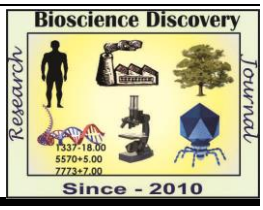
Bioscience Discovery, 8(2):125-128, April - 2017

Print & Online, Open Access, Research Journal Available on http://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)
Research Article



In vitro assay of alpha amylase inhibitory activity of *piper species*

Dipali Thakre, Kiran Damodar, Nazia.D. Khan*, Zia H. Khan, S. M. Mular, Bushra khan

Department of Biochemistry,
Shri Shivaji College of Arts, Commerce and Science, Akola, Maharashtra
*onlynazia247@gmail.com

Article Info

Received: 10-02-2017,
Revised: 25-03-2017,
Accepted: 28-03-2017

Keywords:
Methanol, Piper species, alpha amylase, diabetes mellitus.

Abstract

The genus Piper contains a very large number of species, distributed mainly in the Central and South America, India, Malaysia, Indonesia and Si Lanka. Some of the important species of this family include *P.umbeliatum, P. nigrum, P. chaba, P. betel, P. galeatum, P colubrinum, P. argyrophyllum, P. longum* etc.The objective of the present study, the methanolic extract from three Piper species, namely *P.umbeliatum, P. chaba, and P.betel*, which are used in the Ayurvedic traditional system of medicine to treat diabetes were tested for their inhibitory effect on α-amylase. The assay showed that the methanolic extracts of all piper species at 100 µg/ml exhibited maximum inhibition of alpha amylase. The results revealed that methanolic extract of leaves of P. betel exhibited 77.14% of inhibition at 100µg/ml. The IC_{50} for the leaves extract *P.umbeliatum, P. chaba* and *P. betel* was found to be 42.07%, 43.11% and 43.35% respectively.

INTRODUCTION

Diabetes mellitus (DM) is a multifactorial disease (Ghosh *et al.*, 2012). 171 million people had been affected by diabetes mellitus in 2000 and the ration would be expected to be raised to 366 million in 2030 agents. Diabetes mellitus is a chronic endocrine that affects the metabolic of carbohydrates, proteins, fat, electrolytes and water (Nair *et al*., 2013). It includes a group of metabolic disease characterized by hyperglycemia , in which blood sugar levels are elevated either because the pancreas do not produce enough insulin or cells do not produce enough insulin or cells do not respond to the produced insulin (Nair *et al*., 2013). Therefore a therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia (Chakarbarti and Rajagopalan *et al.*, 2002). This can be achieved by the inhibition of carbohydrate hydrolyzing enzyme like alpha amylase (Bhosale and Hallale, 2011).

Alpha amylase is the important enzymes involved in the digestion of carbohydrate. Alpha amylase is involved in the breakdown of long chain carbohydrates. Alpha amylase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes. Higher plant, animal and microorganism are able to produce different type of alpha amylase to regulate their digestive power (Aresan *et al.*, 2009) (Ganash *et al.*, 2011). In animals alpha amylase inhibitors decrease the high glucose levels that can occur after a meal by slowing the speed with which alpha amylase can convert starch to simple sugar. They need to be given alpha amylase inhibitors in order to keep their glucose levels under control. The plants use alpha amylase inhibitors as a major defective mechanism to prevent infestation (Roger *et al.*, 2000).

Absorption of glucose can be delayed by reducing the rate of digestion of starch. Inhibition of

the mammalian alpha amylase enzyme in the intestine would delay the degradation of starch and oligosaccharides to monosaccharides before they can absorbed. This would decrease the absorption of glucose and consequently reduce postprandial blood glucose level (Karthic and Kirthiram *et al.,* 2008) .

Extensive research has been carried out to screen the bioactivity of these inhibitors because of their significant important in health care and medicine (Tanko and Eze *et al.,* 2012) plant food rich in polyphenols have been reported to cause effects similar to insulin in the utilization of glucose and act as good inhibitor of key enzymes like alpha amylase associated with 2 type diabetes and lipid peroxidation in tissues (Reddy *et al.,* 2010). This is of importance in diabetic people where low insulin levels prevent the fast clearing of extracellular glucose from the blood (Mohammed *et al.,* 2009). Hence diabetics tend to have low alpha amylase levels in order to keep their glucose levels under control. Plants also use alpha amylase inhibitors as a defence mechanism as a protection from insects. These inhibitors alter the digestive action of alpha amylases and proteinases in the gut of insects and inhibit their normal feeding behaviour. Therefore alpha amylase inhibitors have potential roles in controlling blood sugar levels and crop protection (Kumanan *et al.,* 2010). Inhibition of these enzyme systems helps to reduce the rate of digestion of carbohydrates (Bhat *et al.,* 2011). Less amounts of glucose is absorbed because the carbohydrates are not broken down into glucose molecules. In diabetics the short term effect of these enzyme inhibitor drug therapies is to decrease high blood glucose levels. The presently used synthetic enzyme inhibitors cause gastro intestinal side effects such as diarrhoea, flatulence, abdominal bloating etc (Bray and Green way, 1999).Therefore natural alpha amylase from the dietary plants can be used as an effective therapy for treating post prandial hyperglycemia with minimal side effects. The present study was carried out to investigate the inhibitory potentials of the methanolic extracts of piper species (bangali paan –*piper umbeliatum*, kalkatta paan-*piper chaba*, kapuri paan-*piper betel* leaf) on alpha amylase the key enzymes responsible for carbohydrate hydrolysis.

