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In Vitro Evaluation of Antidiabetic Potential of *Vetiveria Zizoniodes* L. [Nash] Root Extracts

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Diabetes mellitus is a chronic metabolic disorder caused by inherited or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It is widely present in all parts of the world affecting nearly 15 % of the population and considered as one of the leading cause of death in humans. In recent years alternative treatment methods are being followed by the diabetic patients in order to avoid side effects of allopathic medicines. Many plant sources are being used to treat diabetes mellitus from time immemorial but all are not validated scientifically. Hence in the present study a commonly available drug source – *Vetiveria zizanioides* L. (Nash) belonging to the family *Poaceae* has been chosen and tested for its *in vitro* antidiabetic potential. Aqueous and ethanolic root extract of *Vetiveria zizanioides* L. (Nash) were prepared and evaluated for its *in vitro* antidiabetic potential. Preliminary phytochemical screening of plant extracts revealed the presence of saponin, terpenoid, alkaloid, coumarin, flavanoids, lignins, glycosides and Phenol. The extracts were studied for *in vitro* antidiabetic activity and its effect on inhibition of glucose diffusion, glycosylation of hemoglobin and glucose transport across yeast cells. From the extracts, ethanol extract exhibited potent inhibition of glucose diffusion, glycosylation of hemoglobin, glucose uptake by yeast cells, significant glucose adsorption capacity, uptake of glucose by rat hemi-diaphragm were found to be in a dose dependent manner. From the results of the study, it is inferred that, *Vetiveria zizanioides* L. (Nash) root ethanol extract possesses excellent antidiabetic activity compared to aqueous extract. However, these effects need to be confirmed using *in vivo* models and clinical trials for its effective utilization as therapeutic agent.

Key words: Vetiveria zizanioides L. (Nash), Antidiabetic, Glucose adsorption, Glycosylation, Glucose diffusion

Diabetes mellitus (DM) is a group of metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. Insulin deficiency in turn leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism **(Rahmawati *et al*., 2019)**. This disease progresses tissue or vascular damages leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular diseases and ulceration. The inappropriate utilization of insulin leads to insulin resistance, which is characterized by the inability of cells to respond to normal levels of circulating insulin **(Adnette *et al*., 2019)**. The number of diabetes mellitus cases has been increasing worldwide in recent years. In 2020, the world health organization (WHO) estimated a total of 260 million of people with diabetes mellitus from the global population, and this report projected to increase 592 million by 2035 **(Cho *et al*., 2018)**.

Antidiabetic medication seeks to maintain a normal glucose level. Currently available allopathic drugs such as sulfonyl urea, glibenclamide and metformin are associated with many side effects and are not cost effective. Prolonged usage of allopathic medicines cause many side effects such as severe headache, weight gain, drug resistance toxicity and hypoglycemic effect **(Shobana *et al*., 2018)**. To reduce modern medicine side effects and consequences to cure diseases by the way of ayurvedic medicinal system of complementary and alternative treatment strategy **(Hu and Jia, 2019)**. Medicinal plants usage has been turned out to be an alternative method for the treatment of diabetes mellitus from our ancient period to till date **(Yakubu *et al*., 2020)**. 80 % of world's population relay on the medicinal plants as a source for the therapeutic mechanism of diseases. Literature reports revealed that, more than 800 plants have been utilized as experiential treatment for diabetes **(Omari *et al*., 2019)**. One tenth of them have been characterized as hypoglycemic plants with active compounds such as mucilage gum, glycan's, flavonoids, triterpenes alkaloids and phenols **(Loodu and Rupasinghe., 2019)**.

