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INHIBITION OF CARBOHYDRATE DIGESTING ENZYMES BY THE METHANOLIC EXTRACTS OF THREE *POGOSTEMON* SPECIES (LAMIACEAE)

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ABSTRACT

One of the anti-diabetic therapeutic strategies is inhibition of carbohydrate digesting enzymes such as α-amylase and α-glucosidase. In the present study were evaluated for their effect on α-amylase and α-glucosidase enzymes in the methanolic extracts of *Pogostemon mollis, Pogostemon vestitus, Pogostemon nilagiricus.* by using *in vitro* assays. Plants extract showed the significant inhibitory activities in α-amylase (IC_{50}values: 901.87 µg/ml, 1020.4 µg/ml and 892.85µg/ml) and α-glucosidase (IC_{50}value: 825.76 µg/ml, 887.19 µg/ml and 819.67 µg/ml). These results suggest that the methanolic extracts of these plants have α-glucosidase and α-amylase inhibitory properties. It could be physiologically useful for treatment of diabetes, although *in vivo* experiments are needed.

KEYWORD: *Pogostemon*, anti-diabetic, carbohydrate, enzymes, α-amylase, α-glucosidase, inhibition.

INTRODUCTION

Diabetes mellitus is one of the chronic, leads to several micro-vascular and macro- vascular complications that affect many organs of the body. Environmental factors such as diet, obesity and sedentary life style increase the risk of diabetes. Other important risk factors include high family aggregation, insulin resistance, nutritional status, age and life style change due to urbanization. The reports of World Health Organization, the diabetic population increase more than 300 million by the year 2025.[1] Diet plays a vital role in the managing of diabetes mellitus. Rather than the diet, the management of diabetes is a global

problem until discovered the successful treatment. The identification of new therapeutic avenues in the treatment of all pathological aspects of this disorder remains a major challenge for current biomedical research. Probably a number of plants present in nature possess noticeable anti-diabetic activity.[2]

The Research works has been found to have definite action on the nervous, circulatory, respiratory, digestive and urinary systems; as well as the sexual organs, the skin, vision, hearing and taste by the medicinal plants.[1] Consequently, the search for safer and easily available antidiabetic agents among medicinal plants continues. The ethnobotanical information reports, almost 800 plants own potential antidiabetic property.[3] Most of the research are carried out to evaluate the therapeutic effect of the plants along with their mode of action i.e.- alteration of glucose metabolism, insulin like effect/ insulinotropic action, improve glucose tolerance, reduction of absorption of glucose from intestine, enhancing insulin signal pathway, hypoglycemia through increase glucose uptake and glycogen synthesis, inhibiting for α -glucosidase and α-amylase, reduction of insulin resistance, reduction of oxidative stress and protecting against tissue damage, generation of beta cell in pancreas.[5] The aim of this study is to investigate and compare the antidiabetic of three plants from the *Pogostemon* genus belonging to the family Lamiaceae.

MATERIALS AND METHODS

α- Amylase Inhibition Assay

The α-amylase inhibition assay were determined by the method of Worthington.[6] Different Concentrations (50-250 µg/ml) of the plant extracts and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing porcine pancreatic α-amylase enzyme (EC 3.2.1.1) (0.5 mg/ml) were incubated at 25˚C for 10 min. After the incubation, 500µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to the reaction mixture. Subsequently, the reaction mixture was incubated at 25◦C for 10 min, followed by addition of 1.0 ml of dinitrosalicylic acid (DNSA). Finally the reaction was stopped by incubation in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 10 ml distilled water and the absorbance was measured at 540 nm in a spectrophotometer. The mixture of all other reagents and the enzyme except the sample was used as a control. The α-amylase inhibitory activity was expressed as percentage inhibition.

$$\text{Inhibition (\%)} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{Control}}} \text{X100}$$

The IC_{50} value was defined as the concentration of the sample extract to inhibit 50% of α-amylase activity under assay condition.

α-Glucosidase Inhibition Assay

The α-glucosidase inhibition assay were done by the method of Apostolidis *et al*.[7] Various amounts of plant extracts (50-250µg/ml) and 100µl of α-glucosidase (0.5 mg/ml) in 0.1 M phosphate buffer (pH 6.9) solution were incubated at 25◦C for 10 min. Then, 50 µl of 5M p-nitrophenyl-α-D-glucopyranoside in 0.1M phosphate buffer (pH6.9) solution was added. Reaction mixtures were incubated at 25°C for 5 min and the absorbance was taken at 405 nm by a spectrophotometer. The mixture of all other reagents and the enzyme except the sample was used as a control and the results of α-glucosidase inhibition activity were expressed in terms of inhibition percentage. The percentage of α-glucosidase inhibitory activity is calculated by the following formula:

$$\text{Inhibition (\%)} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{Control}}} \text{X100}$$

The IC_{50} value was defined as the concentration of the sample extract to inhibit 50% of α-glucosidase activity under assay condition.

