Original Research Article

A Mapping of Diabetes Amelioration Potency of Catharanthus roseus in Alloxan Induced Wistar Albino rat, Under the Light of Geographical Variation Based Contrast.

Abstract

Context: There are many medicinal plants that are utilized in traditional remedies to treat diabetes because diabetes mellitus (DM) is a significant metabolic condition. One of these is the pharmacological herb *Catharanthus roseus*. In the herbal medicine system, the plant has been used to treat diabetes mellitus since ancient times.

Objective: In order to employ the plant material to treat diabetes, the current study set out to investigate the therapeutic effect of leaf extract from *Catharanthus roseus* as well as its safety profile.

Materials and Methods: The *Catharanthus roseus* extract was steeped in ethanol. Diabetes-induced rats with alloxan monohydrate were each given an ethanol extract of C. rosea. In pilot research, metformin plant extract was used in place of the widely prescribed anti-diabetic medication metformin, and it was found to be effective at lowering blood sugar levels while not causing hypoglycemia.

Results: Alloxan monohydrate was injected intraperitoneally into rats to cause diabetes at a dose of 150 mg/kg body weight, and the animals were also given ethanolic extracts of *Catharanthus roseus* leaves at doses of 500, 750, and 1100 mg/kg. Throughout the course of the trial, we checked the blood glucose levels of diabetic and control rats. After testing blood glucose levels, it was shown that all doses of *Catharanthus roseus* reduced the rats' unusually increased blood glucose levels. However, even 750 mg/kg significantly lowers blood sugar levels ($p < 0.05$). In our investigation, group 5 (alloxan + CR. Low Land) outperformed the other extract-treated groups. This shows that variations in geographic location may have an impact on plant ingredients and their bioactivity.

Conclusion: According to the study's findings, metformin and a leaf extract of *Catharanthus roseus* both relieved the pathological condition brought on by diabetes. It clearly shows that the *Catharanthus roseus* leaf extract has significant therapeutic potential for the treatment of diabetes.

Keywords: *Catharanthus roseus,* diabetes mellitus, alloxan, herbal medicine, geographic location

1. Introduction

Diabetes mellitus (DM), commonly referred to as just diabetes, is a set of metabolic illnesses characterized by persistently elevated blood sugar levels. The symptoms of this high blood sugar include frequent urination, increased thirst, and increased appetite. Diabetes can lead to a lot of complications if it is not treated. [1]**.** In 2021, 537 million adults between the ages of 20 and 79 were living with diabetes, according to the tenth edition of the International Diabetes Federation (IDF) Diabetes Atlas, [2].Numerous pharmaceutical drugs have been developed as a result of modern medical advancements. These medications exhibit hypoglycemic effects, however, they are frequently accompanied by several side effects, including fatigue, upset stomach, diarrhea, as well as nephrological disorders [3].

There are currently two classes of injections and six primary classes of contemporary medications that are used to manage blood glucose levels globally. But the majority of contemporary medications include a variety of unwanted side effects, which can lead to serious health issues when using the medication. [4-6].

A plant's chemical composition may be affected by a number of variables, such as the type of plant present, the soil's composition, the climate, and agricultural practices. In essence, geographical variation influences the composition of soil contents, which in turn affects the quality of phytochemical components in plants. We will learn from our study that geographic variation may have an impact on either the established constituent of *Catharanthus roseus* or the novel constituent of *Catharanthus roseus* that exhibits antidiabetic action. It will be regionally dependent if there is any evidence of a change in the qualities of the *Catharanthus roseus* constituents; otherwise, it will be geographically independent [7-9].

Numerous native people still practice traditional medicine (TM) all around the world. *Catharanthus roseus* is also referred to as bright eyes, Cape periwinkle, graveyard plant, old maid, pink periwinkle, and rose periwinkle. [10-12]**.**

Catharanthus roseus includes **anti-oxidant, anti-diabetic, antimicrobial, anticancer, antiulcer, antidiarrheal, hypotensive property, hypolipidimic property, and it also shows enhancement activity** [12].

