Evaluation of Antidiabetic Potential of Andrographis Paniculata

Suparna Deepak
Biotechnology Department, Mahatma Education Society's Pillai College of Arts, Commerce and Science New Panvel, Maharashtra, India

Abstract: **Diabetes mellitus is a serious health problem with continuously increasing rates of incidence and mortality. Many medicinal plants have been used for the treatment of diabetes mellitus in Indian system of medicine and in other ancient systems of the world. *Andrographis paniculata Nees*, a multipurpose tropical plant is believed to have many medicinal properties. The purpose of this study was to evaluate the *in vitro* antidiabetic activity of the Hexane, DCM and Methanol extracts of *Andrographis paniculata Nees*. The extracts were analyzed for antidiabetic potential using a systemic study platform which includes *in vitro* assays namely alpha glucosidase inhibitory activity, alpha amylase inhibitory activity and Glucose uptake by 3T3L1 (mouse fibroblast). All the three extracts showed good dose dependent α-glucosidase and α-amylase inhibitory activity but methanolic extract gave better results than the other extracts. All the three extracts could induce glucose uptake in the 3T3-L1 cells in the absence of insulin.**

Keywords: Medicinal Plants, α-glucosidase, α-amylase, glucose uptake

I. INTRODUCTION

In Ayurvedic Medicine, there are numerous herbs which have been used historically for treating a large variety of ailments. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.*Andrographis paniculata Nees*. is a plant that has been effectively used in traditional Asian medicines for centuries.The plant belongs to the family of Acanthaceae. The common name is Kalmegh or bhuminim. It is an annual, branched, erect- running ½ to 1 meter in height.*A. paniculata* (AP)has been reported as having antibacterial, antifungal, antiviral, choleretic, hypoglycemic hypocholesterolemic, and adaptogenic effects.[1]Andrographolide is a major bioactive phytoconstituent found in various parts of *A. paniculata* but particularly in the leaves[2,3].

Diabetes is an important human ailment afflicting many from various walks of life in different countries. Diabetes mellitus is a complex and a diverse group of disorders that disturbs the metabolism of carbohydrate, fat and protein.The world health organization has estimated a total of 171 million of people with diabetes mellitus from the global population, and this report projected to increase to 366 million by 2030[4].Allopathic drugs used for the treatment of diabetes have their own side effect & adverse effect. like hypoglycaemia, nausea, vomiting, hyponatremia, flatulence, diarrhoea or constipation, alcohol flush, headache, weight gain, lactic acidosis, pernicious anaemia, dyspepsia, dizziness, joint pain[5]. So instead of allopathic drugs, herbal drugs are a great choice which is having more or less no side effect & adverse effects[6].

The lack of perfect models for type II diabetes, coupled with financial restrictions on obtaining and maintaining animals, and social restrictions on extensive use of animals in experimentation, indicate that a more practical approach would involve a series of *in vitro* prescreens before testing a potential new hypoglycaemic agent in animals.

Advantages of *in vitro* models include: 1) Rapid large-scale screening of drug candidates allows promising substances to be identified at an early stage of the drug development process; 2) The time and costs involved in developing active agents are significantly reduced and 3) Cost-intensive and ethically controversial animal experiments can be reduced to a minimum.

The objective of the present study was to evaluate the *in vitro* antihypoglycemic activity of the Hexane, DCM and Methanol extracts of *Andrographis paniculata Nees*.

II. MATERIALS AND METHODS

2.1 Sample Collection and Extraction

Dried aerial parts of *A. paniculata* was obtained from APMC market, Vashi.They were powdered using a grinder, sieved and used for further analysis. The plant material was subjected to Soxhlet extraction using three different solvent successively in order of polarity i.e. from non-polar to polar. The solvents used were hexane, dichloromethane and methanol.

