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IN-VITRO STUDY OF A- GLUCOSIDASE INHIBITION ACTIVITY OF ALSTONIA SCHOLARIS AND CATHARANTHUS ROSEUS

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ABSTRACT

The increasing population of Diabetes Mellitus (DM) in the world has always been concerned by physicians and scientists due to its life-threatening effects including heart diseases and premature death. Out of them, nearly 90-95% population is having type 2 diabetes mellitus (T2DM), characterised by insulin resistance. The available treatments for T2DM includes inhibition of α- glucosidase present on the brush border of human intestine that controls post-prandial hyperglycaemia by slower the releasing of glucose in the blood. Adverse effects of the available α- glucosidase inhibitors (AGI) such as abdominal discomfort and hypoglycaemia has prompted a search for the newer AGIs from natural compounds. The impact of various plant extracts on the management of medical disorders has recently aroused curiosity of researchers. Alstonia scholaris has set its hight reputation in ethnopharmacological world for treating major health issues including skin carcinoma, lung cancer and also T2DM. Though some research supports the antidiabetic activity of the plant, the mode of action responsible for it has yet to be discovered. At the same time, Catharanthus roseus has also been proved to be effective in diabetic conditions but in vitro study of antidiabetic activity of the plant is yet to be encountered. The presented study investigated different extracts of A. scholaris and C. roseus on α- glucosidase isolated from Saccharomyces cerevisiae. The presented research not only gives in-vitro proves of the plants as AGIs but also open ups new pathways in understanding the secondary metabolites as inhibitors.

Keywords: Type 2 diabetes mellitus, α- glucosidase inhibitors, Alstonia scholaris, Catharanthus roseus, Plant extracts

Abbreviations

DM, Diabetes Mellitus; T2DM, type 2 diabetes mellitus; AGI, α- glucosidase inhibitors; BCG, Bromocresol green; pNPG, pera-nitrophenyl α-D-glucopyranoside; PNP, p-nitrophenol; AM, A. scholaris methanolic extract; AP, A. scholaris petroleum ether extract; AC, A. scholaris chloroform extract; CM, C. roseus methanolic extract; CP, C. roseus petroleum ether extract; CC, C. roseus chloroform extract.

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1. INTRODUCTION

The prevalence of chronic diseases is increasing rapidly which eventually shows hazardous effects on human health and life expectancy. Diabetes Mellitus (DM) is one of the most common chronic diseases which has affected 451 million people (from age 18-99 years) in 2017 and a recent study estimated the number to be increased by 693 million by 2045 around the world (Cho et al. 2018). There are mainly two types of diabetes (type 1 and 2) out of which, type 2 diabetes mellitus (T2DM) affects 90-95% diabetic population that is characterised by insulin resistance leading to hyperglycaemia which chronically causes life threatening health issues like retinopathy, coronary and peripheral vascular diseases, neuropathic complications, renal failure and premature death (Sharma et al. 2020). The treatment of T2DM includes medications like sulphonylurea or insulin, combined with healthy diet, meditation and exercise. Inhibition of α- glucosidase enzyme present on the brush border of human intestine is considered as one of the major alternative approaches for diabetes management (Jong-Anurakkun, Bhandari, and Kawabata 2007).

Currently available α- glucosidase inhibitors (AGI) like acarbose, miglitol and voglibose are competing inhibitors of the enzyme that delays the digestion of oligosaccharides leads to controlled post-prandial hyperglycaemia (Martin and Montgomery 1996). The AGIs are also known for its side effects including diarrhoea, abdominal discomfort and weight gaining (Chougale et al. 2009; Coniff et al. 1995). Additionally, at maximum cost, AGIs also show hypoglycaemic effects that makes diabetes management even more unpleasant (Bayraktar, Van Thiel, and Adalar 1996; Mangiagli et al. 2002). Research has been going on worldwide to get newer AGIs, especially from natural compounds like plant secondary metabolites such as flavonoids, alkaloids and other phenols that are more effective and having less side effects (Reza et al. 2020).