Medicinal plants have long been employed as therapeutic remedies in less developed nations with unstable medical care and high prescription costs. In actuality, medicinal plants are significant producers of biologically active substances and are thought to be attractive candidates for drug discovery. Given the high cost of medications and their side effects, as well as the widespread use of medicinal plants with supposedly effective antidiabetic properties [13]. Apparently, we can conclude that the use of medicinal plants (vinca rosea L.) is safer than synthetic drugs. Further vigorous studies involving *Catharanthus roseus* are needed for the control and treatment of diabetes mellitus because here we are just conducting a preclinical trial of this medicinal plant. This study will discuss the antidiabetic activity of *Catharanthus roseus* as well as how variations in geography affect the amount of the constituent in this plant.

2. Materials and Methods

2.1. Drugs, Chemicals, and Instruments

Sigma Aldrich in Germany was used to purchase the ethanol and the alloxan. We received a free sample of the common diabetes medication metformin from Healthcare Pharmaceutical Limited. Plasmatic Laboratory Product Ltd. in the UK was used to purchase the blood serum analysis kits for total cholesterol, HDL, LDL, triglycerides, SGOT, SGPT, and creatinine. The glucometer, Alere GI of Alere Inc., USA, was purchased from Shahbag in Dhaka, Bangladesh, and the Humalyzer 3000 (a semiautomated clinical chemistry analyzer) was utilized to evaluate the biochemical parameters. [improve upon the English highlighted in red to covey the message you want to iterate]

2.2. Plant Collection and Extract Preparation

The leaves of *C. roseus* were gathered in three distinct regions of Bangladesh: North Bengal; a hill track area; and a low land area. The next step involved authentication and taxonomic identification. According to their regulations, the plant specimen was stored at Bangladesh's National Herbarium.

Before being finely ground, the leaves were dried in the shade for seven to ten days. The powdered leaves were violently agitated while steeping in 70% ethanol for 96 hours. After it had finished soaking, the extract was filtered, and the filtered liquid was collected. A rotating evaporator was used to concentrate the extracted solution once it had been moved there. The dried extract was then carefully gathered and kept for further use.

2.3. Experimental Animal Handling

Adult, healthy male Wistar rats weighing between 125 and 200 g were obtained from the Jahangirnagar University Zoology Department in Dhaka, Bangladesh, and housed at the Institute of Nutrition and Food Science, University of Dhaka, where they were maintained in a 12 hour dark/light cycle at a constant temperature of 25 °C. Regular supplies of standard pellet food and clean water were given. Before the inquiry began, the rats were housed there to acclimate. The Institutional Animal Ethics Committee's regulations were followed during all experiments with rats (IEAC). Animals were handled and managed in accordance with the standards set forth by the Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (SCNAT).

2.4. Experimental Guidelines

All experiments were conducted in conformity with the 2013 Declaration of Helsinki's ethical guidelines. The "3R" rules, a cornerstone of Swiss and global regulations governing the exploitation of animals for experimental purposes, were strictly followed during the whole

course of this research. The word "replacement" is represented by the initial "R," which includes both absolute replacements (such as the replacement of animal models with computer-generated models) and relative replacements (replacing live animals by cell or tissue cultures, or replacing vertebrates with invertebrates). Our investigation began with an in silico analysis to ensure that it complied with the idea of "replacement." But this model was unable to produce enough information. An animal model was used in order to do additional research. Rats were used as the test animals because mammalian vertebrates, as opposed to invertebrates, have specific pancreatic and beta cells for antidiabetic potential research. The second "R" stands for "reduction," which refers to any strategy that would result in using fewer animals to collect enough data to address the study questions or in optimizing the information obtained from each animal. Ten rats per group were collected for this study on the basis of the "power analysis method" estimate of the sample size, which was used to ensure compliance with this recommendation. The third "R" stands for "refinement," which suggests reducing the amount of suffering caused to the experimental animals by relieving their misery. In order to make the operation more bearable and decrease the pain from pinching, the tail tips of rats were massaged with isopropyl alcohol before and after each measurement of blood glucose levels. The rats received enough nutrition throughout the whole study, and they were painlessly put to death at the conclusion of the research in accordance with the 2013 revision of the Guidelines for the Euthanasia of Animals.

2.5. Dose Selection

Before the actual study began, a pilot analysis was conducted with the extracts of the same plants which were collected from three different geographical areas of Bangladesh. According to the results of this preliminary study, the plant extract (*C. roseus*)) started to have a pharmacological effect at a dose of 500 mg/kg, indicating that a higher dose would result in a higher MEC (minimum effective concentration). This impact was seen to steadily increase as the dose was raised. When the dose was eventually increased from 1,100 mg/kg to 1,200 mg/kg, no discernible change in the pharmacological action was found. This demonstrated that at a dose of 1,100 mg/kg, the receptors connected to the plant's pharmacological effect started to become saturated.

Since the minimum and maximum effective doses of the plant extract were found to be 500 mg/kg and 1100 mg/kg respectively, we have selected a medium dose for the final study, which was 750 mg/kg. The doses of Metformin (a common medication) were selected in the same manner.

2.6. Biological Sample Collection

For the purpose of measuring blood glucose levels, blood samples were taken by puncturing the tip of the rat's tail. Contrarily, blood was drawn from the sacrificed animal right away following a heart puncture and transferred to a microcentrifuge tube. The supernatant fluid was obtained by centrifuging the collected samples at 5,000 rpm for 5 minutes. After that, this fluid was transferred to another microcentrifuge tube in order to perform biochemical

tests.

After sacrifice, the kidney and liver were immediately dissected from the animal body and carefully cleansed in ice-cold saline for kidney and liver function tests.

2.7. Experimental Design

Rats were weighed individually, divided into groups based on body weight, and then analyzed for anti-hyperglycemic activity (Table 1). The rodents were distributed evenly among the groups according to body weight, with ten rats in each group.

The alloxan control group is represented in Table 1 as rats who only received alloxan treatment. N/A means that the rats in this group did not receive any therapeutic treatment.

Table 1

Anti-hyperglycemic activity analysis.

Group number	**Group status**	**Treatment specimen**	**Dose of treatment specimen (mg/kg)**	**Group abbreviation**
1	Control	Physiological saline	10 mL/kg	C
2	Alloxan control	Alloxan	150 mg/kg	A
3	Alloxan + metformin	Alloxan + metformin	150 mg/kg + 250 mg/60 kg	*A* + *M*250
4	Alloxan + *C. roseus*	Alloxan +*C. roseus*	150 mg/kg + 750 mg/kg	*A* + *CR-Hill Tract*
5	Alloxan + *C. roseus*	Alloxan + *C. roseus*	150 mg/kg + 750 mg/kg	*A* + *CR-Low Land*
6	Alloxan + *C. roseus*	Alloxan + *C. roseus*	150 mg/kg + 750 mg/kg	*A* + *CR-North Bengal*
7	Metformin	Metformin	250 mg/60 kg	*M* 250
8	*C. roseus*	*C. roseus*	750 mg/kg	*CR-Hill Tract*

9	*C. roseus*	*C. roseus*	750 mg/kg	*CR-Low Land*
10	*C. roseus*	*C. roseus*	750 mg/kg	*CR-North Bengal*

2.8. Evaluation of Antidiabetic Properties

The rat model of diabetes was made diabetic using alloxan. Alloxan was initially dissolved in a cold citrate buffer (0.1 M; pH = 4.5). Then, using intraperitoneal methods, this alloxan was administered to the rats at a dose of 150 mg/kg body weight. Following alloxan administration, blood glucose levels were measured four times daily at six-hour intervals to check for hyperglycemia. Within 72 hours after starting alloxan therapy, it was discovered that all of the rats had an average blood glucose level greater than 15 mmol/L, which amply demonstrated their hyperglycemic or diabetic conditions. Metformin and a *C. roseus* extract were administered orally to rats.

2.9. Estimation of Biochemical Parameters

A glucometer was used to measure the blood glucose level. In addition to using Humaluzer 3000, lipid profile, kidney, and liver functioning tests were carried out. Additionally, analyses of the kidney and liver samples' gluconeogenic and glycolytic enzyme activity were conducted.

2.10. Statistical Analysis

The mean and standard deviation (SD) of each study parameter is shown for each group. The "one-way ANOVA test" was used to evaluate the differences between the groups in terms of several biological factors to assess the statistical significance of intergroup heterogeneity. The analysis made use of the "SPSS 16" program. When the "p" value was less than 0.05 (p 0.05), the result was regarded as statistically significant, and when it was less than 0.01 (p 0.01), it was regarded as extremely significant.

3. Result and Discussion

The call of nature has led to an increase in body weight in both the negative control and plant extract-treated groups of our experimental rats. Alloxan had a devastating effect and greatly decreased body weight; however, because of the antioxidant characteristics of the plant (*C.*

roseus), the alloxan-induced treatment group did not exhibit the same level of weight loss. On the other hand, as indicated in Table 2, metformin contributed to a further loss of body weight in Group 3.

To determine how the extract affected blood glucose levels, 100 rats were used and divided into 10 groups. Before administering alloxan to the rats in groups 2–6, blood glucose levels were first measured. Diabetes had been induced after three days. They remained untreated for 14 days. Then, on day 14, the therapy started. The medications and dosages were administered to the rats in groups 3-6 until day 42. The medications were also given to the rats in groups 7 through 10, but alloxan was not administered to these groups. They received treatment for 14 to 42 days. Furthermore, rats in group 1 received regular food and water.

One of the most frequently used substances to cause diabetes in laboratory rodents is alloxan. Our study revealed that the group 1 rats' blood glucose levels were normal, as seen in Figure 1. However, the blood glucose level in the diabetic-controlled group was higher than in all other groups due to blood glucose level in the diabetic-controlled group, however, was higher than in all other groups as a result of beta cell degeneration and an untreated condition. The elevated blood glucose levels decreased in a similar pattern but not to the same degree in all the metformin and extract-treated groups except for group 4. Among the extract-treated groups, group 5 (*C. roseas* collected from low land) showed better results in lowering blood glucose levels, indicating geographical variation in plants is an important factor that cannot be ignored. However, despite being marginally higher than that of the extract-treated group, the metformin-treated group's reduction in blood glucose did not reach statistical significance.

Given that there is no statistically significant difference in the test results between the Metformin group and the extract-treated groups, it is also possible to speculate that further modifying and isolating the therapeutic compound of C. roseus may give us a better result than metformin. However, whereas the plant extract was administered at a dose of 750 mg/kg body weight and contains a number of different compounds, metformin is a single API that was given in doses of 250 mg/kg body weight. As a result, its antidiabetic effects will naturally be less than those of metformin.

As indicated in Table 3, the treatment group's creatinine and urea levels were found to be lower during the kidney function test compared to the alloxan control group. With a creatinine level of 0.93± 0.15, group 5 (Alloxan + CR. Low Land) outperformed the Alloxan Control group. Due to the damaging action of alloxan, the control group had the highest level of creatinine. No significant difference in creatinine deduction was found between the metformin and extract-induced treatment groups.

In the liver functioning test, the serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamate pyruvate (SGPT) levels were also enhanced significantly in alloxan-induced diabetic rats. The SGOT and SGPT levels of treatment groups were lower than all the other groups, including the alloxan control group. In the treatment groups containing the extract,

the protective effect of C. roseus extract declined the liver enzymes. It reduced the abnormally increased level of SGOT and SGPT levels.

The levels of cholesterol, low density lipoprotein (LDL), and triglycerides were lower in the treatment groups than in the alloxan-controlled groups, as indicated in Table 3, because alloxan also raised these markers' levels. According to Table 3, the negative control group's high-density lipoprotein (HDL) level was likewise determined to be the highest, while the alloxan control was the group's lowest. Thus, in both the metformin and plant extract cases, the amount of HDL was raised. All drug-treated groups, particularly group 5 (Alloxan + CR. Low Land), performed marginally better than the diabetic control group in terms of cholesterol, LDL, triglyceride, and HDL levels, but no statistical significance ($P > 0.05$) was discovered when a comparison was made with the healthy control group.

The biological activity or quality of the plant is frequently influenced by a variety of factors, including the location, climate, soil, extraction techniques, etc. that affect a plant's metabolites [14]. In our study, we found that group 5 performed better than the other extract-treated groups (groups 4-6) in the areas of blood glucose-lowering effect, SGOT, SGPT, creatinine, total cholesterol, LDL, triglyceride, and HDL levels. This suggests that differences in geographic location may have an effect on plant constituents and their bioactivity, and previous research has also supported this idea [15, 16].

