chronic hyperglycemia are predisposed to micro and macrovascular complications because of the undisputed association between hyperglycemia and deranged physiological responses. Effective control of hyperglycemia checks the progression of associated complications. Management of hyperglycemia can be done using insulin, glucose-lowering agents, proper nutrition and modification of life-style modification (Sangeetha and Devi, 2018)

A modest approach in the treatment of diabetes is to decrease the postprandial hyperglycemia. The postprandial elevation of glucose can be decreased by inhibiting the carbohydrate hydrolyzing enzymes like alpha-amylase and alpha-glucosidase. Inhibitors of alpha-amylase and glucosidase are the potential targets in the research for the identification and production of lead compounds for the treatment of diabetes (Perera *et al.*, 2015). However, many of these synthetic hypoglycemic agents exert serious side effects, particularly on the

gastrointestinal system. Hence, herbal medicines are sought after in the treatment of diabetes as they are free from side effects and are less expensive when compared to synthetic hypoglycemic agents (Olaokun *et al.*, 2013). In India, natural remedies are used to treat various diseases and disorders because such folklore remedies are effective and inexpensive. These traditional medicines are used in the treatment of diabetes, as well (Reddy *et al.*, 2017). An extensive study of literature shows that more than 1,200 species of plants with hypoglycemic activity have been identified and used for diabetes (Kripa *et al.*, 2011).

Borassus flabellifer, commonly known as Palmyra palm or Asian Toddy palm, is a tall and erect tree with large fan-shaped leaves. The plant possesses various biological activities and medicinal values. The plant parts like inflorescence, fruit pulp and sap of the plant have been reported to be used in the treatment of syphilis, hepatomegaly, diabetes, gastric disorders, and respiratory ailments (Sandhya *et al.*, 2010). This study investigated the anti-diabetic potential of the root of *B. flabellifer* by assessing the inhibition of the enzymes alpha-amylase and glucosidase, in vitro.

MATERIALS AND METHODS

Collection of plant material

The roots of *B. flabellifer* were collected from the Chennai district, Tamil Nadu, India, during the month of January 2019. The plant was taxonomically identified by Dr. P. Jayaraman, Plant Anatomy Research Centre, Tambaram, Chennai (Voucher specimen - PARC/2019/4112).

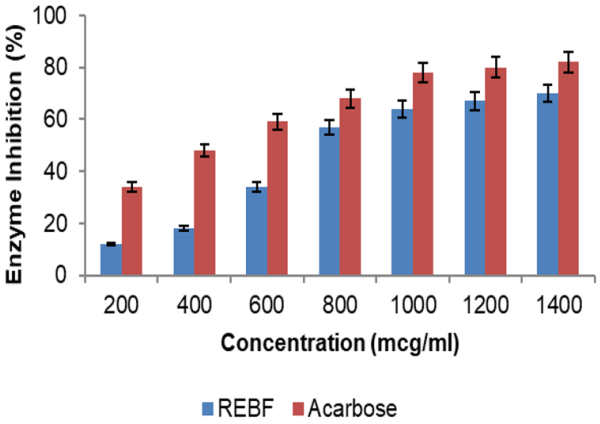

Figure 1: Inhibition of alpha amylase by *B. flabellifer* root extract. [Percentage inhibition assay performed for B. flabellifer rootextract (REBF) and acarbose at concentrations 200 μg to 1400 μg. All values are expressed as Mean $\pm$ S.D (n = 3)]

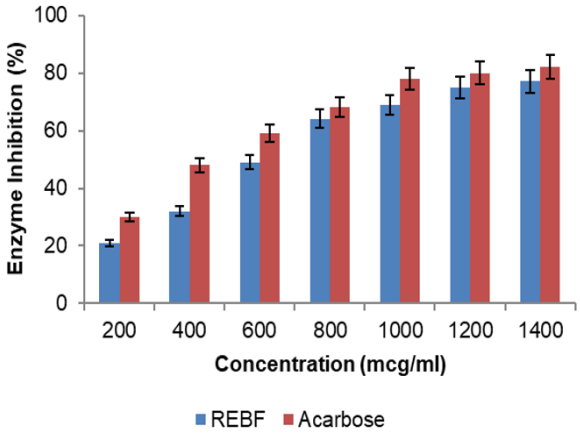

Figure 2: Inhibition of alpha glucosidase by *B. flabellifer* root extract. [Percentage inhibition assay performed for B. flabellifer rootextract (REBF) and acarbose at concentrations 200 μg to 1400 μg. All values are expressed as Mean $\pm$ S.D (n = 3)]