2.2In vitro Antihypoglycemic Assay

A) Alpha glucosidase inhibitory activity

The inhibition of α-glucosidase activity was determined using the modified published method[7]. The yeast alpha glucosidase was dissolved in 100 mM phosphate buffer pH 6.8 and was used as the enzyme extract. P-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Plant extracts were used in the concentration ranging from 2 - 64 mg/ml. Different concentrations of plant extracts were mixed with 320 µl of 100 mM phosphate buffer pH 6.8 at 30 °C for 5 minutes. 2 ml of 50 mM sodium carbonate was added to the mixture to terminate the reaction. α-glucosidase activity was determined spectrophotometrically at 410 nm on spectrophotometer UV-Vis (Shimadzu 265) by measuring the quantity of p-nitrophenol released from p-NPG.The control samples were prepared without any plant extracts. Different concentrations ranging from 0.1 – 10 mg/ml of acarbose was used as positive control. The % inhibition was calculated according to the formula:

% Inhibition = (1–B/A) × 100,
where A is the absorbance of control and
B is the absorbance of samples containing extracts.

The IC 50 values were determined from plots of percent inhibition versus log inhibitor concentration.

B) Alpha amylase inhibitory activity

The assay was carried out following the standard protocol using the 3,5-dinitrosalicylic acid (DNSA) method[8].
A volume of 200 µl of α-amylase solution (2 units/ml) was mixed with 200 µl of the differentconcentrations of extractsranging from 2 - 64 mg/mland was incubated for 10 min at 30 °C. Thereafter 200 µl of the starch solution (0.5% in water (w/v)) was added to each tube and incubated for 3 min. The reaction was terminated by the addition of 200 µl DNSA reagent and was boiled for 10 min in a water bath at 85–90 °C. The absorbance of resulting supernatant was measured at 540 nm using spectrophotometer UV-Vis (Shimadzu 265).Different concentrations ranging from 0.1 – 10 mg/ml of acarbose was used as positive control.
Percent α-amylase inhibition was calculated as follows:

% inhibition = **(1–B/A) × 100**,
where A is the absorbance of control and
B is the absorbance of samples containing extracts.

2.3 Cytotoxicity Assay

The extract of *A.paniculata* leaf was tested for *in vitro* cytotoxicity, using 3T3L1 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The 3T3-L1 adipocytes were treated with concentration of 6.5 to 200 µg/ml of extracts. Herbal extract treated cells were incubated for a day at 37°C in humidified 5% CO_2atmosphere. The adherent cells were washed two times with Phosphate buffered saline (PBS). Then, 20 µl of MTT stock solution (5 mg/ml) was added to each well and the plates were further incubated for 4 hours at 37 °C. Then, 100 µl of DMSO was added to each well. Cells were left for one hour and the absorbance was measured at a wavelength of 570 nm and reference wavelength 630 nm with a micro plate reader (Biotek, USA).

2.4 Glucose Uptake Assay by 3T3L1 Cells

The Inhibition of Glucose Uptake by the extracts were studied in 3T3L1 adipocytes[9]. 3T3L1 cells were seeded (2 × 105 cells/mL) in 6-well plates and treated with Oleic Acid (2 mM) for 24 h. After incubation, cells were incubated in glucose- and serum-free DMEM for another 2 h. Different plant extracts (50 µg/ml) and 7 mM glucose were treated in 3T3L1 cells for 24 h. The supernatant of each group was collected and the glucose level was measured using a *using Abcam* Glucose Uptake Assay Colorimetric Kit (ab 136955). The 2-DG6P is oxidized to generate NADPH, which can be determined by an enzymatic recycling amplification reaction. All the Assays were performed in three replicates and then the measurements were averaged.