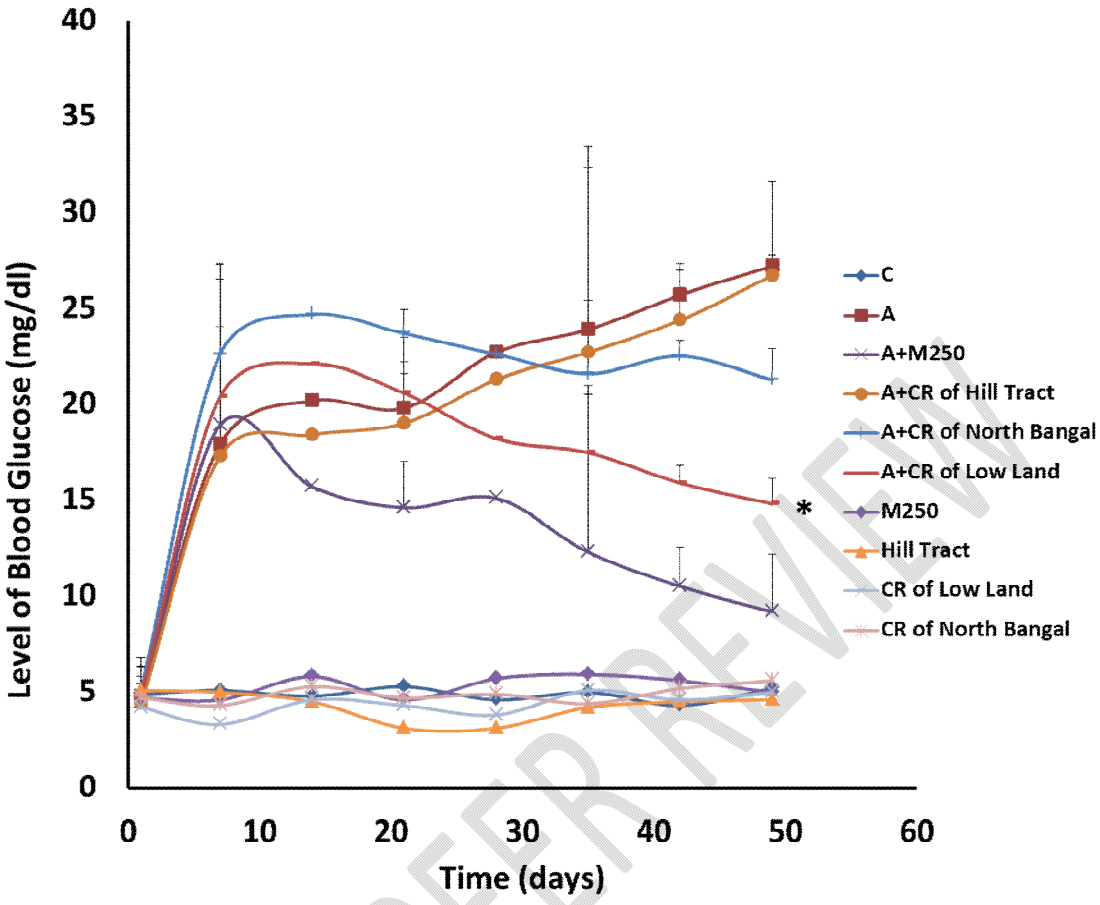

Figure 1

Blood glucose levels of rats in ten groups over the course of treatment. Values were presented as the mean ± SD (n = 10/group). **p < 0.05 and **p < 0.01 indicate significant difference from the disease group (C = control group, A = alloxan-treated group, M = metformin, A + M = alloxan + metformin, A + CR = alloxan +* Catharanthus *roseus, and CR= Catharanthus roseus).*

Table 2

Body weight of rats before the initiation and after the termination of the experiment.

Group number	Group status	Body Weight (gm)	
		Initial	Final
1	Control	135.45± 4.54	162.7± 4.47

2	Alloxan control	141.7± 3.46	124.7±1.85
3	Alloxan + metformin	139.7± 3.78	118.8± 4.58
4	Alloxan + *CR.* Hill Track	137.8± 4.54	125.4± 3.19
5	Alloxan + *CR.* Low Land	135.7± 3.62	132.8± 4.66
6	Alloxan + *CR.* North Bengal	144.7± 7.17	145.8± 6.37
7	Metformin	136.7± 4.05	122.2± 5.08
8	*C. roseus* Hill Track	133.3± 2.34	162.4± 1.06
9	*C. roseus* Low Land	137.7± 3.41	158.4± 3.28
10	*C. roseus* North Bengal	132.4± 2.38	161.1±1.44

Table 3

Effect of C. roseus on the kidney, liver, and lipid function of control and experimental rats.

