Original Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

IN-VITRO ANTIDIABETIC ACTIVITY OF *MALAXIS RHEEDEI* SW (WHOLE PLANT): AN ENDANGERED MEDICINAL ORCHID

RENJINI HARIDAS*[1], SUMATHI P[1], SANGEETH THEKKAN[1]

[1]PG& Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore- 641 029, Tamil Nadu, India.

ABSTRACT

To investigate the *in vitro* anti-diabetic potential of *Malaxis rheedei* SW whole plant methanol extract. This study evaluates the anti-diabetic potential of *M. rheedei* whole plant via *in vitro* inhibition of α-amylase and α-glucosidase using the methanol extract. The methanol extract of *M. rheedei* displayed the most effective inhibition of both α-amylase and α-glucosidase activities with half-maximal inhibitory concentration (IC_{50}) of 407.56 µg/ml (α-amylase inhibitory activity) and 380.66 µg/ml (α- Glucosidase inhibitory activity).The observed inhibitions of α-amylase and α-glucosidase proved that the whole plant extract of *M. rheedei* have the potential to manage Diabetes mellitus but it is not a cure for Diabetes.

KEYWORDS: ***Malaxis rheedei SW, Diabetes, α-Amylase, α-Glucosidase, Hyperglycemia***

RENJINI HARIDAS*

PG & Research Department of Botany,
Kongunadu Arts and Science College (Autonomous),
Coimbatore- 641 029, Tamil Nadu, India.

***Corresponding Author**

Received on: 21-11-2016
Revised and Accepted on : 06-03-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.2.p130-133

INTRODUCTION

Diabetes mellitus is one of the common metabolic disorders and 2.8% of the population suffers from this disease throughout the world[1]. In olden days it was considered a disease of minor significance. In the 21st century, it is one of the main threats to human health. It is estimated that 300 million people will be affected by diabetes mellitus in the year 2025[2]. This disease characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. Chronic hyperglycemia causes damage to eyes, kidneys, nerves, heart and blood vessels[3]. Plant materials which are being used as traditional medicine for the treatment of diabetes are considered one of the good sources for a new drug or a lead to make a new drug[4]. Several drugs are available to decrease hyperglycaemia but these drugs have side effects. Alpha amylase and alpha glucosidase inhibitors are used to achieve greater control over hyperglycemia in type 2 diabetes mellitus[5]. So the search for new drug/compound is essential to overcome the diabetic problems. It is still a challenge to medical community[6].The genus *Malaxis* (Orchidaceae) consists of about 300 species that are widespread in the tropics and subtropics of the old and new worlds and this genera used as traditional medicines[7, 8]. 'Ashtavarga' is a composition of eight medicinal herbs and this includes four orchid plants, in that *Malaxis* is one of the important genus among them[9, 10]. Ashtavarga plants are also reported to restore health immediately and work as antioxidants in the body[11] and these plants have been threatened since ancient times, and are difficult to obtain in the required quantity[12]. The first record of Indian orchid used in ayurvedic medicine is *Malaxis rheedei* SW., which was discussed in 'Charaka Samhita', a classic ancient Indian medicinal treatise written by Charaka in Sanskrit, a few thousand years ago[13 - 14]. And *Kattunayakans* tribes used this plant externally for snake poison[15]. Because of these medicinal properties, *M. rheedei* have been selected for the evaluation of anti-diabetic potential.

MATERIALS AND METHODS

Plant Materials

Wild plant species were collected from Malappuram district, Kerala, India. The plant was authenticated by the Taxonomist Dr. Binu Thomas, Department of Botany St. Joseph's College, Devagiri, Kozhikode – 673008, Kerala, India. The Specimen voucher is maintained in the Institute.

Preparation of Plant Extract

The fresh whole plant parts of *M. rheedei* were washed with tap water and shade dried for two month and powdered coarsely. Then they were finely powdered mechanically using pulverizer and passed through 40 mesh sieve and stored in airtight containers. About 250g of powdered whole plant were extracted in soxhlet apparatus with methanol. The extract was dried under reduced pressure at low temperature (40-50°C). The last traces of the solvent was removed under vacuum drier and the solid mass obtained was dissolved in DMSO and made into stock solution.

Protocol

α-Amylase Inhibition Assay[16]

Reagents

- 0.02 M Sodium phosphate buffer (pH 6.9)
- 0.006 M NaCl
- 1% Starch
- Dinitro salicylic acid

Procedure

Different concentrations (50-250µg/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 bought from precision scientific com. (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 dinitro salicylic 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.