***Vetiveria zizanioides* L. (Nash)** is belongs to the family *Poaceae* (Grass family) and is mostly distributed in all over the India. Only in someplaces of South India is the grass systematically cultivated. The grass is known by several local names in different regions in India **(Mishra *et al*., 2013)**. It is a densely tufted grass with the culms arising from an aromatic rhizome up to 2m tall; the roots are stout, dense in nature. Roots are mainly used for fever, stimulant, diuretic, cooling, inflammations, irritation, tonic, stomachic, antispasmodic and emmenagogue. *Vetiveria zizanioides* L. (Nash) was selected for this study on the basis of its various phytoconstituents and its active metabolites role in pharmacological effects for curing many diseases. The phytochemical constituents of *Poaceae* family plant has some of the bioactive secondary metabolites such as coumarin, alkaloids, flavonoids, quinine, lignin, glycosides and phenol are responsible for its hypoglycemic activity and used as a potent drug for diseases like diabetes mellitus **(Jothi and Brindha, 2014)**. It contains many bioactive chemical constituents such as vetiverol, vetivone, khusimone, khusimol, vetivene, khositone, terpenes, benzoic acid, tripene-4-ol, ß-humulene, epizizianal, vetivenylvetivenate, iso khusimol, vetiver oils, vetivazulene, zizaene, prezizaene and bvetispirene. It is commonly called as Khas-Khas grass in English, Usheer in Hindi, vettiver in tamil, Ushira in Sanskrit, Vattivellu in Telugu, Ramacham in Malayalam. It has been used as an ayurvedic medicine to cure many diseases such as diabetes mellitus **(Sanjay *et al*., 2012)**, rheumatism, inflammation, diarrhea, epilepsy and removal of helminthes. It has possess anti-tubercular **(Dharmendra *et al*., 2012)**, antioxidant **(Subhadradevi *et al*., 2010)**, antifungal **(Devprakash *et al*., 2011)**, antibacterial, hepatoprotective **(Chaudhary *et al*., 2010)** and antidepressant activity. Various tribes use the special parts of the grass for many of their ailments such as mouthulcer, fever, boil, epilepsy, burn, snakebite, scorpion sting, fever, and headache. It has scientific evidence for the hypoglycemic effect through literature survey and has not been validated effectively. Hence *Vetiveria zizanioides* L. (Nash) was chosen as a test drug to analyze the blood sugar control mechanism using *in vitro* parameters.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals were purchased from the Sisco Research Laboratories (SRL) Pvt. Ltd., India. Organic solvents were procured from Merck (Chennai, India). All other chemicals of the reagent grade were obtained from local suppliers (SRL, Himedia & Biosystems, Chennai, India). All other reagents were of analytical grade.

Collection, Identification and Authentication

Plant *Vetiveria zizanoides* (L) Nash. selected for the present study was collected from medicinal plant shop at Trichy, identified with the help of Flora of Presidency of Madras, **(Gamble, 1997)** and authenticated by Dr. S. John Britto, with the specimen deposited at RAPINAT Herbarium **(Specimen No. SP001)**, Centre for Molecular Systematics, St. Joseph's college (Autonomous), Tiruchirapalli, India.

Physicochemical Parameters (Annonymous, 1996)

The determination of various physicochemical parameters such as foreign matter, moisture content, total ash, acid insoluble ash, water soluble ash, preliminary phytochemical analysis **(Brindha *et al*., 1981; Evans, 2009)** and fluorescence analysis **(Chase and Pratt, 1949)** were analyzed as per standard textual procedure of Indian Pharmacopoeia.

Determination of Foreign Matter

The collected plant material was taken and spread it in thin layer and the pieces of foreign matter were stored out by visual inspection. All portion of the foreign matter was pooled and weighed.

Preparation of Aqueous Extract

Dry root of *Vetiveria zizanoides* L. (Nash) was powdered coarsely using electric blender. 100g of root powder was taken and boiled with six parts of water till the content was reduced to one third. Filtered the content and evaporated to dryness. Paste form of the extract obtained was used for further analysis **(Molnar *et al*., 2017)**.

Preparation of Ethanol Extract

Dry root of *Vetiveria zizanoides* L. (Nash) was powdered coarsely using electric blender. The root powder 100g of *Vetiveria zizanoides* L. (Nash) was taken and soaked with alcohol (100% Ethanol) for 48 hours. The plant material was filtered and the filtrate was evaporated to dryness. Paste form of the obtained extract was used for further analysis **(Rao *et al*., 2003)**.