Group no.	Group status	Kidney Function test	Liver Function test	Lipid Profile Function Test

		Creatinine (mg/dl)	Urea	SGOT (u/l)	SGPT (u/l)	Cholesterol (mg/dl)	Trigliseride (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
1	Control	0.54± 0.11	26.28± 1.49	45.14± 3.27	33.40± 4.57	103.86± 2.12	55.36± 7.03	40.84±1.05	72.18± 3.87
2	Alloxan control	2.87± 0.23	83.74± 7.16	89.06± 6.08	79.9± 4.95	184.34± 4.34	115.20± 5.85	75.62±5.20	39.87±0.91
3	Alloxan + metformin	1.32± 0.24	57.44± 5.92	78.20± 1.86	53.40± 7.33	133.22± 5.10	84.64± 6.72	57.90±3.04	56.66±0.78
4	Alloxan + *CR.* Hill Track	2.29± 0.22	76.12± 3.54	84.75± 3.97	72.10± 5.91	177.04± 17.12	110.38± 6.86	71.48± 6.56	43.14±1.87
5	Alloxan + *CR.* Low Land	0.93± 0.15	61.00± 7.8	74.44± 4.36	60.40± 2.54	99.30± 2.41	71.16± 2.41	48.96± 3.75	66.9± 3.79
6	Alloxan + *CR.* North Bengal	1.88± 0.13	68.81± 13.7	84.08± 3.98	74.80± 2.15	175.16± 2.84	106.26± 2.51	70.27± 3.56	40.28±2.21
7	Metformin	0.71± 0.11	29.62± 3.41	43.38± 4.65	32.40± 3.25	102.24± 2.31	52.94± 4.93	40.42± 2.18	72.32±2.91
8	*C. roseus* Hill Track	0.90± 0.13	27.30± 2.72	46.44± 4.18	33.40± 3.95	100.54± 3.41	57.70± 5.09	42.16± 3.01	72.66±4.92
9	*C. roseus* Low Land	0.59± 0.11	29.48± 4.27	42.78± 3.51	27.80± 2.81	97.90± 4.22	59.84± 3.76	42.58± 3.47	71.94± 1.97
10	*C. roseus* North Bengal	0.63± 0.15	28.60± 1.52	44.24± 8.38	30.80± 2.85	99.02± 3.88	57.84± 7.08	42.82± 1.09	72.08± 1.95

Conclusion

Using our findings as a guide, it is clear that the plant can be quite useful as an anti-diabetic. The location, climate, soil, extraction methods, etc. that alter a plant's metabolites can, however, frequently have an impact on the biological activity or quality of the plant. This shows that the bioactivity of plant components and their relationship to geographic location may vary. Consequently, it can be concluded that more thorough research and the accurate

isolation of anti-diabetic components from extracts can increase the likelihood of *Catharanthus roseus* being included in the diabetes care system.

Conflict of interest statement – include conflict of interest statement.

Acknowledgement – include acknowledgement thanking any individual or group that has helped the authors.

Funding – declare the source of funding for the purchase of all items and the study.

[Write the references properly and consistently to attain publishable standards]

REFERENCES:

1. Shouip, Hossam. (2014). Diabetes mellitus. [provide details of the reference including the link]

2. International Diabetes Federation. IDF Diabetes Atlas, 10th ed. Brussels, Belgium: International Diabetes Federation, 2021. [complete the writing of the reference in full and provide the link please]

3. Bindu Jacob, Narendhirakannan R T. Role of medicinal plants in the management of diabetes mellitus: a review. 3 Biotech. 2019 Jan;9(1):4-include last page of the article] doi: 10.1007/s13205-018-1528-0. Epub 2018 Dec 12. PMID: 30555770; PMCID: PMC6291410.