Preparation of plant extract

The roots of *B. flabellifer* were shade dried and mechanically ground to a coarsely powder. The coarse powder was subjected to exhaustive cold maceration in 70% ethanol for 72 h, filtered, concentrated in a rotary evaporator and stored at 4 °C for further study (REBF)

Phytochemical analysis

Preliminary phytochemical screening of REBF for the presence of primary and secondary plant metabolites was carried out using standard procedures.

Assay of alpha-amylase inhibition

In vitro amylase inhibition was studied by the method of Bernfeld (1955). About 100 μL of the extract, of varying concentrations, was preincubated with 200 μL of α- amylase enzyme (Hi media Rm 638) and 100 μL of phosphate buffer (2mM, pH 6.9). After 20 min incubation, 100μL of 1% starch solution was added. 200 μL of the buffer served as the control. About 500μL of dinitrosalicylic acid reagent was added to both control and test and incubated at 60 °C for 5 min. Acarbose (Sigma) was used as the standard inhibitor. The absorbance was recorded at 540 nm, and the experiment was done in triplicate.

Assay of alpha-glucosidase inhibition

In vitro α-glucosidase inhibition was performed by pre-incubating equal volumes of REBF (of varying concentrations) sodium phosphate buffer (1 mM, pH 6.9) and α-glucosidase enzyme (Sigma) for 5 min and then the addition of 0.1 ml of *p*-nitrophenyl-α -*D*-glucopyranoside (Sigma), followed by incubation

at 25 °C for 10 min. Acarbose was used as the standard inhibitor. The absorbance was recorded at 405 nm, and the percentage of inhibition was calculated. The experiment was done in triplicate, and the values were recorded.

Assay of aldose reductase inhibition

The assay of aldose reductase inhibition was carried out Following the method of Hayman and Kinoshita (1965). Briefly, 1.8 ml of reaction mixture consisted of 50 mM sodium phosphate buffer (pH 6.2), 0.125 mM NADPH, 400 mM lithium sulphate, 3 mM glyceraldehyde, aldose reductase enzyme (Sigma) and REBF. The addition of NADPH to the reaction mixture initiated the reaction. The mixture was incubated for 30 min, and sodium bicarbonate was added at the end of the incubation period. The absorbance of the reaction mixture at 350 nm was recorded before and after the incubation period. Quercetin, the standard aldose reductase inhibitor, was used as the standard. The experiment was done in triplicate, and the values were recorded.

Calculation of the percentage of inhibition

The percentage inhibition of amylase, glucosidase and aldose reductase enzymes was calculated using the formula

$$Inhibition(\%) = \frac{100 \times (Absorbance\ of\ Control - Absorbance\ of\ test)}{Absorbance\ of\ Control}$$

Statistical analysis

Values are expressed as a mean of three independent experiments $\pm$ standard deviation. The IC_{50} values were calculated by regression analysis.

RESULTS AND DISCUSSION

Diabetes mellitus, a metabolic disorder affecting millions around the globe, is reported to cause morbidity and mortality. It is characterized by elevated blood glucose levels, clinically termed hyperglycemia. This clinical condition interferes with various metabolic pathways and causes several micro and macrovascular complications. Hyperglycaemia can be controlled by alpha-amylase and alpha-glucosidase inhibitors, and thus, these inhibitors can exert control over type 2 diabetes mellitus (Sangeetha and Vedasree, 2012). The present study assesses the alpha-amylase and alpha-glucosidase inhibitory potential of the root of *B. flabellifer*.

Preliminary phytochemical analysis of REBF was carried out. The analysis revealed the presence of primary metabolites such as carbohydrates, proteins, and lipids, and secondary metabolites such as flavonoids, tannins, and saponins.

The alpha-amylase inhibition assay showed that the hydroalcoholic extract of the root of *B. flabellifer* exhibited potential inhibition at concentrations above 200 μg/ml (Figure 1). The enzyme inhibition was found to increase gradually with increasing concentrations of REBF, and the increase was less proportionate at concentrations above 1600 μg/ml. Acarbose, the standard inhibitor of amylase, exhibited a maximum of 85.5% inhibition in this study.

The alpha-glucosidase inhibition assay showed that the hydroalcoholic extract of the root of *B. flabellifer* exhibited potential inhibition at the concentration of 100 μg/ml. The enzyme inhibition was proportionate with the concentration of extract, and maximum inhibition was observed to be 77.5%. The maximum inhibition of Acarbose, the standard inhibitor, exhibited in this study was 87.4% (Figure 2).

The IC_{50} values of the extracts were calculated using regression analysis, and the values were found to be 652.37 and 607.06 μg/ml, respectively, for amylase and glucosidase inhibition (Figure 3). The IC_{50} values of acarbose in the inhibition of amylase and glucosidase were 612.55 and 550.38 μg/ml, respectively. The results of the study reveal that the hydroalcoholic extract of the root of *B. flabellifer* exhibits appreciable inhibitory potential against both the enzymes. The hydroalcoholic extract inhibited 50% of the activity of amylase and glucosidase at concentrations of 200 μg/ml and 110 μg/ml, respectively. The enzyme inhibitory potential of REBF was be comparable to the standard inhibitor acarbose.

The aldose reductase inhibition by REBF was also studied. The effect of REBF was compared with quercetin, the standard inhibitor. The extract showed 50 % inhibition of the enzyme at a concentration of 1 mg/ml. The inhibition was not proportional to the concentration of the extract, and concentrations less than 600 μg/ml showed minimal inhibition (less than 10 %). Quercetin exhibited remarkable potential and exhibited 50 % inhibition at 400 μg/ml. The results reveal that the extract needs to be purified for better investigation of aldose reductase inhibition.

Natural products are excellent resources of therapeutic agents owing to the presence of various metabolites such as flavonoids, alkaloids, terpenoids, phenolics, and tannins. These bioactive substances possess antioxidant, hypolipidemic, and hypoglycemic activities and hence are used in the treatment of diabetes mellitus (Hammeso *et al.*, 2019). The inflorescence of *B. flabellifer* has been reported to possess hypoglycemic activity, and its efficacy in reducing blood glucose and Lipid levels

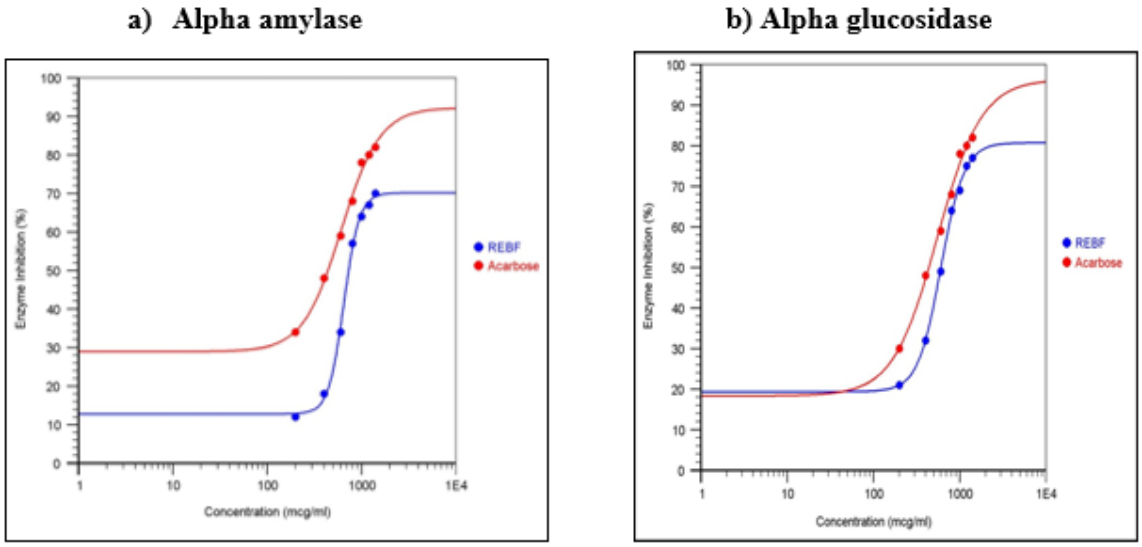

Figure 3: Regression analysis of amylase and glucosidase inhibition by *B. flabellifer* root extract (REBF)

have been reported (Goyal *et al.*, 2014). Similarly, the leaves and root of *B. flabellifer* have been found to possess Antioxidant potential, as reported by Sudhakar *et al.* (2011).

CONCLUSIONS

The present study reveals the amylase, glucosidase, and aldose reductase inhibitory potential of the hydroalcoholic root extract of *B. flabellifer*. Hyperglycemia in diabetes can be effectively managed by the inhibitors of amylase and glucosidase. These naturally available inhibitors do not pose side effects that are generally indicated in the chronic usage of synthetic anti-hyperglycemic agents, and hence, the hydroalcoholic extract of the root of *B. flabellifer* proves to be a potential hypoglycemic drug in *invitro* conditions, and the same can be validated *in vivo* using animal models.

REFERENCES

Bernfeld 1955. Colowick and N. O. Kaplan (eds,), New York, NY, USA,. In Methods in Enzymology, Academic Press. . pages 149–158.

Dutta, D., Mukhopadhyay, S. 2016. Intervening at prediabetes stage is critical to controlling the diabetes epidemic among Asian Indians. *Indian Journal of Medical Research*, 143(4):401–404.

Goyal, P., Agarwal, A. K., Lakashminarasimhaiah, S. G. K. 2014. Antidiabetic and Antihyperlipidemic effect of Borassus flabellifer in Streptozotocin (STZ) induced diabetic rats. *World Journal of Pharmacy and Pharmaceutical Sciences*, 4(1):1172–1184.

Hammeso, W. W., Emiru, Y. K., Getahun, K. A., Kahaliw, W. 2019. Antidiabetic and Antihyperlipidemic Activities of the Leaf Latex Extract of Aloe megalacantha Baker (Aloaceae) in Streptozotocin-Induced Diabetic Model. *Evidence-Based Complementary and Alternative Medicine*, pages 1–9.

Hayman, S., Kinoshita, K. H. 1965. Isolation and properties of lens aldose reductase. *Journal of Biological Chemistry*, 240:877–882.

Kripa, K. G., Sangeetha, R., Madhavi, P., Deepthi, P. 2011. Phytochemical Screening and in vitro Amylase Inhibitory Effect of the Leaves of Breynia retusa. *Pakistan Journal of Biological Sciences*, 14(19):894–899.

Olaokun, O. O., Mcgaw, L. J., Eloff, J. N., Naidoo, V. 2013. Evaluation of the inhibition of carbohydrate hydrolysing enzymes, antioxidant activity and polyphenolic content of extracts of ten African Ficus species (Moraceae) used traditionally to treat diabetes. *BMC Complementary and Alternative Medicine*, 13(1).

Perera, H. K. I., Premadasa, W. K. V. K., Poongunran, J. 2015. α-glucosidase and glycation inhibitory effects of costus speciosus leaves. *BMC Complementary and Alternative Medicine*, 16(1).

Reddy, N., Ramanjaneyulu, K., Sabbani, V., Choday, V. 2017. In Vitro and in Vivo Antidiabetic Activity of Rumex Vesicarius Leaves Extract in Streptozo-

tocin Induced Diabetic Albino Wister Rats. *Journal of Diabetes & Metabolism*, (06):8–8.

Sandhya, Sudhakar, S., Banji, K., Knv, D., ., R. 2010. Pharmacognostical standardization of Borassus flabellifer root. *Ann Biol Res*, 1(4):85–94.

Sangeetha, R., Devi, N. 2018. In-vitro amylase and glucosidase inhibitory activity of the extracts of the leaves of Madhuca longifolia. *International Journal of Research in Pharmaceutical Sciences*, 9(3):980–983.

Sangeetha, R., Vedasree, N. 2012. Vitro α -Amylase Inhibitory Activity of the Leaves of Thespesia populnea. ISRN Pharmacology. pages 1–4.

Sudhakar, K., Chiluka, V. L., Shankar, N. L. G., Matsyagiri, L., Shankar, M., Hya, S. 2011. Anti oxidant activity of methanolic extracts of female Borassus flabellifer leaves and roots. *Der Pharmacia Sinica*, 2(3):193–199.