RESULTS

In the present study, methanolic extracts of *P. mollis, P. vestitus* and *P. nilagiricus* were evaluated for their inhibition effect on α- glucosidase enzymes using *in vitro* assays. The percentage inhibition at the concentration of 200, 400,600, 800 and 1000 µg/ml by methanolic extracts of *P. mollis, P. vestitus* and *P. nilagiricus* showed a concentrations dependent reduction in Table-1. The maximum inhibitions were found at a concentration of 1000 µg/ml. The IC_{50} values of methanol extracts of *P. mollis, P. vestitus* and *P. nilagiricus* 825.76 µg/ml, 887.19 µg/ml and 819.67 µg/ml at $p<0.05$ respectively. The inhibitions of α-amylase by the *P. mollis, P. vestitus* and *P. nilagiricus* methanolic extracts at concentrations (200, 400, 600, 800 and 1000 µg/ml) were significantly different ($p > 0.05$) from one another. The maximum inhibition was also found at a concentration of 1000 µg/ml and the lowest in 200µg/ml. The IC_{50} values of methanol extract of *P. mollis* (901.87 µg/ml),

P. vestitus (1020.4 µg/ml) and *P. nilagiricus* (892.85µg/ml) at p<0.05 respectively. The final results suggest that all tested plants have potential diabetic properties via inhibiting α-amylase and α- glucosidase enzymes (Fig-1).

Table 1: Inhibition of α-amylase inhibitory activity

Plant name	Sample concentration (µg/ml)	% of inhibition	IC_{50} value (µg/ml)
Pogostemon mollis	200	8.19 ± 0.13	901.87
	400	19.23 ± 0.54	
	600	31.52 ± 0.23	
	800	46.14 ± 0.75	
	1000	55.44 ± 0.22	
Pogostemon nilagiricus	200	9.25±0.16	1020.4
	400	17.36±0.37	
	600	28.04±0.21	
	800	41.74±0.27	
	1000	49.58±0.47	
Pogostemon vestitus	200	10.24±0.11	892.85
	400	21.37±0.02	
	600	38.47±0.36	
	800	45.37±0.44	
	1000	53.84±0.27	

Values are expressed as mean ± SD (n=3).

Table 2: Inhibition of α- Glucosidase inhibitory activity

Plant name	Sample concentration (µg/ml)	% of inhibition	IC_{50} value (µg/ml)
Pogostemon mollis	200	13.28 ± 0.77	825.76
	400	24.94 ± 0.91	
	600	37.33 ± 0.57	
	800	48.93 ± 0.11	
	1000	60.55 ± 0.84	
Pogostemon nilagiricus	200	12.92±0.09	887.19
	400	20.4±0.04	
	600	34.68±0.33	
	800	45.67±0.12	
	1000	57.36±0.26	
Pogostemon vestitus	200	14.47±0.08	819.67
	400	26.48±0.45	
	600	31.24±0.22	
	800	49.85±0.13	
	1000	62.47±0.34	

Values are expressed as mean ± SD (n=3).

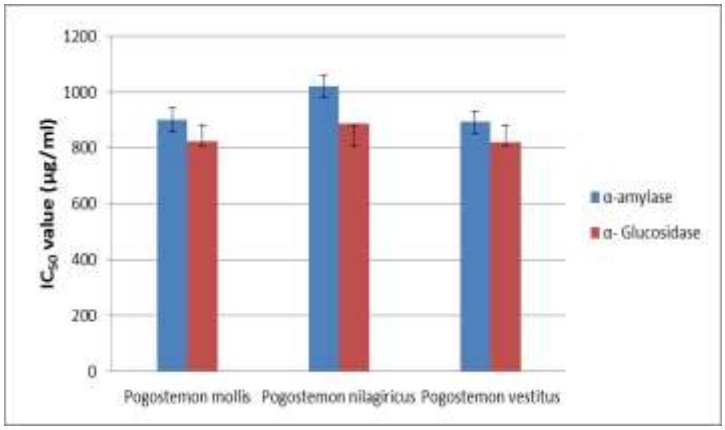

Figure 1: comparative inhibition of carbohydrate digesting enzymes by three *Pogostemon* species