4. Liyanagamage, D. S. N. K., Jayasinghe, S., Attanayake, A. P., & Karunaratne, V.. Medicinal plants in management of diabetes mellitus: an overview. Ceylon Journal of Science. 2020; 49(1), 03–11. DOI: http://doi.org/10.4038/cjs.v49i1.7700

5. Shukia R, Sharma SB, Puri D, Prabhu KM, Murthy PS. Medicinal plants for treatment of diabetes mellitus. Indian J Clin Biochem. 2000 Aug;15(Suppl 1):169-177. doi: 10.1007/BF02867556. PMID: 23105280; PMCID: PMC3454085.

6. Tran N, Pham B, Le L. Bioactive Compounds in Anti-Diabetic Plants: From Herbal Medicine to Modern Drug Discovery. Biology (Basel). 2020 Aug 28;9(9):252 – include last page of reference]. doi: 10.3390/biology9090252. PMID: 32872226; PMCID: PMC7563488.

7. Radwan H, et al. Complementary and alternative medicine use among patients with type 2 diabetes living in the United Arab Emirates. BMC. Complementary Med. Ther. 2020;20(1):1–12. [7] Radwan H, Hasan H, Hamadeh R, Hashim M, AbdulWahid Z, Hassanzadeh Gerashi M, Al Hilali M, Naja F. Complementary and alternative medicine use among patients with type 2 diabetes living in the United Arab Emirates. BMC Complement Med Ther. 2020 Jul 10;20(1):216 1 - 12. doi: 10.1186/s12906-020-03011-5. PMID: 32650773; PMCID: PMC7350641. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7350641/pdf/12906_2020_Article_3011.pdf

8. Bnouham, Mohamed & Ziyyat, Abderrahim & Mekhfi, Hassane & Tahri, Abdelhafid & Abdelkhaleq, Legssyer. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990-2000). Int J Diabetes & Metabolism. 2006; 14. 1-25. 10.1159/000497588.

9. Aziz, S., Saha, K., Sultana, N., Nur, H. P., Ahsan, M. A., Ahmed, S., Hossain, M. K. Comparative studies of elemental composition in leaves and flowers of Catharanthus roseus growing in Bangladesh. Asian Pacific Journal of Tropical Biomedicine, 2016; 6(1), 50-54. https://doi.org/10.1016/j.apjtb.2015.10.003

10. Marles, R. J, Farnsworth, N. R. Antidiabetic plants and their active constituents. Phytomedicine. 1995; 2: 137–189.

11. "*Catharanthus roseus*". Germplasm Resources Information Network (GRIN). Agricultural Research Service (ARS), United States Department of Agriculture (USDA). Retrieved 4 August 2019. [write the reference properly please]

12. Mishra J N, & Verma, N K. A brief study on Catharanthus Roseus: A review. International Journal of Research in Pharmacy and Pharmaceutical Sciences. 2017 March; 2(2): 20 – 23.

https://www.researchgate.net/publication/319007421_A_brief_study_on_Catharanthus_Roseus_A_review .

13. Gushiken LF, Beserra FP, Rozza AL, Bérgamo PL, Bérgamo DA, Pellizzon CH. Chemical and Biological Aspects of Extracts from Medicinal Plants with Antidiabetic Effects. Rev Diabet Stud. 2016 Summer-Fall;13(2-3):96-112. doi: 10.1900/RDS.2016.13.96. Epub 2016 Aug 10. PMID: 28012277; PMCID: PMC5553761.

14. Xu W, Cheng Y, Guo, Y, Yao, W, Qian H. Effects of geographical location and environmental factors on metabolite content and immune activity of Echinacea purpurea in China based on metabolomics analysis. Industrial Crops and Products, 2022; 189, 115782. https://doi.org/10.1016/j.indcrop.2022.115782.

15. Kumar A, Singhal K, Sharma R, Vyas G, Kumar V. Total Phenolic and Antioxidant Activity of in Different Geographical Locations of Rajasthan Catharanthus Roseus. Asian Journal of Experimental Biological Sciences. 2013; 4. 155-158.

16. Aziz S, Saha, K, Sultana N, Ahmed, Shamim Al-Mansur, Muhammad. Phytochemical and elemental screening on leaves and flowers of Catharanthus roseus: An important medicinal plant of Bangladesh. International Journal of Chemical Sciences. 2014; 12. 1328-1336. [please check and write this reference correctly and be consistent with the reference system]