MATERIAL AND METHODS

Source of plant material:

Leaves of piper species (*piper umbeliatum, piper chaba, piper betal*) were collected locally and used for the preparation of extracts.

Preparation of plant extract :

The leaves of *piper umbeliatum ,piper chaba , piper betal* were obtained and washed with distilled water and dried at 50^0C the crude methanolic extract was obtained by extracting 10 grams of dried plant powder in 100 ml methanol.

Assay for alpha amylase inhibition:

The assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme and the plant extracts in concentration range 20-100 µg/ml were incubated for 10 minutes at room temperature followed by addition of 200µl of starch in all test tubes. The reaction was terminated with the addition of 400 µl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any plant extracts. For standard, acarbose was used instead of leaves extract at same concentration.

Formula

$$\text{Inhibition (\%)} = \frac{\text{Abs 540 (control)} - \text{Abs 540 (extract)}}{\text{Abs 540(control}} \times 100$$

RESULTS AND DISCUSSION

The inhibitory activity of methanolic extracts of *P. Umbeliatum,P. chaba,* and *P. betel* on alpha amylase was investigated in this study and the results are shown in Table 1. In this assay, % inhibition for alpha amylase was directly proportional to be increasing concentration of the *P. umbeliatum, P. chaba,* and *P. Betel* extract with maximum inhibition was 45%, 50% and 77.14% at 100µg/ml respectively. Acarbose was used as a standard drug which showed 80.12% inhibition at 100µg/ml.

Table 1: The percent inhibition of alpha amylase by methanolic extracts of *P. umbeliatum*, *P. chaba,* and *P. betel* leaves.

Sr. no	Name of piper species	Concentration (µg/ml)	Inhibition of alpha amylase (%)	IC_{50} (µg/ml)
1	*Piper umbeliatum*	20 µg/ml 40 µg/ml 60 µg/ml 80 µg/ml 100 µg/ml	28.33 35 37.77 41.81 45	42.07
2	*Piper chaba*	20 µg/ml 40 µg/ml 60 µg/ml 80 µg/ml 100 µg/ml	33.33 36.25 40 43.63 50	43.11
3	*Piper betel*	20 µg/ml 40 µg/ml 60 µg/ml 80 µg/ml 100 µg/ml	36.66 42.5 44.44 50.90 77.14	43.35
4	Acarbose	20 µg/ml 40 µg/ml 60 µg/ml 80 µg/ml 100 µg/ml	40.52 48.3 51.71 56.8 80.12	48.48

Percent alpha amylase inhibition of the three plant extracts was plotted as a function ofconcentration in comparison with acarbose and IC_{50} values are as shown in Figure 1.The results indicate that out of the three methanolic plant extracts, *P. Betel* exhibited good anti alpha amylase activity, *P. Chaba* showed appreciable inhibition activity and *P. umbeliatum* showed the least inhibitory activity. The plant extracts of *P. umbeliatum*, *P. chaba*, and *P. betel* showed an IC_{50}value of 42.07, 43.11 and 43.35µg/ml respectively in the alpha amylase inhibition assay. Fig.1. *P. Betel* showed the greater % inhibition of the alpha amylase enzyme compared to other plant extracts. .From the results, it can be concluded that use of *P. betel* leaves extract will be greatly beneficial to reduce the rate of digestion and absorption of carbohydrates and thereby contribute for effective management of diabetes by decreasing the post-prandial hyperglycemia.

The results of the present study indicate that, methanolic extracts of *Piper betel* showed the maximum alpha amylase inhibitory activity when compared with *P. umbeliatum*, *P. chaba*. The leaves may essentially contain herbal bioactive compounds inhibiting enzyme activity and further structural elucidation and characterization methodologies have to be carried out in order to identify the bioactive constituents. In conclusion, more research is required for developing a potential and valuable anti diabetic therapy using alpha amylase inhibitors of plant origin.

REFERENCES

Arasan Elaya Raja, M. Vijayalakshmi and Garikapati Devalarao, **2009**. *Acrous calamuslinn* ,Chemisty and Biology , Research Journal of Pharmacy and Technology.,**2** (2):256-261.

Bhat M, Zinjarde SS, Bhargava SY, Kumar AR and Joshi BN, **2011**. *Evi Based Complement Alternate Med*., PHI learning Pvt. Ltd. New Delhi. **20**:81- 89.

Bhosale UP and Hallale BV, **2011**. *Gastroenterology.*, Notion press Chennai, India. **111** (13) 13-20.

Bray GA and Greenway FL, **1999**. *Endocr Rev.*, Taxmann Publications pvt. Ltd. New Delhi, India. **20**:805-879.