III. RESULTS AND DISCUSSION

3.1 Inhibitory activity of different AP extracts against α-glucosidase

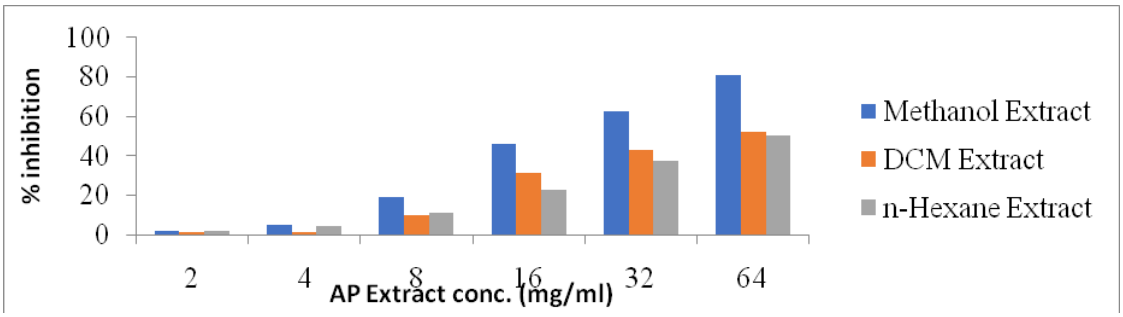

Figure 1. The α-glucosidase inhibitory activity different extracts of *A. paniculata*.
Fig 1 shows that all the extracts of *A. paniculata* showed a dose dependent inhibitory action against α-glucosidase. Negligible inhibition was observed at 2 to 4 mg/ml concentrations of all the three extracts but a considerable increase was observed at 16 to 64 mg/ml concentrations. Methanolic extract showed the best inhibitory effect.

3.2 Inhibitory activity of different AP extracts against α-amylase

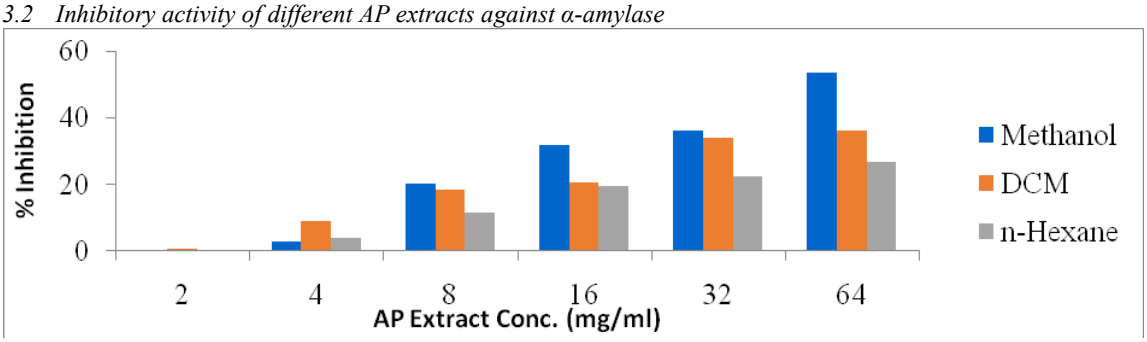

Figure 2. The α-amylase inhibitory activity different extracts of *A. paniculata*.
Fig 2 shows the effect of different extracts of AP on α-amylase which was also found to be dose dependent for all the three extracts. Similar to the results of α-glucosidase inhibitory activity, Methanolic extract showed the best inhibitory effect on α-amylase. At lower concentrations DCM and methanolic extracts showed a similar activity but at higher concentration, the methanolic extract showed a greater inhibitory activity.

Inhibitory activity of different concentration of Acarbose against α-glucosidase and α-amylase