Alstonia scholaris, also known as Devil's tree or Saptaparni (in Sanskrit) is an evergreen buttressed tree that has been used in Ayurveda, Unani as well modern medical practices to treat many medical conditions like chronic diarrhoea, dysentery, malaria, cough and cold, headache, menstrual disorders and asthma (Kala 2005; Khan and Singh 1970; Khyade, Kasote, and Vaikos 2014; Sajem, Rout, and Nath 2008; Shanmugam, Rajendran, and Annadurai 2011). Different extracts of its leaves and bark have been identified to be anti-cancerous against lung cancer, skin carcinoma; anti-inflammatory, anti-diabetic as well as effective against cataract (Arulmozhi et al. 2010; Jagetia and Baliga 2006; Jahan, Chaudhary, and Goyal 2009; Soni, Choudhary, and Bodakhe 2019; Sultana, Qazi, and Kamal 2019; Wang et al. 2017). While, Catharanthus roseus, commonly known as bright eyes or old maid is a flowering plant of Apocynaceae family that is ethnopharmacologically used in cancer management and psoriasis treatment (Omara et al. 2020; Rai, Tandon, and Khatoon 2014). Vindolin, isolated from C. roseus shows antihyperlipidemic effect on diabetic mice (Oguntibeju, Aboua, and Goboza 2019). Even though the plant is known for its ethnopharmacological values, very few studies have been conducted to explore its effect on various diseases.

Though, A. scholaris and C. roseus have been proven to be effective in some health conditions, in vitro study of antidiabetic activity of the plants has not been encountered significantly. In this study, different extracts of plant leaves have been encountered for its α- glucosidase inhibition activity. To the best of our knowledge, α-glucosidase inhibition by C. roseus is being documented for the first time that open ups newer pathway of research in its chemical compound study.

2. MATERIAL AND METHODS:

2.1 Collection of plant and preparation of plant extracts:

Fresh leaves of Alstonia scholaris and Catharanthus roseus were collected from St. Xavier's college campus, Ahmedabad, Gujarat, India. Leaves were washed with distilled water and allowed to shade dry completely. Following methods were performed to get different extracts of the leaves. To get the methanolic extract, powdered leaves (10gms) of each plant were extracted in 1L methanol on rotary shaker for 72 hrs. On the other hand, chloroform extract was made by 10gms of powdered leaves, extracted in 1L chloroform in Soxhlet apparatus for 6 hrs (or 4 cycles). Both the extracts were filtered by Whatman filter paper 1 followed by rotary evaporator drying method. Finally, small aliquots of the extracts were dissolved in methanol for further studies.

2.2 Phytochemical assay:

Total flavonoid content present in each extract was estimated by aluminium chloride method using quercetin (150µg/ml methanol) as standard flavonoid (Woisky and Salatino 1998). In brief, different aliquots of quercetin (50-250µl) or 100µl of sample extracts were made up to 1 ml using methanol followed by addition of 2 ml of 2% aluminium chloride. The tubes were incubated for 10 minutes at room temperature and read at 415 nm. Sample control for each extract was made without addition of aluminium chloride to nullify the color of extracts. The Blank tube was having no sample or standard to nullify colour of methanol and aluminium chloride. Amount of flavonoid for each extract was calculated with the help of standard curve of quercetin concentration.

Total alkaloid content present in extracts of each plant was estimated by Bromocresol green (BCG) colorimetric assay using Berberian (5mg/ml) as standard alkaloid (Ajanal, Gundkalle, and Nayak 2012). Briefly, different aliquots (100-500µl) of Berberian or 200µl of sample extract (dissolved in methanol) were washed 3

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times by chloroform. The pH of solution was adjusted neutral using 0.1N sodium hydroxide. The solution was mixed with 5 ml BCG and 5 ml of sodium phosphate buffer (pH 4.7) and transferred to separatory funnel. The colored solution was extracted by mixing the solution vigorously with 4 ml of chloroform and allowed to be settled down. Chloroform layer was taken out in test tube and volume was made up to 5 ml using chloroform. The solution was mixed well and read at 470 nm. Blank was not having sample or standard to nullify the coloured extracted from only BCG and sodium phosphate buffer solution. Amount of alkaloid in sample extract was estimated by standard curve of Berberian concentration.