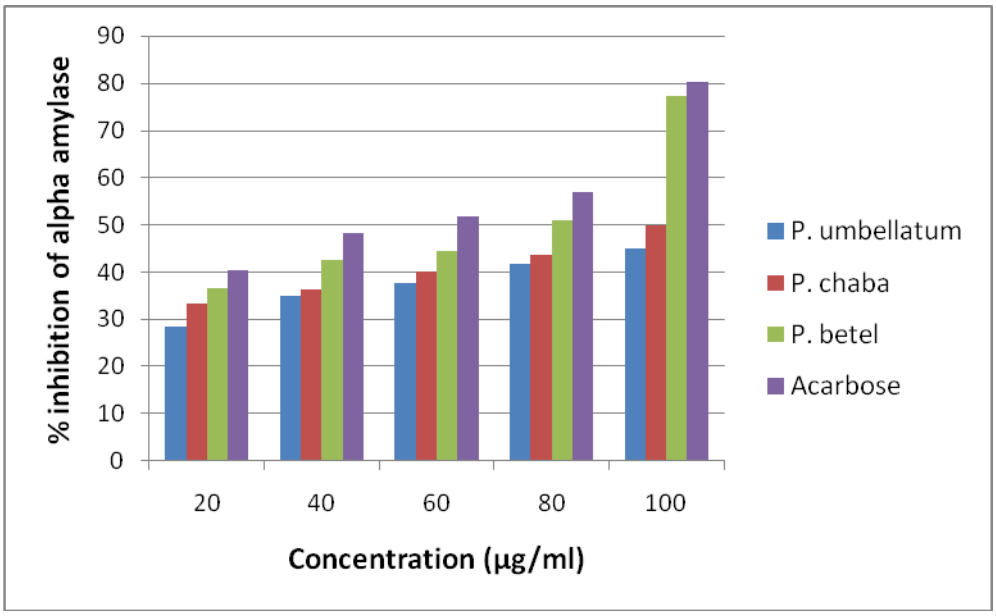


Fig. 1. % inhibition of alpha amylase by methanolic extract of *P. umbeliatum, P. Chaba, P. betel* and reference alpha amylase inhibitor, acarbose.

Chakrabarti R and Rajagopalan R , **2002**.*Current Science*., Current science association with collaboration with the Indian Academy of Science, India. **83** (12):1533-1538.

Ghosh S, Ahire M, Patil S, Jabgunde A, Bhat M, Bimba D. Joshi N, Pardesi K, Dhavale DD. and Chopade BA, **2012**. Antidiabetic Activity of *Gnidiaglauca* and *Dioscoreabulbifera*: Potent Amylase and Glucosidase Inhibitors, Evidence-Based Complementary and Alternative Medicine, **10** (1) 12-14.

Karthic K , Kirthiram KS, Sadasivam S, Thayumanavan B. and Palvannan T, **2008**. Identification of alpha amylase inhibitors from Syzygium cumini Linn. Seeds. *Journal of Experimental Biology* , **46** (9):677-680.

Kumanan R, Manimaran S, Saleemulla K, Dhanabal SP and Nanjan MJ, **2010**. Evaluation of antibacterial properties of extracts of piper betel leaf. *Int J Pharmaceutical Biomed Res.*,**1** (2):69-72.

Mohammed A, Adelaiye AB, Bakari AG and Mabrouk MA, **2009**. Inhibition of food intake in obese subjects by peptide YY3. *International Journal of Medicine and Medicinal Sciences*., **1**(12):530-535.

Morton RL, Schroeder HE, Bateman KS, Chrispeels MJ, Armstrong E and Thomas JV, 2000. Bean alpha amylase inhibitor 1 in transgenic peas. Provides complete protection from pea weevil under field conditions. Proceeding of National Acadamy of science. USA. **97**(8):3820-3825.

Nair SS , Kavrekar V and Mishra A, **2013**.In vitro studies on alpha amylase and alpha glucoside inhibitory activities of selected plant extract. In European Journal of Experimental Biology.,**3**(1):128-132.

Reddy NV, Anarthe SJ and Raghavendra NM, **2010**. Pharmaceutical polymeric controlled drug delivery system. *J Res. Biomed. Sci.*, **1**(2):72-75.

Sharma GN, Dubey SK, Sharma P and Sati N, **2011**. Medicinal values of Bael . *International Journal of Current Pharmaceutical Review and Research*, **1**(3):12-22.

Tanko Y, Eze ED, Jimoh A, Yusuf K, Mohammed KA, Balarabe F and Mohammed A, 2012. Haemostatic effect of aquous extracts of mushroom (*Ganoderma lucidum*) *European Journal of Experimental Biology*, **2** (6):2015-2018.

How to Cite this Article:
Dipali Thakre, Kiran Damodar, Nazia D Khan, Zia H Khan, SM Mular, Bushra khan, 2017. *In vitro* assay of alpha amylase inhibitory activity of *piper species*. *Bioscience Discovery*, **8**(2):125-128.