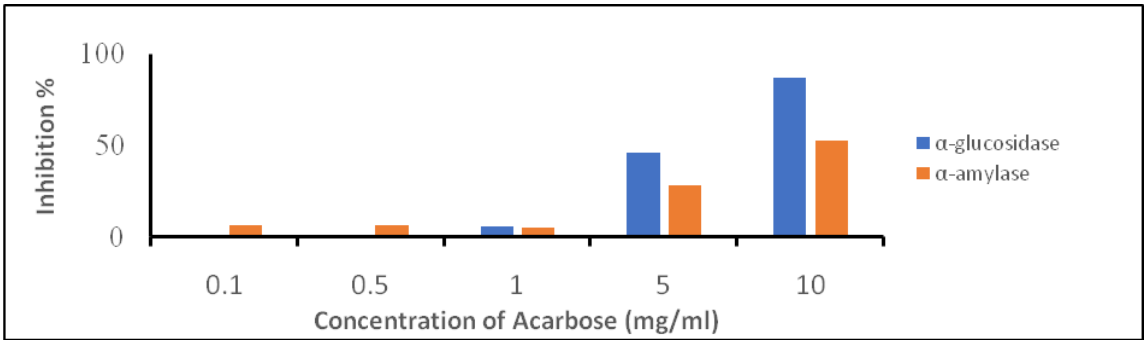

Figure 3. The α-glucosidase inhibitory and α-amylase inhibitory activity different extracts of Acarbose.
Fig 3 shows the effect of acarbose, which was used as the positive control, on α-glucosidase and α-amylase. Acarbose also showed very less inhibition of both the enzymes at lower concentrations but showed good inhibition at 5mg/ml and 10 mg/ml concentration. Highest inhibition for α-glucosidase was found to be 87% at 10 mg/ml concentration and for α-amylase highest inhibition was 52.6% at the same concentration. Acarbose showed dose dependent inhibition for α-glucosidase. However, against α-amylase, dose dependent inhibition was not observed at lower concentrations.

Analyte	IC_{50}	
	α-glucosidase	α-amylase
Acarbose	5.36 ± 0.28	9.49 ± 0.15
Methanol AP extract	27.97 ± 0.35	55.10 ± 0.26
DCM AP extract	54.12 ± 0.48	105.40 ± 0.73
n-Hexane AP extract	61.32 ± 0.39	153.25 ± 0.53

Table 1. IC_{50} values for *in vitro* α-glucosidase and α-amylase inhibition by acarbose and different AP extracts

All the analyses were done in triplicates. At least four serially diluted solutions of each analyte were taken for the calculation of the IC_{50} values.

3.3 Cytotoxicity Assay

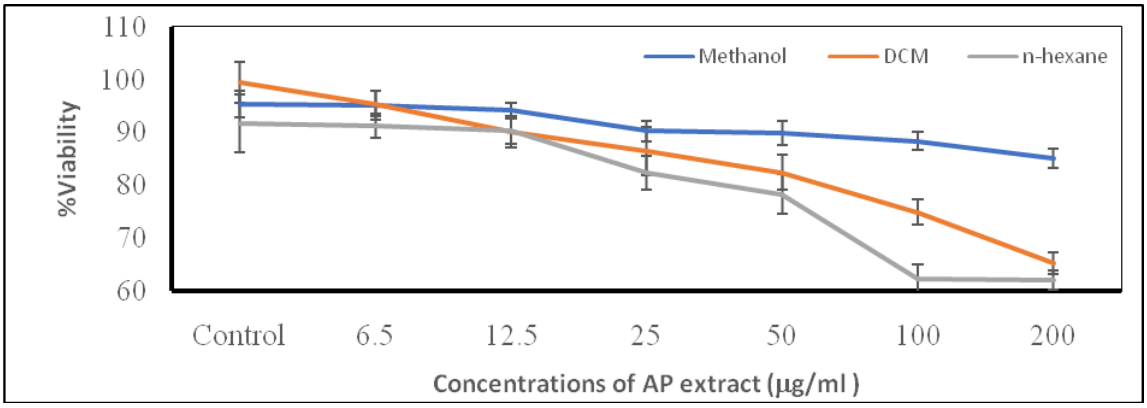

Figure 4: The cell viability effect of AP extracts on 3T3L1 cells.

Fig 4 shows the effect of the crude extracts on the cell viability of the 3T3-L1 cell lines. The methanolic extract did not show a considerable decrease in the viability till 200 µg/ml. DCM extract showed a dose dependent decrease in the viability. N-hexane extract did not show a decrease in the viability at low concentrations but above 25 µg/ml concentration, the viability decreased as the concentration increased.