2.3 Purification of α- glucosidase from Saccharomyces cerevisiae:

Purification of α- glucosidase from Saccharomyces cerevisiae (baker's yeast) was carried out by Halvorson et al., protocol with appropriate changes (Halvorson 1957). The method includes culturing of yeast in a broth containing 2.5gm peptone, 1.5gm yeast extract, 1gm ammonium sulphate, 1.25gm potassium dihydro phosphate, 0.125gm calcium chloride, 0.125 magnesium sulphate and 20gm maltose for 500ml of total volume. 5% of total volume of broth was inoculated by yeast cells and was kept at 37ºc on shaker for 14 hours subsequently addition of 3% maltose to induce the enzyme as α- glucosidase is an inducible enzyme. It was incubated for 4 hours at 37ºc on shaker and the cells were pallet down by centrifuging at 7000rpm for 10 minutes at 4º C.

Ethyl acetate was added that is $1/10^{th\ volume}$ of total weight of the pellet and incubated for 30 minutes with vigorous shaking at room temperature. Addition of distilled water (150ml/100gm pellet) was followed by neutral pH adjustment using ammonium water. The mixture was incubated for 15hrs at room temperature and centrifuged at 6000 rpm for 10 minutes at 4º C. Cell free solution was kept at -20º C overnight (crude extract).

The crude extract was slowly thawed at 4º C and centrifuged in falcon tube at 6000 rpm, 4º C for 10 minutes. The supernatant was dialysed in 0.1 M potassium phosphate buffer (pH 7.0) at 4º C for 24 hours. Dialysed extract was centrifuged in 4º C at 6000 rpm for 10 minutes and supernatant (enzyme extract) was collected for further examinations.

2.4 Calculation of α- glucosidase specific activity:

A reaction system containing enzyme extract (150µl) and 5mM, 50µl synthesised specific substrate pNPG (para-nitrophenyl α-D-glucopyranoside), was made up to 500µl by potassium phosphate buffer (pH 6.8) and incubated at 37º C for 15 minutes. Addition of 2 ml sodium carbonate gives coloured reaction which was read at 400nm (Ur. Rehman et al. 2018).

To calculate the specific activity (in mM/mg/hr), protein estimation of enzyme extract by Folin Lawry method and standard curve of p-nitrophenol (PNP) were performed as PNP is the final product released by enzyme after acting on the substrate.

2.5 Inhibitory action of plant extracts on α- glucosidase:

To check the inhibition of each plant extract on partially purified enzyme, estimation of enzyme activity with pNPG in the presence of plant extract was carried out. In brief, different aliquots of each plant extract (substituted in methanol) was mixed with 100µl enzyme extract to which 100µl of pNPG (5mM) was added. The mixture was incubated at 37º C for 15 minutes. Sodium carbonate (2ml) inhibited the enzyme, giving colored reaction that was read at 400nm. Acarbose was standard inhibitor used in this method while control tubes to nullify the reading of only methanol as well as plant extracts were also made. Percentage of inhibition by each plant extract of both the plants were calculated by below equation,

$$\%\text{Inhibition} = \frac{\text{Abs [control]} - \text{Abs [sample]}}{\text{Abs [control]}}$$

The results were compared with acarbose inhibition to give concrete data to the inhibitory action of each plant extract.

3. RESULT AND DISCUSSION:

3.1 Phytochemical analysis of extracts:

Quantitative analysis of flavonoid and alkaloid contents in different extract of both the plants were performed using appropriate methods.

3.1.1 Estimation of total flavonoid and alkaloid content:

Ammonium chloride method was performed to estimate total flavonoids in each extract of plants using quercetin as standard flavonoid. Figure 1 shows standard curve of quercetin concentration which was used to calculate flavonoids in sample extracts.

