ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

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

In-vitro amylase and glucosidase inhibitory activity of the extracts of the leaves of *Madhuca longifolia*

Sangeetha R* and Devi N

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: 07.01.2018
Revised on: 15.06.2018
Accepted on: 17.06.2018

Keywords:

Anti-hyperglycemic,
Amylase,
Diabetes mellitus,
Glucosidase,
Madhuca longifolia

ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, a clinical condition which ensues mostly in micro and macrovascular complications. Management of diabetes involves the control of hyperglycemia using agents which can inhibit enzymes like amylase and glucosidase. This study was aimed at investigating the naturally available inhibitors of amylase and glucosidase found in the leaves of *Madhuca longifolia*. The hexane, chloroform, petroleum ether, ethanol and hydroalcoholic extracts of the leaves of *M. longifolia* were studied for their inhibitory potential against amylase and glucosidase. The extracts were found to exhibit potential equivalent to that exhibited by the standard inhibitor, acarbose. The hydroalcoholic extract was the most potent with an IC_{50} of 1.8 mg/ml, much comparable to that of acarbose (IC_{50} = 0.9 mg/ml). These findings suggest that the leaves of *M. longifolia* may be a promising source for the development of oral hypoglycemic agents.

* Corresponding Author

Name: Dr. R. Sangeetha
Phone: +91-9884204394
Email: sara_dna@yahoo.co.in

ISSN: 0975-7538
DOI: https://doi.org/10.26452/ijrps.v9i3.1618

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

Diabetes is a metabolic disorder that affects several million people worldwide and is one of the prime causes of morbidity and mortality in the world. Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia which occurs due to deficiency of insulin secretion with or without insulin resistance. Chronic hyperglycemia is associated with micro and macro-vascular complications and an association between hyperglycemia and deranged physiological responses always exist. Regardless of the type of diabetes, effective control of glycemia slows down the progression of associated complications. Glycemic management involves insulin, glucose-lowering medications, appropriate nutrition and lifestyle modifications (Olokoba *et al*., 2012; Baynes, 2015; Mohanasundaram *et al*., 2016).

A therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia. This can be achieved by the inhibition of 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. 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. In India, since ancient times, traditional medicine has used plants in the treatment of diabetes (Veerabhadrappa *et al*., 2017). Ethnobotanical studies of herbal remedies used for diabetes have identified more than 1,200 species of plants with hypoglycemic activity (Kripa *et al*., 2011; Perera *et al*., 2016).

Madhuca longifolia is a plant belonging to the family Sapotaceae and is distributed throughout India. It has been traditionally used to treat ulcer, rheumatism, tonsillitis and diabetes mellitus. The medicinal value of the leaves of *M. longifolia* can be attributed to the presence of flavonoids and other bioactive compounds present in them (Singh and Singh, 2009; Annalakshmi *et al*., 2013). This study was designed to investigate the anti-diabetic potential of the leaves of *M*. *longifolia* in vitro by assessing the inhibition of the enzymes alpha-amylase and glucosidase.

MATERIALS AND METHODS

Collection of plant material

The leaves of *M. longifolia* were collected from local suburban areas of Kancheepuram district, Tamilnadu, India during January 2017. The plant was taxonomically identified by Dr. S. Aravind, Associate Professor, Department of Botany, National Institute of Siddha, Tambaram, Chennai (Voucher specimen - NISMB2212017).

Preparation of plant extracts

The leaves of *M. longifolia* were shade dried and coarsely powdered. The coarse powder was subjected to successive extraction in solvents of increasing polarity (n-hexane, chloroform, ethyl acetate, ethanol) by using cold maceration technique for 72, 48 and followed by 24 h. Also, 1 kg of the coarse powder was subjected to exhaustive cold maceration in 70% ethanol for the same duration. The solvents were filtered, distilled and dried in vacuum desiccators to obtain the extracts.

Assay of alpha-amylase inhibition

In vitro amylase inhibition was studied by the method of Bernfeld, 1955. In brief, 100µL of the extract was allowed to react with 200µL of α- amylase enzyme (Hi-media Rm 638) and 100µL of phosphate buffer (2mM, pH 6.9). After 20 min incubation, 100µL of 1% starch solution was added. 200µL 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-incubation of equal volumes of extract, sodium phosphate buffer (1 mM, pH 6.9) and α-glucosidase enzyme (Sigma) for 5 min and 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.