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

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[17]

Reagents

- α-glucosidase (0.5 mg/ml)
- 0.1 M phosphate buffer (pH 6.9)
- 5M p-nitrophenyl-α-D-glucopyranoside

Procedure

Various amounts of plant extracts (50-250µg/ml) and 100µl of α-glucosidase bought from precision scientific com. (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:

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

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

STATISTICAL ANALYSIS

For *in vitro* antidiabetic activity of the extracts, the results were expressed as mean± standard deviation (SD) (n=3) and subjected to one-way analysis of variance (ANOVA) by using SPSS software version 10.

RESULTS AND DISCUSSION

Diabetic disease is a serious medical threat of public health. There is a strong need of new drugs for the treatment and prevention of this disease. The recent advances in understanding the activity of intestinal enzymes (α-amylase and α-glucosidase) have led to the development of newer pharmacological agents. A high postprandial blood glucose response is associated with micro and macro-vascular complications in diabetes and more strongly associated with the risk for cardiovascular diseases[18]. The aim of this study is to gather scientific evidence and knowing the effectiveness of natural constituents of plants that are used for treatment of diabetes. The exhibited properties of anti-diabetic in methanolic extract *of M. rheedei* attributes the presence of flavonoid, tannin, glycoside, resin, steroids, terpenoids, cardiac glycosides and triterpenoids etc.The crude methanolic extract of *M. rheedei* inhibits the enzymes like salivary, amylase and glucosidase and shows potential activities against diabetes mellitus disease with IC_{50} value of 407.56 **µ**g/ml (α-amylase inhibitory activity) and 380.66 **µ**g/ml (α- Glucosidase inhibitory activity) respectively (Table 1 and 2). The present finding reveals that methanolic extract of *M. rheedei* efficiently manage both alpha amylase and alpha-glucosidase enzymes in a concentration dependent manner. Many herbal extracts are used in ayurveda for the treatment of diabetes and have been reported to have antidiabetic activity in the inhibition potential towards alpha amylase and glucosidase activity. Crude methanol extract of *Psidium guajava* leaves[19] and *Caesalpinia digyna* root[20] and the crude ethanolic extract of *C. auriculata* flowers and of *C. angustifolia* whole plant part and leaves[21] have been reported inhibition potential towards *in vitro* antidiabetic assays. Therefore methanol extract of *M. rheedei* is capable of effectively inhibiting the α- amylase and glucosidase activity and the plant based inhibitory potential offers a prospective therapeutic approach for the management diabetes

Figure 1
Figure showing the species Malaxis rheedei SW

Table 1
In vitro antidiabetic activity of M. rheedei methanol extract through inhibition of α-amylase inhibitory activity

Sample concentration (µg/ml)	Inhibition of α-amylase	IC_{50} value (µg/ml)
50	10.82 ± 0.34	
100	23.76 ± 0.56	
150	37.89 ± 0.13	**407.56**
200	49.12 ± 0.58	
250	61.34 ± 0.95	

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

Table 2
In vitro antidiabetic activity of M. rheedei methanol extract through inhibition of α- Glucosidase inhibitory activity

Sample concentration (µg/ml)	Inhibition of α- Glucosidase	IC_{50} value (µg/ml)
50	14.56 ± 0.43	
100	27.69 ± 0.14	
150	40.12 ± 0.86	**380.66**
200	52.54 ± 0.34	
250	67.87 ± 0.62	

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

CONCLUSION

The antidiabetic studies shows that the crude methanolic extract exhibited the most potent α-amylase and α-glucosidase inhibitory activity which is an indication that the solvent is capable of extracting the active constituents in *M. rheedei.* However, these data suggests that the bioactive compounds are responsible for biological activities. Therefore the study suggests the future use of this species in pharmaceutical industries. In addition to these, the phytochemical activities of *M. rheedei* justify the traditional usage of this species for health care system of both tribal as well as local inhabitants in the Western Ghats region.

CONFLICTS OF INTEREST

Conflict of interests declared none.