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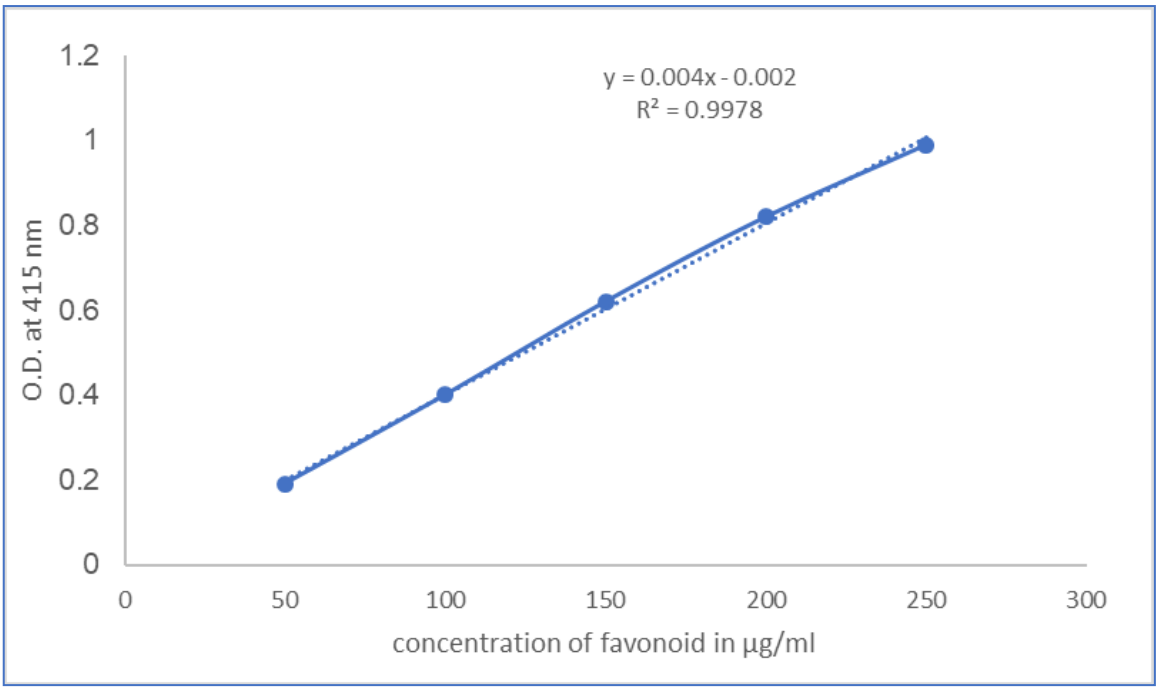

Figure 1 Standard curve of flavonoid concentration in quercetin

Concentration of flavonoid in different extracts of the plant were calculated using the regression formula (y = mx + c) obtained from the graph.

On the other hand, quantitative assay for total alkaloid content of plant extracts was carried out using Bromocresol green (BCG) colorimetric method considering standard curve of Berberian concentration shown in figure 2.

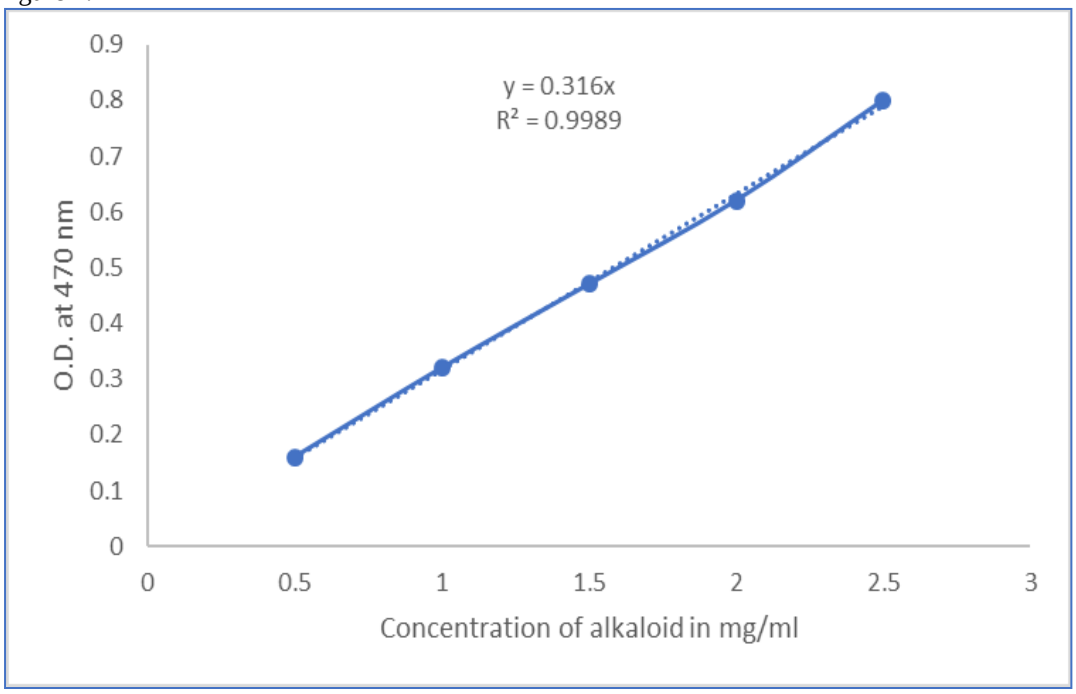

Figure 2 standard curve of concentration of Berberian in mg/ml

Total alkaloid concentration in plant extracts was estimated by similar equation method (using regression formula) as followed in flavonoid estimation.