Calculation of the percentage of inhibition

The percentage inhibition of α-amylase enzyme was calculated using the formula,

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

Statistical analysis

Values are expressed as the mean of six experiments ± standard deviation. The IC_{50} values were calculated by regression analysis.

RESULTS AND DISCUSSION

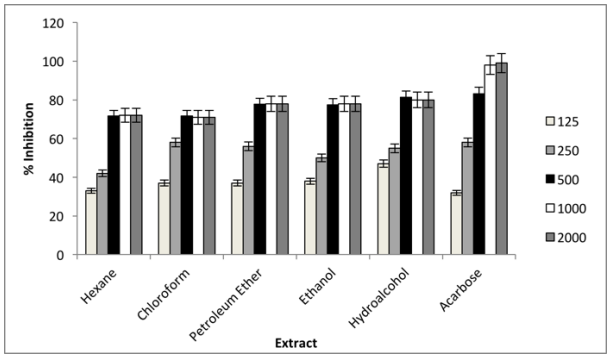

Figure 1: Amylase Inhibition Assay Percentage inhibition assay performed for extracts and acarbose at concentrations of 125 µg to 2000 µg. All values are expressed as Mean ± S.D (n = 6).

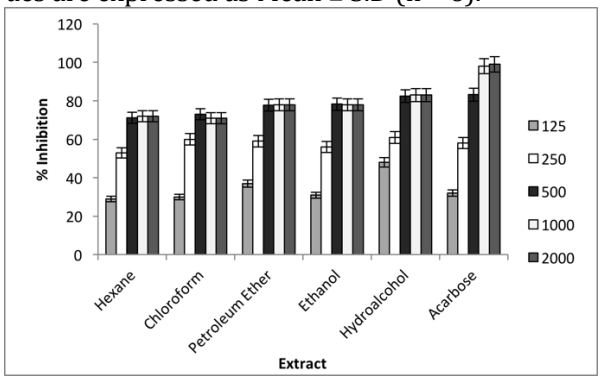

Figure 2: Glucosidase Inhibition Assay Percentage inhibition assay performed for extracts and acarbose at concentrations of 125 µg to 2000 µg. All values are expressed as Mean ± S.D (n = 6).

Table 1: IC50 values of extracts of the leaves of Madhuca longifolia in the inhibition of alpha-amylase and glucosidase

Extract	Amylase	Glucosidase
n-hexane	297.66	345.33
Chloroform	186.2	198.92
Petroleum ether	181.97	187.2
Ethanol	198.6	201.5
Hydroalcoholic	123.59	132.71
Acarbose	113.76	114.65

IC_{50} values of extracts are expressed in microgram/millilitre

All values are expressed as Mean ± S.D (n = 6)Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, a clinical condition which interferes with many metabolic pathways, causing several micros and macrovascular complications. Alpha-amylase and alpha-glucosidase inhibitors are used to exert control over hyperglycemia in type 2 diabetes mellitus. The present study intends to assess the alpha amylase and alpha-glucosidase inhibitory potential of the leaves of *M. longifolia*, in order to minimize the toxicity and side effects of the inhibitors currently used to control hyperglycemia.

The alpha-amylase inhibition assay showed that all the extracts of *M. longifolia* leave exhibited potential inhibition at the concentration of 500 µg/ml (Figure 1). The range of inhibition was between 71.7% to 81.3%. Acarbose, the standard inhibitor of amylase exhibited 83.2% inhibition in this study.

The alpha-glucosidase inhibition assay showed that all the extracts of *M. longifolia* leave exhibited potential inhibition at the concentration of 500 µg/ml. The range of inhibition was between 71.2% to 82.4%. Acarbose, the standard inhibitor of amylase exhibited 84.2% inhibition in this study (Figure 2).