3.4 Glucose Uptake Assay by 3T3L1 Cells

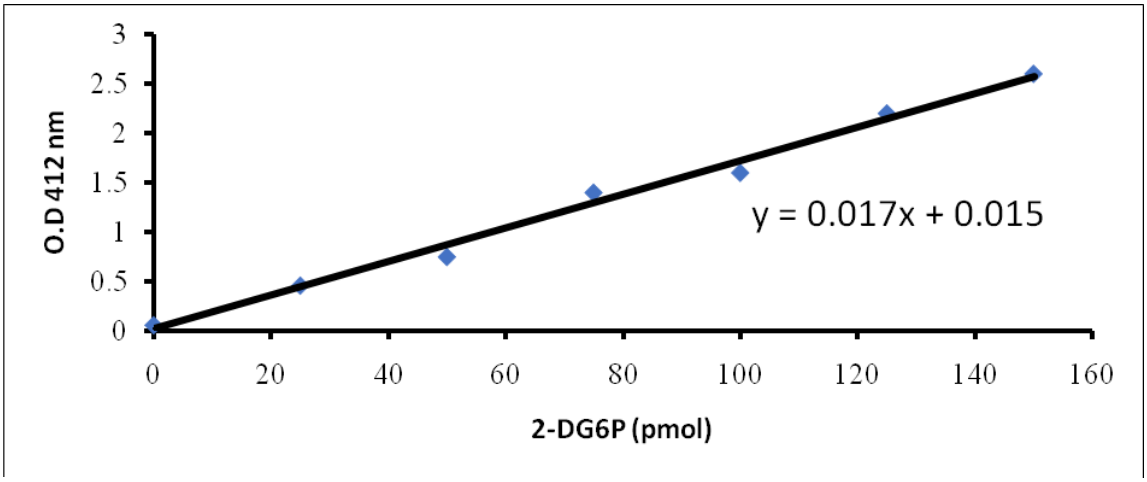

Figure 5: 2-DG2P standard Graph

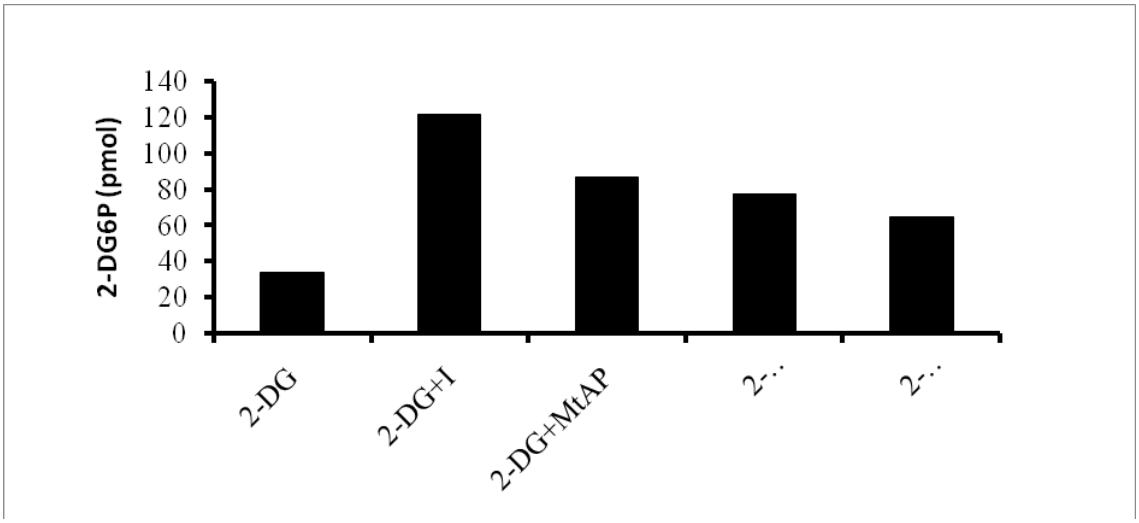

Figure 6: 2-DG Uptake in 3T3-L1 Cell Lines

Fig 5 depicts the standard graph obtained for 2-DG2P *using Abcam* Glucose Uptake Assay Colorimetric Kit. This graph was used to determine the glucose uptake by 3T3-L1 cell lines in the presence of the AP extracts which is shown in Fig 6. All the three extracts were able to increase the glucose uptake in the cells as compared with the control. However, when compared with insulin, the glucose uptake in presence of the AP extract was low but comparable.

Plants which in phytochemicals like flavonoids, tannins alkaloids, phenolic compounds have various therapeutic applications. These bioactive compounds could be isolated and further standardized to be used in treatment of many diseases without any side effects which are observed with various synthetic drugs. Our previous study[10], it was reported that the phytochemical screening of AP extracts prepared in methanol, DCM and n-hexaneusing HPTLC showed the presence of flavonoids in higher amounts besides other phytochemicals like alkaloids and tannins. These phytochemicals were responsible for good radical scavenging, antibacterial and antifungal activities shown by these extracts.

In the present study, we have tried to study the activity of these three extracts on *in vitro*α-glucosidase and α-amylase activities. α-amylase and α-glucosidase are the enzymes responsible for digestion of carbohydrates and increasing the postprandial glucose levels in diabetic patients. Inhibiting their activity could help in controlling postprandial hyperglycemia[11]. The effect of AP extracts was compared with the commercial available acarbose which is an α-glucosidase and α-amylase inhibitor but have gastrointestinal side effects[12].It was found that all the three extracts could inhibit both enzymes in a dose dependent manner. However, all the three extracts showed a higher α-glucosidase inhibitory activity as compared to inhibitingα-amylase activity. Moreover, as depicted in Table No.1 summarising the IC_{50}values of all the analytes, methanol extract was found to be a strong inhibitor of enzymes withIC_{50} being 27.97 mg/ml and 55.1 mg/ml for the α-glucosidase and α-amylase respectively. Although acarbose appears to be more effective inhibitor in comparison with these extracts, further purification of the extract may give better inhibition of enzymes.

The glucose uptake by the adipose tissue stimulated by insulin is responsible for decreasing the postprandial blood glucose levels, thus critical in managing diabetes mellitus[13]. For studying the effect of crude extracts, 3T3-L1 cells act as an excellent system. The cell cytotoxicity assay show that the AP extracts didn't have considerable effect on the viability of the cells at lower concentrations of all extracts. However, the cell viability was reported to decline with increase in the concentration of DCM and n-hexane extracts. In the current study, it was demonstrated that the AP extracts could significantly increase glucose uptake in the 3T3-L1 cells. The effect was comparable to the effect of insulin. As stated earlier, further purification of the extracts could enhance the insulin like activity also.

IV. CONCLUSION

In this study,*in vitro* studies were carried out to evaluate the antidiabetic potential of different extracts of *Andrographis paniculata*. It was found that methanolic extract gave the better enzyme inhibitory and glucose uptake activity as compared to DCM and n-hexane extracts.

REFERENCES

[1] Agbonlahor O, Joyce E F, Osayemwenre E, Vincent I,Abiodun F and Peter L. Harnessing the medicinal properties of *Andrographis paniculata*for diseases and beyond: a review of its phytochemistry and pharmacology.Asian Pac J Trop Dis. 2014 Jun; 4(3): 213–222.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4032030/

[2] Thanasekaran J, Cheng-Ying H, Jie-Jen L, and Joen-Rong S. Experimental and Clinical Pharmacology ofAndrographis paniculataand Its Major BioactivePhytoconstituent Andrographolide. Evidence-Based Complementary and Alternative Medicine. 2013, 846740https://www.hindawi.com/journals/ecam/2013/846740/

[3] Md. Sanower H, Zannat U, Abubakar S, and K. M. Hafizur R. Andrographis paniculata (Burm. f.) Wall. ex Nees: A Review of Ethnobotany, Phytochemistry, and Pharmacology. Scientific World Journal Volume 2014, Article ID 274905, 1 -28.

[4] Sarah W, Gojka R, Anders G, Richard S and Hilary K. Global Prevalence of Diabetes. Diabetes Care. 2004; 27: 1047-1053.https://www.who.int/diabetes/facts/en/diabcare0504.pdf

[5] Tabish, S A. Is Diabetes Becoming the biggest Epidemic of the twenty-first Century? International Journal of Health Sciences. 2007, 1 (2) 2, 5–8.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3068646/

[6] Manikandan R, Vijaya A A and Durai M G. Phytochemical and in-vitro anti-diabetic activity of methanolic extract of Psidium guajava leaves. Int.J.Curr. Microbiol. App. Sci., 2 (2), 15-19.https://pdfs.semanticscholar.org/c340/aef22828e79cf43477eeadf42c5a19032022.pdf

[7] Sindhu. S. N, Vaibhavi K and Anshu M. In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts European Journal of Experimental Biology, 2013, 3(1):128-132 http://www.imedpub.com/articles/in-vitro-studies-on-alpha-amylase-and-alpha-glucosidase-inhibitory-activities-of-selected-plant-extracts.pdf

[8] M. N Wickramaratne, J. C. Punchihewa, and D. B. M. Wickramaratne. In-vitro alpha amylase inhibitory activity of the leaf extracts of Adenanthera pavonina BMC Complement Altern Med. 2016; 16: 466.https://www.ncbi.nlm.nih.gov/pubmed/27846876

[9] Kyung-A H, Yu-Jin H, Ga Ram K and Jeong-Sook C. Extracts from Aralia elata (Miq) Seem alleviate hepatosteatosis via improving hepatic insulin sensitivity. BMC Complementary and Alternative Medicine. 2015. 15:347. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4595215/

[10] Suparna D, Asmita P and Punam S. Study of antioxidant and antimicrobial activities of Andrographis paniculata. Asian J. Plant Sci. Res., 2014, 4(2):31-41.http://www.imedpub.com/articles/study-of-antioxidant-and-antimicrobial-activities-of-andrographis-paniculata.pdf

[11] Poovitha, S., Parani, M. In vitro and in vivo α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). *BMC Complement Altern Med* **16,** 185 (2016). https://doi.org/10.1186/s12906-016-1085-1

[12] Van de Laar FA. α-glucosidase inhibitors in the early treatment of type 2 diabetes. Vasc. Health Risk Manag. 2008; 4:1189–95.https://www.ncbi.nlm.nih.gov/pubmed/19337532

[13] V. S Deepa, K. Rajaram, P. Suresh Kumar. In vitro and in vivo antidiabetic effect of Andrographis lineata Wall. Ex.Nees and Andrographis serphyllifolia Wt.Ic leaf extracts. Afr. J. Pharm. Pharmacol. 2013. Vol. 7(29). 2112-2121 https://www.academicjournals.org/app/webroot/article/article1380729424_Deepa%20et%20al.pdf

[14] Dzatil A Md B, Mohammad J S, Siti H S,Md. Mukhlesur R, and Siti Z M So'ad. α-Glucosidase Inhibitory Activity of Selected Malaysian Plants. J Pharm Bioallied Sci. 2017 Jul-Sep; 9(3): 164–170.

[15] Nuraniza A, Md Muzaffar A K K, Md Taher and Solachuddin J A I. Herbal extracts exhibit anti-diabetic activities in 3T3-L1 adipocytes model. Progress in Nutrition .2015; Vol. 17, N. 4: 301-310.10.4103/jpbs.JPBS_35_17

[16] Iniyan G. Tamil, B. Dineshkumar, M. Nandhakumar, M. Senthilkumar, and A. Mitra In vitro study on α-amylase inhibitory activity of an Indian medicinal plant, Phyllanthus amarus. Indian J Pharmacol. 2010; 42(5): 280–282.http://www.ijp-online.com/article.asp?issn=0253-7613;year=2010;volume=42;issue=5;spage=280;epage=282;aulast=Tamil

[17] Li H, Wu G, Fang Q, Zhang M, Hui X, Sheng B, Wu L, Bao Y, Li P, Xu A, Jia W. Fibroblast growth factor 21 increases insulin sensitivity through specific expansion of subcutaneous fat.Nat Commun. 2018; 9: 272. https://www.ncbi.nlm.nih.gov/pubmed/29348470

[18] Rammohan S, M. Zaini A and Amirin S. In vitro α-glucosidase and α-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide.2008 Acta Biochimica Polonica, Vol 55. 2, 391-398.http://www.actabp.pl/pdf/2_2008/391.pdf