Final concentration of total alkaloids and flavonoids in plant extracts estimated in performed phytochemical analysis has been listed in table 1 which suggests methanol as favourable solvent for extraction of

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flavonoid and alkaloid followed by chloroform and petroleum ether. Conclusively it can be dictated that dry leaves of Catharanthus roseus has higher phytochemical content than that of Alstonia scholaris.

Name of plant	Solvent used for extraction	Concentration of flavonoid (gm/gm of dry leaves)	Concentration of alkaloid (gm/gm of dry leaves)
Alstonia scholaris	Methanol	3.55	0.079
	Chloroform	1.56	0.075
	Petroleum ether	0.04	0.023
Catharanthus roseus	Methanol	5.50	0.167
	Chloroform	1.42	0.113
	Petroleum ether	0.08	0.061

3.2 Estimation of specific activity from enzyme extract:

Total protein estimation by Folin Lawry of the enzyme extract was performed that resulted in having

Table 1 Concentration of flavonoid and alkaloid (gm/gm of dry leaves) in different solvents

0.198 mg/ml total protein. The enzyme activity of α- glucosidase was performed as a colorimetric assay that evaluate the release of PNP as product. Figure 3 shows standard curve of PNP by colorimetric estimation of the same.

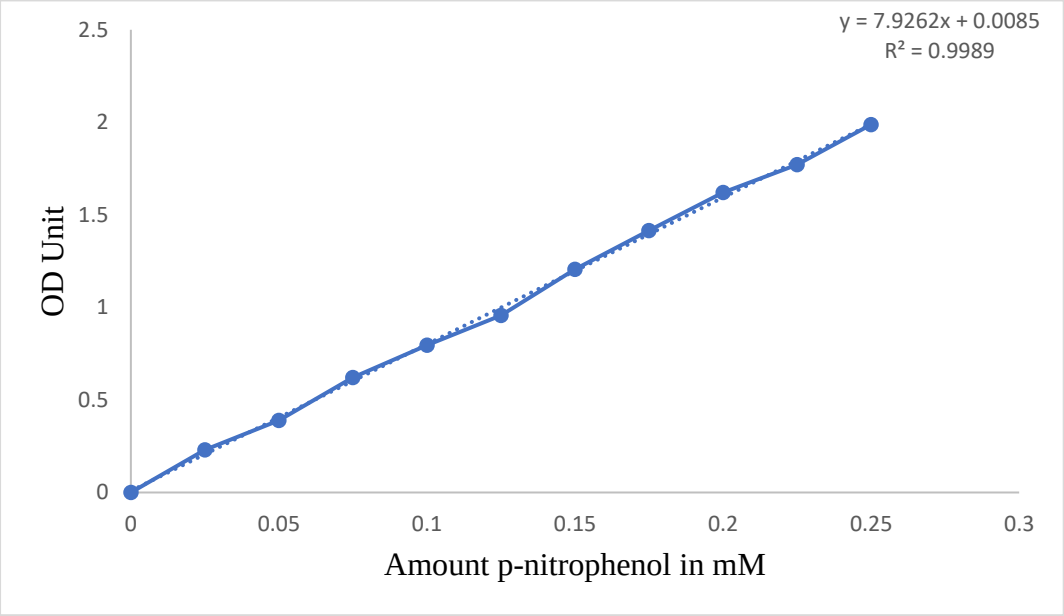

Figure 3 standard curve of p-nitrophenol (PNP)

Production of PNP by enzyme extract (mM/ml) was calculated by regression formula (y = mx + c) obtained from PNP standard curve. Specific activity of enzyme extract, considering production of PNP and amount of protein was 0.404 mM/mg/hr.

3.3 Inhibitory action of plant extracts:

Results of inhibitory action of methanolic, chloroform and petroleum ether extracts of Alstonia scholaris and Catharanthus roseus are shown in Table 2.