In Vitro Antidiabetic Studies

In vitro antidiabetic activities were carried out on the basis of various methodologies such as glucose adsorption capacity, glucose uptake by isolated rat hemi-diaphragm **(Jith and Jayakumari, 2017)**, glucose diffusion assay **(Sathiyavelu *et al*., 2013)**, non enzymatic glycosyaltion of hemoglogin **(Adisa *et al*., 2004)** and glucose uptake in yeast cells **(Bhutkar and Bhise, 2013).**

Statistical Analysis

All the experiments were done in triplicate, and the results were expressed as mean ± standard error mean (SEM).

RESULTS AND DISCUSSION

Total ash, acid insoluble ash and water soluble ash content were determined by standard textual procedure of Indian Pharmacopoeia. From the result **(Table-1)** it was found that the total ash content of the plant material was 3.45 %, acid insoluble ash was found to be 0.31 % and water soluble ash found to be 3.14 % which indicates the purity of the test drug taken under study. Ash value aids to decide quality and purity of crude drugs. The results showed that there is higher value of total ash and lesser acid in soluble ash indicating the purity of selected drug **(Kokateck *et al*., 2002)**.

The preliminary phytochemical screening of the plant extracts were tabulated **(Table-2)**, which revealed the presence of saponin, coumarin, quinone, lignin, glycosides, sugar and phenol in the aqueous extract and the presence of terpene, flavonoids, coumarin, quinone, lignin, alkaloids, glycosides, sugar and phenol in ethanol extract. Phenols possess rich antioxidant activity as well as hypoglycemic effect in managing type 2 diabetes mellitus **(Lin *et al*., 2016)**. Both the extracts revealed the active secondary metabolites in the plant sample which possess good pharmacological effects on curing diseases through its diverse mechanism of action.

The fluorescence behaviour of the drug powder **(Table-3)** with the above mentioned chemicals were

observed in the day light and UV light. It was found to give various shades of green, red, pink, blue, yellow and colourless. The brown and yellow fluorescence indicates the presence of alkaloids and flavonoids. Green fluorescence indicates the presence of sterol. It is an important parameter to evaluate the nature of chemical constituents present in drug.

The adsorption capacity of the aqueous and ethanolic extract of *Vetiveria zizanoides* L. (Nash) were found to be **(Table-4)** directly proportional to the molar concentration of glucose **(Fig.1).** Higher amount of glucose was bound with the plant extract with increase in its concentration **(100 mM)**. This result revealed that, the plant extract under study could bind with glucose even at lower concentration **(5 mM)** also, thereby reducing the amount of glucose available for transport across the intestinal lumen, consequently blunting the postprandial hyperglycemia **(Kalidoss *et al*., 2017)**. This may be due to their both insoluble and soluble constituents and fiber content present in the plant extracts.

The effect of the plant extracts on retarding glucose diffusion across the dialysis membrane is shown in **Table-5**. The rate of glucose diffusion was found to increase with the time from 30 to 180 min. In the present study, the movement of glucose across the dialysis membrane was monitored once in 30 min till 180 min and it was found that, the aqueous and ethanol extracts of the selected plant demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane compared to control. There was higher inhibitory potential expressed by ethanol extract compared to aqueous extract.

GDRI is a useful *in vitro* index to predict the effect of fiber content for the delay in glucose adsorption in the gastrointestinal tract **(Hariharan *et al*., 2023)**. The decrease in glucose diffusion could be attributed to the inhibition of the enzyme α-amylase, which prolongs the glucose release from starch **(Proenca *et al*., 2019)**. From the results obtained, it was clear that the aqueous and ethanol extract of *Vetiveria zizanoides* L. (Nash) possess hypoglycemic potential effect by inhibiting the entry of glucose thereby controlling the postprandial blood glucose level for the management of diabetes mellitus **(Basha *et al*., 2012)**.