DISCUSSION

Management of diabetes without side effects is still one of the health challenges to the medical community. Synthetic hypoglycaemic drugs like acarbose, miglitol and voglibose are used in conjunction with other antidiabetic drugs, but these inhibitors have been found to possess gastrointestinal side effects like abdominal discomfort and flatulence.[8] It was proposed that inhibition activity of such α-amylase and α-glucosidase would delay the degradation of carbohydrate. These result in the reduction of postprandial blood glucose level elevation.[9] As a result of this, there is growing interest in discovering new and effective α-amylase and α-glucosidase inhibitors from plants with minimal or no side effects.[8] Therefore, α-amylase and α-glucosidase inhibition are control of hyperglycemia as they delay carbohydrate digestion and consequently reduce the postprandial plasma glucose level.[10]

The present study has been carried out to evaluate the *in vitro* antidiabetic potential of methanolic extracts of *P. mollis*, *P. vestitus* and *P. nilagiricus* in inhibiting α-glucosidase and α-amylase enzymes. These findings of *P. mollis*, *P. vestitus* and *P. nilagiricus* efficiently inhibit both α-amylase and α-glucosidase enzymes *in vitro* in a dose dependent manner. The methanolic extracts showed the most potential α-amylase inhibition activity at all the concentrations tested also in α-glucosidase. However, at high concentration (1000 mg mL^{-1}), both α-amylase and α-glucosidase inhibition percentage was found to be much stronger in all the tested plants.

Similar *in vitro* diabetic studies have been analyzed in *Ocimum basilicum* [11], *O. sanctum*[12] and there are several plants used as antidiabetic remedies, the hypoglycemic effect has been confirmed and the mechanisms of hypoglycemic activity of these plants are being studied.[13]

CONCLUSIONS

In conclusion, methanolic extracts of these plants demonstrate a good α-glucosidase and α-amylase inhibitory activity. Further, studies are required to elucidate *in vitro* antidiabetic potential of *P. mollis*, *P. vestitus* and *P. nilagiricus* for validating their traditional medicinal claim.

REFERENCES

1. Mamun-or-Rashid ANM, Shamim Hossain MD, Hassan N, Dash BK, Sapon Md A, Sen MK. A review on medicinal plants with antidiabetic activity. Journal of Pharmacognosy and Phytochemistry, 2014; 3(4): 149-159.
2. Bhatt UP, Sati SC, Chandra S, Kumar S., Anthwal A, Singh R, Singh D, Kumar N, Kumar A, Bahuguna RP. Evaluation of *in vivo* and *in vitro* anti-diabetic activity of *Roylea cinerea*. Int. J. Pharm. Sci. Rev. Res., 2015; 32(2): 210-213.
3. Patience O. Osadebe, Estella U. Odoh, Philip F. Uzor. The search for new hypoglycemic agents from plants. Afr. J. Pharm. Pharmacol., 2014; 8(11): 292-303.
4. Husnia I. Marrif, Salma I. Al-Sunousi. Pancreatic β Cell Mass Death. Front Pharmacol, 2016; 7: 83.
5. Hosseini A, Shafiee-Nick R, Ghorbani A. Pancreatic beta cell protection/regeneration with phytotherapy. Braz. J. Pharm. Sci., 2015; 51(1): 1-16.
6. Worthington V. Alpha amylase, in: V. Worthington (Ed.), Worthington Enzyme Manual Freehold, Worthington Biochemical Corp, NJ, 1993; 36- 41.
7. Apostolidis E, Kwon YI, Shetty K. Inhibitory potential of herb, fruit and fungal enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innovative Food Science and Emerging Technologies, 2007; 8: 46-54.
8. Sridevi FH, Jayaraman P, Pachaiyappan P. Evaluation of α-Glucosidase Inhibitory Action of Isolated Compound beta Amyrin from *Memecylon umbellatum* Burm. Inter J Pharma Phyto Res., 2015; 7(6): 1033-1038.
9. Arasan E, Vijayakanth R, Balaji S, Mageswaran R, Rahaman SA. *In vitro* antidiabetic activity of plant extracts of *Pergularia extensa* Chiov. Int. J. Pharm. Sci. Rev. Res., 2015; 33(1): 18-19.
10. Telagari M, Hullatti K. *In-vitro* α-amylase and α-glucosidase inhibitory activity of *Adiantum caudatum* Linn. and *Celosia argentea* Linn. extracts and fractions. Indian J Pharmacol, 2015; 47(4): 425-429.

11. El-Beshbish H, Bahashwan S. Hypoglycemic effect of basil (*Ocimum basilicum*) aqueous extract is mediated through inhibition of a-glucosidase and a-amylase activities: An *in vitro* study. Toxicol Ind Health, 2012; 28(1): 42- 50.
12. Anil Rege AA, Chowdhary AS. Evaluation of Alpha-Amylase and Alpha-Glucosidase Inhibitory Activities of *Ocimum sanctum* Linn. Int. J. Pharm. Sci. Rev. Res, 2014; 25(1): 130-133.
13. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed. 2012; 2(4): 320- 330.