The IC_{50} values of the extracts have been tabulated in Table 1. The study reveals that the hydroalcoholic extract of the leaves of *M. longifolia* exhibits significant inhibitory potential against both the enzymes. The hydroalcoholic extract inhibited 50% of the activity of amylase and glucosidase at concentrations 123.59 µg/ml and 132.71 µg/ml respectively. The effect exerted by the hydroalcoholic extract was similar to the standard inhibitor acarbose. Plant extracts serve as excellent medicines owing to the presence of flavonoids, alkaloids, terpenoids, phenolics and tannins (Mohod and Bodhankar, 2013). Polyphenols, tannins, anthocyanins and flavonoids have been proved to possess inhibitory effects towards amylase and glucosidase (Coman *et al*., 2012).

Similarly, the leaves of *M. longifolia* have been found to possess several biomedical compounds which contribute to the various medicinal values reported in the literature (Annalakshmi *et al*., 2013). Dahake *et al*., 2010 had earlier reported the antihyperglycemic activity of the bark of *M. longifolia*. The methanolic extract of the bark was found to decrease blood sugar levels in streptozotocin-induced diabetic rats.

CONCLUSION

The present study reveals the amylase and glucosidase inhibitory potential of the extracts of *M. longifolia* leaves. The inhibitors of amylase and glucosidase are promising targets for the management of hyperglycemia in diabetics. Naturally available inhibitors can overrule the side effects produced by synthetic anti-hyperglycemic agents and hence the hydroalcoholic extract of the leaves of *M. longifolia* can be studied for the identification of lead compounds that may help in the therapy of diabetes mellitus.

REFERENCES

Annalakshmi, R., Mahalakshmi, S., Charles, A. and Savariraj Sahayam., C. GC-MS and HPTLC analysis of leaf extract of Madhuca longifolia (Koenig) Linn. Drug invention today, 2013; 5, pp.76-80.

Baynes, H.W. Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus. Journal of Diabetes Metabolism, 2015; 6, pp. 541.

Coman C., Rugina, O. D. and Socaciu, C. Plants and Natural Compounds with Antidiabetic Action. Notulae Botanicae Horti Agrobotanici, 2012; 40(1), pp.314-325.

Dahake, A.P., Chakma, C.S., Chakma, R. C. and Bagherwal, P. Antihyperglycemic activity of methanolic extract of *Madhuca longifolia* bark. Diabetologia Croatica, 2010; 39, pp.3-8.

Kripa, K.G., Sangeetha, R., Madhavi, P. and Deepthi, P. Phytochemical screening and in vitro amylase inhibitory effect of the leaves of Breynia retusa. Pakistan Journal of Biological Sciences, 2011; 14(9), pp.894-899.

Mohanasundaram.S, Victor Arokia Doss, Prasad Maddisetty. Hepato-renal protective effects of a hydroethanolic extract of Senna alata on enzymatic and non-enzymatic antioxidant systems in streptozotocin-induced diabetic rats. Integr Med Res 2016; 5: 276–283

Mohod, S. M. and Bodhankar, S. L. Antiulcer activity of aqueous extract of leaves of Madhuca indica J. F. Gmel against naproxen-induced gastric mucosal injury in rats. Journal of Acute Disease, 2013; 2(2), 127-133

Olokoba, A.B., Obateru, O.A. and Olokoba, L.B. Type 2 Diabetes Mellitus: A Review of Current Trends. Oman Medical Journal, 2012; 27(4), pp.269-273

P. Bernfeld. Amylase, α and β. In: S. P. Colowick and N. O. Kaplan (eds,), *Methods in Enzymology*, Academic Press, New York, NY, USA, 1955, pp. 149–158,

Perera, H. K. I., Premadasa, W. K.V. K. and Poongunran, J. α-glucosidase and glycation inhibitory effects of costus speciosus leaves. BMC Complementary and Alternative Medicine, 2016; 16:2

Singh, A. and Singh, P.K. An ethnobotanical study of medicinal plants in Chandauli District of Uttar Pradesh, India. Journal of Ethnopharmacology, 2009; 121, pp.324–329.

Veerabhadrappa, K.V. and Raveendra Reddy, J. Antidiabetic activity of ethanolic extract of *Sesbania grandiflora*. International Journal of Research in Pharmaceutical Science, 2017, 8(4), 650-655.

Worthington, V. Worthington Enzyme Manual Freehold, Worthington Biochemical Corporation, New Jersey: 1993, pp. 36–261.