Effect of aqueous and ethanol extract of *Vetiveria zizanoides* L. (Nash) were given in the **Table-6**. An increase in the glycosylation of Hb was observed on incubation of hemoglobin with the increasing concentration of the glucose over a period of 72 hrs. However, the plant extracts significantly inhibited the haemoglobin glycosylation which is indicated by the presence of increasing concentration of haemoglobin. The ethanol extract exhibited higher inhibition of glycosylation (91.4 % in 250µg/ml) than aqueous extract (82.6 % in 250µg/ml). The plant extracts possess the inhibition of haemoglobin glycosylation at different physiological concentrations of the glucose over the period of 72 hrs, indicating the efficiency of plant extracts $\mathbf{IC_{50}}$ in decreasing the formation of the glucose-haemoglobin complex and thus amount of free haemoglobin increases, thereby reducing the glycosylation of blood cell component.

Some of the studies reported that the human bodies possess enzymatic and nonenzymatic antioxidative mechanisms which minimize the generation of reactive oxygen species, responsible for many degenerative diseases including diabetes **(Madhuri *et al*., 2016)**. Increased concentration of glucose in the blood leads to its binding capacity with hemoglobin which may result in the formation of the reactive oxygen species. Plant extracts play an important role the inhibition of the glycosylation end products **(Baily *et al*., 1989)**.

Effect of aqueous and ethanol extract of *Vetiveria zizanoides* L. (Nash) on Glucose uptake by yeast cells were revealed in the **Table-7**. This assay is based on the movement of glucose across the membrane of yeast cells, with the help of the plant extracts. The yeast cells were suspended in plant extracts at various concentrations of glucose (1mg/ml to 5mg/ml).The amount of glucose remaining in the solution after incubation was observed. This determines the glucose uptake by the yeast cells **(Suhashini *et al*., 2014)**. From the results, it was found that the percentage inhibition in glucose uptake by yeast cells at 5mM glucose concentration of aqueous (45.3 %) and ethanol extract (48.7 %) and maximum inhibition of glucose at 25mM glucose concentration of aqueous (62.4 %) and ethanol

(84.7 %) extracts revealed its glucose uptake nature by yeast cells. The result suggested that ethanol extract exhibited maximum level of inhibition (84.7%) than aqueous extract.

Glucose uptake by isolated rat diaphragm is studied inorder to determine the pheripheral uptake of glucose by tissues **(Jijith *et al*., 2017)**. The effect of *Vetiveria zizanoides* L.(Nash) on glucose uptake by isolated rat-hemidiaphragm were shown in **Table-8**. Ethanol extract enhanced the glucose uptake effectively (7.25mg/g of tissue) compared to aqueous extract (6.44mg/g tissue). The results were compared with positive control (insulin).

Table 1: Physicochemical Parameters of *Vetiveria zizanioides* L. (Nash)

S.No.	PARTICULARS	VALUE (%)
1	Foreign matter	2.0 ± 0.02
2	Moisture content	8.5 ± 0.01
3	Total ash	3.45 ± 0.12
4	Acid insoluble ash	0.31 ± 0.01
5	Water soluble ash	3.14 ±0.02

Table 2. Preliminary Phytochemical Screening of *Vetiveria zizanioides* L. (Nash)

S.No.	Tests	Result	
		Aqueous Extract	Ethanol Extract
1	Saponins	+	-
2	Tannins	-	-
3	Sterol	-	-
4	Terpene	-	+
5	Flavonoids	-	+
6	Coumarin	+	+
7	Quinone	+	+
8	Lignin	+	+
9	Alkaloids	-	+
10	Glycosides	+	+
11	Sugar	+	+
12	Phenol	+	+

Table 3. Fluorescence Analysis of *Vetiveria zizanioides* L. (Nash)