Name of plant	Name of solvent	%Inhibition of α- glucosidase
Alstonia scholaris	Methanol (AM)	74
	Chloroform (AC)	74

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	Petroleum ether (AP)	45
Catharanthus roseus	Methanol (CM)	36
	Chloroform (CC)	61
	Petroleum ether (CP)	17

Table 2 %inhibition of different plant extract on α- glucosidase

(A. scholaris methanolic extract-AM, A. scholaris petroleum ether extract- AP, A. scholaris chloroform extract-AC; C. roseus methanolic extract- CM, C. roseus petroleum ether extract- CP, C. roseus chloroform extract- CC) Methanolic and chloroform extract of Alstonia scholaris leaves gave maximum inhibition (74%) followed by petroleum ether extract (45%). In case of Catharanthus roseus, chloroform extract showed higher inhibition of α-glucosidase (61%) followed by methanol (36%) and petroleum ether extract (17%).

4. CONCLUSION

Inhibition of α- glucosidase, especially using natural compounds is definitely one of the newest approaches of T2DM management to control post prandial hyperglycaemia (He et al. 2022). Despite the fact that C. roseus has been proven to be antidiabetic by ethnopharmacological and Ayurvedic studies, the mode of action has not been dug deeper (Alkreathy and Ahmad 2020). The presented study proposed the same for C. roseus. At the best of our knowledge, chloroform and petroleum extracts of A. scholaris and effect of C. roseus leaf extracts on yeast α- glucosidase is a novel report. From all the examined extracts, AM and AC shows highest inhibition (74%), followed by CC (61%). Other extracts like, AP, CM and CP show less than 50% inhibition of the enzyme. Quantification of alkaloids and flavonoids from the investigated extracts shows higher flavonoid and alkaloid presence in methanolic extracts compared to others. Though quantity of methanol extracted flavonoid in C. roseus is higher than A. scholaris, lower α- glucosidase inhibition activity might light up to the specific flavonoids present in A. scholaris that may be responsible for the specific activity. The fact that CC has a higher inhibitory effect than other C. roseus extracts suggests that some unique secondary metabolites are involved in the activity. Outcomes of the presented investigation offers a new approach for identify the specific secondary metabolites present in the plants responsible of the activity. Some in-silico studies of the specific metabolites may help understanding their interactions of it with the protein. Overall, the research not only indicates and investigates the plant's method of action on α-glucosidase, but it also paves the way for future research to delve deeper into the subject.

5. REFERENCES

1. Ajanal, Manjunath, MahadevB Gundkalle, and ShraddaU Nayak. 2012. “Estimation of Total Alkaloid in Chitrakadivati by UV-Spectrophotometer.” Ancient Science of Life 31(4):198. doi: 10.4103/0257-7941.107361.
2. Alkreathy, Huda Mohammed, and Aftab Ahmad. 2020. “Catharanthus Roseus Combined with Ursolic Acid Attenuates Streptozotocin-Induced Diabetes through Insulin Secretion and Glycogen Storage.” Oxidative Medicine and Cellular Longevity 2020. doi: 10.1155/2020/8565760.
3. Arulmozhi, Sinnathambi, Papiya Mitra Mazumder, Sathiyanarayanan Lohidasan, and Prasad Thakurdesai. 2010. “Antidiabetic and Antihyperlipidemic Activity of Leaves of Alstonia Scholaris Linn. R.Br.” European Journal of Integrative Medicine 2(1):23–32. doi: 10.1016/j.eujim.2009.12.001.
4. Bayraktar, Miyase, David H. Van Thiel, and Nezaket Adalar. 1996. “A Comparison of Acarbose versus Metformin as an Adjuvant Therapy in Sulfonylurea-Treated NIDDMI Patients.” Diabetes Care 19(3):252–54. doi: 10.2337/diacare.19.3.252.
5. Cho, N. H., J. E. Shaw, S. Karuranga, Y. Huang, J. D. da Rocha Fernandes, A. W. Ohlrogge, and B. Malanda. 2018. “IDF Diabetes Atlas: Global Estimates of Diabetes Prevalence for 2017 and Projections for 2045.” Diabetes Research and Clinical Practice 138:271–81. doi: 10.1016/j.diabres.2018.02.023.
6. Chougale, Ashok D., Varsha A. Ghadyale, Shrimant N. Panaskar, and Akalpita U. Arvindekar. 2009. “Alpha Glucosidase Inhibition by Stem Extract of Tinospora Cordifolia.” Journal of Enzyme Inhibition and Medicinal Chemistry 24(4):998–1001. doi: 10.1080/14756360802565346.
7. Coniff, Robert F., Jo Ann Shapiro, Timothy B. Seaton, and George A. Bray. 1995. “Multicenter, Placebo-Controlled Trial Comparing Acarbose (BAY g 5421) with Placebo, Tolbutamide, and Tolbutamide-plus-Acarbose in Non-Insulin-Dependent Diabetes Mellitus.” The American Journal of Medicine 98(5):443–51. doi: 10.1016/S0002-9343(99)80343-X.
8. Halvorson. 1957. “Saccharomyces i t a l i c u S.” BIOCHIMICA ET BIOPHYSICA ACTA 3.
9. He, Ming, Yuhan Zhai, Yuqing Zhang, Shuo Xu, Shaoxuan Yu, Yingxin Wei, Haifang Xiao, and Yuanda Song. 2022. “Inhibition of α-Glucosidase by Trilobatin and Its Mechanism: Kinetics, Interaction Mechanism and Molecular Docking.” Food & Function 13(2):857–66. doi: 10.1039/D1FO03636J.