REFERENCES

1. Mukesh R, Namita P. Medicinal Plants with Antidiabetic Potential - A Review. Am.-Eurasian J. Sustain. Agric. 2013. 13 (1): 81-94.
2. Vaiyapuri Sivajothi, Shruthi Shirur Dakappa. In vitro and in silico antidiabetic activity of pyran ester derivative isolated from *Tragia cannabina*. Asian Pac J Trop Biomed. 2014. 4 (1): 454-459.
3. Malviya E, Jain S and Malviya S. Antidiabetic potential of medicinal plants. Acta Pol. Pharm. 2010. 67 (2): 113-118.
4. Maurya umashanker and Srivastava shruti. Traditional Indian herbal medicine used as antipyretic, antiulcer, anti-diabetic and anticancer: a review, Int. j. res. pharm. Chem. 2011. 1(4).
5. Sindhu. S. Nair, Vaibhavi Kavrekar and Anshu Mishra. In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. Eur J Exp Biol. 2014. 3(1):128-132
6. Sabeeha Shafi, Nahida Tabassum. Survey on Anti-Diabetic Plants in Kashmir [India]. J. Adv.Pharm. Edu. & Res. 2013. 3 (4): 306 - 318.
7. Yufang Meng, Aiqun Hu, Qifei Yi, Fuwu Xing. *Malaxis malipoensis* (Malaxideae: Orchidaceae), a new species from Yunnan, China. J Torrey Bot Soc. 2014. 141(1): 91-94.
8. Ramesh Marasini and Susan Joshi. Antibacterial and Antifungal Activity of Medicinal Orchids Growing in Nepal.J. Nepal Chem. 2012. 29:104-109.
9. Abishkar Subedi1, Bimal Kunwar, Young Choi, Yuntao Dai, Tinde van Andel, RamP Chaudhary, Hugo J de Boer, and Barbara Gravendeel,. Collection and trade of wild-harvested orchids in Nepal. J Ethnobiol Ethnomed. 2013. 9 (1):1-10.
10. Renjini Haridas, Sangeeth Thekkan and Manorama S. Determination of total antioxidant activity of *Malaxis rheedei* Sw (Orchidaceae). J Pharmacogn Phytochem. 2016. 5 (4): 132-135.
11. Rajiv K. Vashistha, Ajay Sing, Vikas Jaidon, Parun Saxena. Astavarga plants- threatened medicinal herbs of the North-West Himalaya. Int. J. Med. 2012. 2(4):661-676.
12. Lalit Giri, Indra D. Bhatt, Ranbeer S. Rawal. Popularization of 'Ashtavarga' plants for conservation and sustainable utilization. Curr. Sci. 2015. 108 (7):1197-1198.
13. Sahaya Shibu, Chitra Devi, Sarmad Moin, Servin Wesley. Evaluation of bioactive potential of *Coelogyne nervosa* a.rich. - An endemic medicinal orchid of Western Ghats, India. Asian J Pharm Clin Res. 2013. 6(1): 114-118.
14. Renjini Haridas, Manorama S and Binu Thomas.Phytochemical characterization of potential bioactive compounds from medicinal orchid: *Malaxis rheedei* sw. IJPSR. 2016. 6(2): 53-56.
15. Renjini Haridas, Thangapandian V and Binu Thomas. Ethnomedicinal knowledge of tribe-*Kattunayakans* in Nilambur forests of Malappuram district, Kerala, India. *IJPR.* 2015. 5(2):76-85.
16. Worthington, V. Alpha amylase. In: Worthington Enzyme Manual. Worthington Biochemical Corp. Freehold. *NJ.* 1993. 36-41.
17. Apostolidis, E, Y.I.I. Kwon, K. Shetty.Potential of cranberry-based herbal synergies for diabetes and hypertension management. Asia Pac J Clin Nutr. 2006. 15: 433-441.
18. Vasundhara C.C.S and Gayathri Devi S. *In vitro* antidiabetic activity of leaves and seeds of *Boerhavia diffusa. JPBR*, 2014, 2 (2): 184-189.
19. Manikandan. R., Vijaya Anand A., and Durai Muthumani G. Phytochemical and in vitro anti-diabetic activity of methanolic extract of *Psidium guajava* leaves. Int.J.Curr.Microbiol.App.Sci. 2013. 2(2): 15-19.
20. M. B. Narkhede, P. V. Ajimire, A. E. Wagh, Manoj Mohan and AT Shivashanmugam. *In vitro* antidiabetic activity of *Caesalpina digyna* (R.) methanol root extract. Asian J. Plant Sci. Res. 2011. 1(2): 101-106.
21. Shravan Kumar Nanumala, Tulasi P and Errabelli Sujitha. *In vitro* anti-diabetic activity of seed extracts of *Cassia auriculata* and *Cassia angustifolia*. Euro. J. Exp. Bio. 2015. 5(5):12-17

Reviewers of this article

DR. R. MURUGESWARAN

Research officer (Botany) and HOD,
Survey of Medicinal Plants Unit,
Regional Research Institute of Unani Medicine, Royapuram,
Chennai - 600013, India.

G. Bakhya Shree M.S. (Research)

Coordinator and Trainer, Department of Biotechnology and Life Sciences, Dexter Academy, Madurai, Tamilnadu

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript