IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

Preliminary Phytochemical & *In-vitro* Anti-diabetic Activity of Different Extracts of *Plumbago zeylanica* Linn. Root

Praveen Kumar Soni [1 & 2*], Ashish Agrawal [3]

1. PhD Research Scholar, Faculty of Pharmacy, Mandsaur University, Mandsaur
2. School of Pharmacy, Sangam University, Bhilwara, Rajasthan, 311802
3. Faculty of Pharmacy, Mandsaur University, Mandsaur, M.P., 458001

ABSTRACT

AIM- The aim of the present investigation is to study Preliminary Phytochemical & *In-Vitro* Evaluation of Anti-diabetic Activity of Roots of *Plumbago zeylanica*. **MATERIAL & METHODS-** The roots of the selected medicinal plant was procured from the local market ayurvedic shop. The root part of selected plant was dried under shade and processed for the successive solvent extraction method. All the extracts were dried under vacuum and subjected to determination of percentage yield. All the extracts were subjected to preliminary phytochemical screening for the presence of various active Phytoconstituents. Anti-diabetic potential of different extracts were determined by α-amylase and Glucose Diffusion Assay. The dilution of different extracts were prepared in concentration of 10-100µg/ml. **RESULTS-** The crude drug was extracted by different solvents and solvents were selected according to polarity index i.e. solvents with lower polarity to higher polarity. The dried powders of root were extracted by using Petroleum ether, DCM, Ethyl acetate, Methanol and finally water. On the completion of preliminary phytochemical screening, petroleum ether extract showed presence of steroids and fatty acids. The DCM and ethyl acetate extracts showed presence of alkaloids, flavonoids, and terpenoids. The methanolic and water extracts showed the presence of some flavonoids, glycoside compounds. The anti-diabetic activity of DCM and ethyl acetate extracts showed maximum activity in comparison to other extracts. **CONCLUSION-** The phytochemical screening of root part showed the presence of various alkaloids and flavonoids.

KEYWORDS-

In-Vitro Evaluation, Anti-diabetic Activity, Roots, *Plumbago zeylanica*, Plumbagin

INTRODUCTION

Diabetes mellitus is a serious complex multifactorial disorder characterized by hyperglycemia (very high blood glucose level) and glucose intolerance, either due to the relative deficiency in

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

insulin secretion or impaired the effectiveness of insulin's action to enhance glucose uptake. If left untreated, it can lead to severe complications. These complications include hyperlipidemia (abnormal high level of lipid in the blood), oxidative stress, and enzymatic glycation of protein [1]. Considering the fact that diabetes is regarded as a chronic metabolic disease, numerous antidiabetic therapies with conventional drugs are often not a single-dose program as most drugs require frequent injections, sometimes for the entire life of the diabetic patient. However, many of these conventional drugs have been reported for their inefficiency with prominent adverse side effects [2]. These limitations have largely prompted the exploration of management strategies involving the use of medicinal plants reported to be cost effective antidiabetic agents with fewer reported side effects [3]. Traditional use of herbal medicine is the basis and integral part of various cultures, which was developed within an ethnic group before the developed and spread of modern science. Herbal drugs constitute a major part in all the traditional systems of medicine. These have made a great contribution in maintaining human health. A majority of the world's population still rely on herbal medicines to meet its health needs. The practice continues today because of its biomedical benefits and its place in culture beliefs in many part of world. Therefore, different medicinal systems are using the active plant constituents, which discovered as natural hypoglycemic medicine came from the virtue of traditional knowledge. Asia's large population and rapid economic development have made it an epicenter of the epidemic. Asian populations tend to develop diabetes at younger ages and lower BMI levels. Several factors contribute to accelerated diabetic epidemic in Asians, including the "normal-weight metabolically obese" phenotype, high prevalence of smoking and heavy alcohol use; high intake of refined carbohydrates (e.g. white rice) and dramatically decreased physical activity levels [4-6]. The present study was therefore undertaken to investigate the antidiabetic activity and mechanism of action using various *in vitro* models designed to stimulate specific antidiabetic targets of selected medicinal plant.

MATERIALS & METHODS

Collection and authentication of the plant: The *Plumbago zeylanica* (root) were collected from outfield Medicinal garden and procured from local market, India, during the month of July

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

that shows the green colour with rough surface. The plant leaves were washed thoroughly in tap water, dried in shade, finely powdered and used for successive extraction methods. Plants were identified by the senior botanist, Botany department (Scientist C) at Central Council for Research in Ayurveda, Govt. of India and herbarium specimen was submitted in respective department of Pharmacognosy.

Determination of Physicochemical parameters [7,8]

A. Ash Values

Ash values are helpful in determining the quality and purity of crude drug, especially in the powder form. The total ash of a crude drug reflects the care taken in its preparation. The acid insoluble ash is a part of the total ash which is insoluble in dilute hydrochloric acid. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. The procedures given in Indian Pharmacopoeia were used to determine the different ash values.

a. *Determination of total Ash value*

Accurately weight about 3 gm of air dried drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug. The determination of total ash value was calculated by using following formula.

b. *Determination of acid insoluble Ash value*

The ash obtained as directed under total ash was boiled with 25 ml of HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

c. *Determination of water soluble Ash value*

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

The total ash obtain was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water ignited for 15 minutes. The weight of insoluble matter was subtracted from the weight of total ash. The differences in weight represent the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

B. Determination of Loss on Drying

Weight about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100^0c or 105^0c. Cool in a desiccators and watch. The loss in weight is recorded as moisture.

C. Determination of Moisture Content

About 10g of leaves (without preliminary drying), after accurately weighing (weight to within 0.01g) was placed in a tarred evaporation dish. It was then dried at 105^oC for 5 hours and weighed. Drying was continued and the root was weighed at 1 h interval until the difference between two successive weighing corresponded to not more than 0.25 percent. Constant weight was reached when two consecutive weighing after drying for 30min. and cooling for 30min. in a desiccators, did not show more than 0.01g difference.

D. Determination of Swelling Index (WHO, 2000)

Introduce the specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed, into a 25 ml glass stoppered measuring cylinder. Add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour. Allow to stand for 3 hours at room temperature.

Successive extraction methods:

Powdered drug 100gm was weighed and packed in soxhlet. The drug was continuously extracted with petroleum ether for about 72 hours. Complete defatting was ensured by placing a drop form the thimble on a filter paper give any oily spot. The mare was dried in air to remove traces of petroleum ether. Defatted drug was subjected to extraction with dichloromethane in soxhlet apparatus, the extraction was completed in 17-18 hrs. The extract was dried & stored in dark

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

place. Drug was subjected to extraction with ethyl acetate in soxhlet apparatus, the extraction was completed in 17-18 cycles. The extract was dried & stored in dark place. Similarly, the dried extracts were treated by using methanol, butanol and finally water to get their crude extracts [9-11].

Phytochemical Screening

Preliminary phytochemical screening was performed for the presence of various phytoconstituents i.e. alkaloids, flavonoids, phenolic compounds, terpenoids, fatty acids, steroids, sugars etc [11].

***IN VITRO* ANTIDIABETIC EVALUATION**

Ability of the plant materials to retards the movement of glucose from the intestine into the blood was evaluated by physical methods *in vitro*. The following are the convenient models for assessing the materials which affect the absorption of glucose *in vitro*.

Glucose Diffusion Assay [12]

Plant extracts were mixed with glucose and placed in the sealed dialysis membrane and kept in the orbit shaker bath at 37ºC, at 150rpm. The movement of glucose across the membrane into the external solution was measured at periodic intervals using commercial GOD-POD kit.

Requirements

Dialysis membrane

0.15M sodium chloride solution

D- Glucose (25Mm in sodium chloride solution)

Orbit shaker

GOD - POD kit

Dialysis membrane containing 2ml of 25mM glucose solution was mixed with 1ml of different plants extracts and was placed in the centrifuge tube containing 45ml 0.15M NaCl and then kept in orbit shaker bath at 37ºC at 150rpm. The movement of glucose into the external solution was monitored at set of time intervals using GOD- POD kit. Glucose concentration in the external solution was expected as mg/dl/hr.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

Inhibition of Alpha-Amylase Enzyme [13]

A starch solution (1%) was obtained by stirring 0.1g of potato starch in 100ml of 16Mm of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5mg of alpha-amylase in 100ml of distilled water. The colorimetric reagents is prepared by mixing sodium potassium tartarate solution and 3, 5 di-nitro salicylic acid solution 96Mm. Both control and plant extracts (10-100µg/ml) were added with starch solution and left to react with alpha-amylase solution under alkaline conditions at 25ºC. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 di-nitro salicylic acid to 3-amino-5-nitro salicylic acid. This reaction is detectable at 540nm.

RESULTS

Determination of physicochemical parameters

Table No. 1: Physico-chemical parameters of *Plumbago zeylanica* (root)

S. No.	Determination	% ash content
1	Total ash	7.5
2	Acid insoluble ash	2.55
3	Water soluble ash	4.16
4	Loss on Drying	6.94

Determination of Moisture content

The study on selected plant materials showed that the difference of two consecutive weighing after drying for 30min. and cooling for 30min. in a desiccators- 0.05 gm for *Plumbago zeylanica* (root).

Determination of Swelling Index

The Experimental results on selected plant material shows – volume occupied by 1 gm of plant material 2.45 ml respectively for *Plumbago zeylanica* (root).

% Yield Determination and Characteristic views of Extracts

Table No.2: %Yield (w/w) of extracts *Plumbago zeylanica* (root)

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

S No.	Solvent	%Yield (w/w)
1	Petroleum ether	3.59
2	Dichloromethane	4.43
3	Ethyl Acetate	3.55
4	Methanol	15.67
5	Butanolic	7.68
6	Aqueous	8.23

Phytochemical Screening

Phytochemical screening of different extracts showed the presence of different Phytochemical.

Table No. 3: Phytochemical test for different extracts of root of *Plumbago zeylanica* (root)

S.No.	Test	Petroleum ether	Dichloromethane	Ethyl acetate	Methanol	Butanolic	Aqueous
1.	Carbohydrate ➢ Molish test ➢ Felling test	 - -	 - -	 - -	 - -	 + +	 + +
2.	Glycosides ➢ Bronteger test	-	-	+	-	++	+
3.	Alkaloid ➢ Mayer test ➢ Hager test	 - -	 + +	 + +	 - -	 + +	 - -
4.	Phytosterol + Triterpenoids ➢ Salkowaski test	-	+	-	+	+	-
5.	Protein + Amino acid ➢ Biuret test ➢ Ninhydrin test	 - -	 - -	 - -	 - -	 - -	 - -
6.	Phenolic test ➢ Ferric test ➢ Lead acetate test	 - -	 + +	 + +	 + +	 ++ ++	 - -
7.	Flavonoids ➢ Alkaline test	-	-	+	+	+++	+
8.	Saponin ➢ Foam test	-	-	-	-	+	+

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

9	Mucilage ➢ Iodine test ➢ Ethanol test	-	-	-	-	-	+ +

Note: (+) *ve* indicates positive result, whereas (–) *ve* indicates negative result

GLUCOSE DIFFUSION ASSAY

Different extracts the concentration of glucose into the external solution by retarding its diffusion through membrane. After 27 hrs the control showed 316.66mg/dl of glucose in the external solution whereas the ethyl acetate extract after 27 hrs showed 232mg/dl of glucose. Ethyl acetate extract possessed a good effect in glucose diffusion and was showed highest activity.

Table No.4: Mean glucose intensity in the external solution of various extracts at different time intervals

Time in hours	Control (in the absence of extract)	Pet Ether	DCM	Ethyl acetate	Methanol	Butanolic	Water
1h	134.56±1.22	105.34±1.11	94.33±1.56	83.55±2.68	97.33±1.11	92.39±1.11	98.99±1.11
3h	201.31±1.45	184.45±1.45	155.33±2.22	116.31±3.55	156.33±2.34	157.31±2.24	159.78±2.26
5h	244.12±1.33	216.56±1.33	193.62±3.45	157.68±2.34	192.62±3.44	197.67±3.56	191.61±3.78
24h	312.37±2.54	293.11±1.56	256.33±2.24	228.45±2.45	258.33±2.55	259.56±2.11	260.78±2.34
27h	316.33±1.12	303.33±1.27	262.33±3.33	232.55±2.18	260.33±3.11	267.11±3.47	269.66±3.37

Glucose values are expressed as mean ±SEM, -p<0.001- compared to control

INHIBITION OF ALPHA-AMYLASE ENZYME

DCM & Ethyl acetate extract were a dose dependent increase in percentage inhibitory activity against alpha-amylase enzyme. At a concentration of 100 µg/ml of plant extract showed a maximum percentage inhibition.

Table No.5: *In-vitro* anti diabetic activity of root in alpha amylase enzyme inhibition method

S. No	Concentration of sample (µg/ml)	% Inhibition					
		Pet Ether	DCM	Ethyl acetate	Methanol	Butanol	Water

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

1	10	10.02	31.11	33.33	23.22	31.33	30.31
2	20	14.21	36.22	39.67	26.56	36.21	31.53
3	40	18.50	39.45	41.65	28.12	39.45	34.55
4	60	22.19	41.41	48.87	32.78	41.41	39.67
5	80	26.34	51.56	55.77	34.54	46.53	41.11
6	100	30.63	57.11	61.88	36.66	49.44	44.19

DISCUSSION

Diabetes is a chronic metabolic disorder affecting a major proportion of the population worldwide. A sustained reduction in hyperglycemic will decrease the risk of developing micro vascular disease and reduce their complications [14]. The conventional therapies for diabetes have many shortcomings like side effects and high rate of secondary failure. On the other hand herbal extracts are expected to have similar efficacy without side effects as that of conventional drugs. The present investigation reports that antidiabetic effect of *Plumbago zeylanica* Linn. by *In-vitro* models [15]. Medicinal plants could be considered as potential sources for providing a reasonable amount of the required elements other than diet to the patients of diabetes mellitus. Several controlled clinical trials of trace element supplements for glycemic control revealed the beneficial role for supplementation for the control and management of diabetes [16-18].

The present finding reveals that *Plumbago zeylanica* L. efficiently inhibit alpha- amylase enzyme *in vitro* in a dose dependent manner. The DCM & Ethyl acetate extracts from *Plumbago zeylanica L* root showed a dose dependent inhibitory effect of alpha –amylase activity. There was a dose-dependent increase in percentage inhibitory activity against alpha-amylase enzyme. At a concentration of 100µg/ml of plant extract showed a maximum percentage inhibition of 57.11% and 61.88%. The *in vitro* antidiabetic evaluation was assessed by glucose diffusion assay. *Plumbago zeylanica L*. ethanolic extract showed maximum decrease in glucose diffusion when compared to control values. Glucose diffusion is useful *in vitro* index to predict the effect of plant fibers on the delay in glucose absorption in GI tract. In addition to glucose adsorption, the retardation in glucose diffusion might be attributed to the

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

physical obstacle presented by fiber particles towards glucose molecules and entrapment of glucose within the network formed by fibers. The effect of various extracts on glucose diffusion inhibition was depicted. At the end of 27 hrs, glucose movement of control (without plant extract) in the external solution had reached a plateau with a mean glucose concentration above 300mg/dl. It was evident that the ethyl acetate extract were found to be potent inhibitor of glucose diffusion ($p < 0.001$) compared to control. The ethyl acetate extract was found to be more potent than other extracts showing the lowest mean glucose concentration of 232.55±2.18 mg/dl at the end of 27 hrs. When compared with pet ether and DCM extract of *Plumbago zeylanica L.*, methanolic extract showed good activity in alpha-amylase enzyme inhibition and glucose diffusion method. In diabetic blood glucose is not utilized by tissue resulting in hyperglycemia. The fatty acids from adipose tissue are mobilized for energy purpose. The reduction in weight is due to loss in muscle adipose tissue, protein and fatty acids. Studies have also reported significant weight reduction in untreated diabetic rats [19-20].

CONCLUSION

From the present study we conclude that the preliminary phytochemical analysis of *Plumbago zeylanica L.* indicated the presence of flavonoids, Glycosides. In this present study was exhibited significant inhibition activity of DCM & Ethyl acetate root extract of *Plumbago zeylanica* L. by using *in vitro* amylase and glucose diffusion assay, so further the compound isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of antidiabetic agent.

REFERENCES

1. H. M. Al-Hourani and M. F. Atoum, “Body composition, nutrient intake and physical activity patterns in young women during Ramadan,” *SingaporeMedical Journal*, vol. 48, no. 10, pp. 906–910, 2007.
2. P. Gupta, M. Bala, S. Gupta et al., “Efficacy and risk profile of anti-diabetic therapies: Conventional vs traditional drugs—A mechanistic revisit to understand their mode of action,” *Pharmacological Research*, vol. 113, pp. 636–674, 2016.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

3. A.G. Atanasov, B. Waltenberger, and E. M. Pferschy-Wenzig, "Discovery and resupply of pharmacologically active plantderived natural products: a review," *BiotechnologyAdvances*, vol. 33, no. 8, pp. 1582–1614, 2015.
4. M. S. Deutschl¨ander, *Isolation and identification of a novel antidiabetic compound [Ph.D. thesis]*, Department of Plant Science, Faculty of Nature and Agricultural Sciences, University of Pretoria, 2010.
5. B. Van Wyk and P. VanWyk, *Field Guide to Trees of Southern Africa*, Struik Publishers, Cape Town, 1997.
6. P. Coates, *Trees of Southern Africa*, Struik, Cape Town, South Africa, 1984.
7. J. Sagbo, *Phytochemical analysis and antibacterial properties of aqueous and ethanolic extracts of Brachylaena elliptica and Brachylaena ilicifolia. MSc Dissertation [Dissertation, thesis]*, University of Fort Hare, 2015.
8. T.Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *Journal of Immunological Methods*, vol. 65, no. 1-2, pp. 55–63, 1983.
9. M. van de Venter, S. Roux, L. C. Bungu et al., "Antidiabetic screening and scoring of 11 plants traditionally used in South Africa," *Journal of Ethnopharmacology*, vol. 119, no. 1, pp. 81–86, 2008.
10. S.Oyedemi, G. Bradley, andA.Afolayan, "Antidiabetic activities of aqueous stem bark extract of strychnoshenningsii Gilg in streptozotocin-nicotinamide type 2 diabetic rats," *Iranian Journal of Pharmaceutical Research*, vol. 11, no. 1, pp. 221–228, 2012.
11. D. Janjic and C. B.Wollheim, "Islet cell metabolism is reflected by the MTT (tetrazolium) colorimetric assay," *Diabetologia: Clinical and Experimental Diabetes and Metabolism*, vol. 35, no. 5, pp. 482–485, 1992.
12. Gallagher AM, Flatt PR, Duffy G, Abdel-Wahab YH A. "The effects of Traditional Antidiabetic plants on *in vitro* Glucose Diffusion". Nutrition Research. 2003; 23:43-424.
13. Manikandan R, Vijaya Aanad A and Durai Muthumani G. "Phytochemical and *in vitro* anti-diabetic activity of methanolic extract of *Psidium guajava* leaves".

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

International Journal of Current Microbiology and Applied Sciences. 2013; 2(2): 15-19.

14. Kim SH, Hyun SH, Choung SY. "Antidiabetic effect of *cinnamon* extract on blood glucose in db/db mice". Journal of Ethanopharmacology. 2006; 104:119-123.
15. Marles RJ, Fransworth NR. "Antidiabetic plants and their active constituents". Phytomedicine. 1995; 2: 137-189.
16. Halberstan M, Cohen N, Shlimovich P. "Oral vanadyl sulfate improves insulin sensitivity in NIDDM but not in obese non diabetic subjects". Diabetes 1996; 45: 659-666.
17. Anderson AA, Polansky MM, Bryden NA. "Effects of supplemental chromium on patients with symptoms reactive hypoglycemia". Metabolism 1987; 36: 351-355.
18. Paolisso G Sgambo S, Gambardella A. "Daily magnesium supplements improve glucose handling in elderly subjects". American Journal of clinical Nutrition 1992; 55: 1161-1167.
19. Momoh Johnson, Longe Adeteju Olufunmilayo, Campbell Charles Adegboyega, Omotayo Mutiat Adetayo. "Discussion-Body weight changes: Evaluation of Antidiabetic and the Effect of Methanolic Leaf Extract of *Jatropha curcas* on some Biochemical parameters in Alloxan induced Diabetic Male Albino Rats". European Journal of Medicinal Plants, 18th August 2014; 4, 1 (12): 1503-1512.
20. Suryawanshi NP, Bhutey AK, Nagdeote AN, Jadhav, *et al*. "Discussion-Body weight changes: Study of lipid peroxide and lipid profile in diabetes mellitus". Indian Journal of Clinical Biochemistry. 2006; 1 (21): 126-130.