10. Jagetia, Ganesh Chandra, and Manjeshwar Shrinath Baliga. 2006. "Evaluation of Anticancer Activity of the Alkaloid Fraction of Alstonia Scholaris (Sapthaparna) in Vitro and in Vivo." Phytotherapy Research 20(2):103–9. doi: 10.1002/ptr.1810.
11. Jahan, Swafiya, Ranu Chaudhary, and Pradeep Kumar Goyal. 2009. "Anticancer Activity of an Indian Medicinal Plant, Alstonia Scholaris, on Skin Carcinogenesis in Mice." Integrative Cancer Therapies 8(3):273–79. doi: 10.1177/1534735409343590.
12. Jong-Anurakkun, Nilubon, Megh Raj Bhandari, and Jun Kawabata. 2007. "α-Glucosidase Inhibitors from Devil Tree (Alstonia Scholaris)." Food Chemistry 103(4):1319–23. doi: 10.1016/j.foodchem.2006.10.043.
13. Kala, Chandra Parakash. 2005. "Ethnomedicinal Botany of the Apatani in the Eastern Himalayan Region of India." Journal of Ethnobiology and Ethnomedicine 1:1–8. doi: 10.1186/1746-4269-1-11.
14. Khan, J. B., and G. P. Singh. 1970. "Ethno-Medicinal Active Plants for Treating Cold and Cough in the Vicinity of Nahargarh Wildlife Sanctuary, Jaipur, India." Our Nature 8(1):225–30. doi: 10.3126/on.v8i1.4332.
15. Khyade, Mahendra S., Deepak M. Kasote, and Nityanand P. Vaikos. 2014. "Alstonia Scholaris (L.) R. Br. and Alstonia Macrophylla Wall. Ex G. Don: A Comparative Review on Traditional Uses, Phytochemistry and Pharmacology." Journal of Ethnopharmacology 153(1):1–18. doi: 10.1016/j.jep.2014.01.025.
16. Mangiagli, Antonino, Salvatore Italia, Vincenzo De Sanctis, and Saveria Campisi. 2002. "Impaired Glucose Homeostasis in Young Adult Thalassemic Patients: A Pilot Study with Acarbose." Journal of Pediatric Endocrinology and Metabolism 15(2):205–10. doi: 10.1515/JPEM.2002.15.2.205.
17. Martin, Anne E., and Patricia A. Montgomery. 1996. "Acarbose: An a-Glucosidase Inhibitor." 53(X):2277–90.
18. Oguntibeju, Oluwafemi, Yapo Aboua, and Mediline Goboza. 2019. "Vindoline—A Natural Product from Catharanthus Roseus Reduces Hyperlipidemia and Renal Pathophysiology in Experimental Type 2 Diabetes." Biomedicines 7(3):59. doi: 10.3390/biomedicines7030059.
19. Omara, Timothy, Ambrose K. Kiprop, Rose C. Ramkat, Jackson Cherutoi, Sarah Kagoya, Decrah Moraa Nyangena, Tsedey Azeze Tebo, Papias Nteziyaremye, Lucy Nyambura Karanja, Abigael Jepchirchir, Alfayo Maiyo, Betty Jematia Kiptui, Immaculate Mbabazi, Caroline Kiwanuka Nakiguli, Brenda Victoria Nakabuye, and Margaret Chepkemoi Koske. 2020. "Medicinal Plants Used in Traditional Management of Cancer in Uganda: A Review of Ethnobotanical Surveys, Phytochemistry, and Anticancer Studies." Evidence-Based Complementary and Alternative Medicine 2020. doi: 10.1155/2020/3529081.
20. Rai, Vartika, Pramod Kumar Tandon, and Sayyada Khatoon. 2014. "Effect of Chromium on Antioxidant Potential of Catharanthus Roseus Varieties and Production of Their Anticancer Alkaloids: Vincristine and Vinblastine." BioMed Research International 2014. doi: 10.1155/2014/934182.
21. Reza, Md Sharif, Md Sadikur Rahman Shuvo, Md Mahadi Hassan, Mohammad Anwarul Basher, Md Amirul Islam, Nura Ershad Naznin, Sarah Jafrin, Khondoker Shahin Ahmed, Hemayet Hossain, and A. F. M. Shahi. Ud Daula. 2020. "Antidiabetic and Hepatoprotective Potential of Whole Plant Extract and Isolated Compounds of Aeginetia Indica." Biomedicine and Pharmacotherapy 132:110942. doi: 10.1016/j.biopha.2020.110942.
22. Sajem, Albert L., Jayashree Rout, and Minaram Nath. 2008. "Traditional Tribal Knowledge and Status of Some Rare and Endemic Medicinal Plants of North Cachar Hills District of Assam, Northeast India." Ethnobotanical Leaflets 12:261–75.
23. Shanmugam, S., K. Rajendran, and M. Annadurai. 2011. "Ethnomedicinal Plants Used to Cure Diarrhoea and Dysentery in Pachalur Hills of Dindigul District in Tamil Nadu, Southern India." Journal of Applied Pharmaceutical Science 1(8):94–97.
24. Sharma, Priyanka, Tushar Joshi, Tanuja Joshi, Subhash Chandra, and Sushma Tamta. 2020. "In Silico Screening of Potential Antidiabetic Phytochemicals from Phyllanthus Emblica against Therapeutic Targets of Type 2 Diabetes." Journal of Ethnopharmacology 248:112268. doi: 10.1016/j.jep.2019.112268.
25. Soni, Pranay, Rajesh Choudhary, and Surendra H. Bodakhe. 2019. "Effects of a Novel Isoflavonoid from the Stem Bark of Alstonia Scholaris against Fructose-Induced Experimental Cataract." Journal of Integrative Medicine 17(5):374–82. doi: 10.1016/j.joim.2019.06.002.
26. Sultana, Nighat, Muhammad Saleem Qazi, and Mustafa Kamal. 2019. "New Anti-Inflammatory Triterpene Esters and Glycosides from Alstonia Scholaris." Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry 19(4):370–86. doi: 10.2174/1871523018666190724122406.
27. Ur. Rehman, Najeeb, Ajmal Khan, Ahmed Al-Harrasi, Hidayat Hussain, Abdul Wadood, Muhammad Riaz, and Zahra Al-Abri. 2018. "New α-Glucosidase Inhibitors from the Resins of Boswellia Species with Structure–Glucosidase Activity and Molecular Docking Studies." Bioorganic Chemistry 79(April):27–33. doi: 10.1016/j.bioorg.2018.04.020.
28. Wang, Chao Min, Kuei Lin Yeh, Shang Jie Tsai, Yun Lian Jhan, and Chang Hung Chou. 2017. "Anti-Proliferative Activity of Triterpenoids and Sterols Isolated from Alstonia Scholaris against Non-Small-Cell Lung Carcinoma Cells." Molecules 22(12):1–13. doi: 10.3390/molecules22122119.

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29. Woisky, Ricardo G., and Antonio Salatino. 1998. "Analysis of Propolis: Some Parameters and Procedures for Chemical Quality Control." Journal of Apicultural Research 37(2):99–105. doi: 10.1080/00218839.1998.11100961.