S.No.	Test Chemicals	*Vetiveria zizanioides* L. (Nash)			
		Day Light		UV Light	
		24 hrs	48 hrs	24 hrs	48 hrs
1	Drug powder	Brown	Brown	Brown	Brown
2	Drug powder + 1 N NaOH	Yellowish Red	Yellowish Red	Yellowish Red	Yellowish Red
3	Drug powder +Alc 1 N NaOH	Yellow	Yellowish Red	Yellow	Yellowish Red
4	Drug powder + 1 N Hcl	Colourless	Colourless	Colourless	Colourless
5	Drug powder + 50 % H2SO4	Light Green	Light Green	Light Green	Light Green
6	Drug powder + Chloroform	Light Brown	Light Brown	Light Brown	Light Brown
7	Drug powder + Hexane	Colourless	Colourless	Colourless	Colourless
8	Drug powder +Ethylacetate	Light Yellow	Light Yellow	Light Yellow	Light Yellow
9	Drug powder + Acetone	Light Yellow	Light Yellow	Light Yellow	Light Yellow
10	Drug powder + Benzene	Colourless	Colourless	Colourless	Colourless
11	Drug powder + Alcohol	Colourless	Colourless	Colourless	Colourless
12	Drug powder + Water	Colourless	Colourless	Colourless	Colourless

Table 4. Effect of Aqueous and Ethanol extracts of *Vetiveria zizanoides* L.(Nash) on Glucose adsorption capacity

Sample	Glucose concentration (mM)				
	5 mM	**10 mM**	**25 mM**	**50 mM**	**100 mM**
1 % Plant Extract **(Aqueous)**	0.206 ± 0.02	0.304 ± 0.02	0.474 ± 0.03	1.05 ± 0.01	2.71 ± 0.03
1 % Plant Extract **(Ethanol)**	0.214 ± 0.01	0.298 ± 0.01	0.453 ± 0.01	2.73 ± 0.02	3.65 ± 0.03

Values are expressed in Mean ± SEM (n=3)

Table 5. Effect of Aqueous and Ethanol extracts of Vetiveria zizanoides L. (Nash) on Glucose Diffusion

Sample	Glucose content in the Dialysate (mM)				
	30 min	**60 min**	**90 min**	**120 min**	**180 min**
Control	1.26 ± 0.15	2.78 ± 0.23	2.47 ± 0.12	3.67 ± 0.27	4.19 ± 0.31
Aqueous	0.59 ± 0.10	1.16 ± 0.16	1.03 ± 0.18	1.96 ± 0.21	2.44 ± 0.27
GDRI %	**52.97**	**40.44**	**36.62**	**26.59**	**23.30**
Ethanol	0.29 ± 0.02	0.78 ± 0.14	0.94 ± 0.18	1.69 ± 0.19	2.41 ± 0.24
GDRI %	**76.48**	**56.17**	**45.34**	**36.68**	**24.46**

Values are expressed in Mean ± SEM (n=3)

GDRI – Glucose Diffusion Retardation Index

Table 6. Effect of Aqueous and Ethanol extracts of *Vetiveria zizanoides* L. (Nash) on Non Enzymatic Glycosylation of Hemoglobin

S.No.	Concentration of Plant Extracts (µg/ml)	% Inhibition	
		Aqueous Extract	**Ethanol Extract**
1	50	15.0 ± 0.01	22.0 ±0.04
2	100	36.6 ± 0.03	47.8 ± 0.06
3	150	68.5 ± 0.02	71.8 ± 0.03
4	200	75.1 ± 0.04	84.6 ± 0.02
5	250	82.0± 0.02	91.4± 0.05
IC_{50}		**125**	**105**

Values are expressed in Mean ± SEM (n=3)

Table 7. Effect of Aqueous and Ethanol extracts of *Vetiveria zizanoides* L. (Nash) on Glucose Uptake by Yeast Cells

Concentration of Plant Extracts (µg/ml)	% of increase in glucose uptake					
	Aqueous Extract			**Ethanol Extract**		
	5mM	**10mM**	**25mM**	**5mM**	**10mM**	**25mM**
100	18.5±0.01	22.6±0.01	27.1±0.06	15.3±0.01	28.9±0.02	35.3±0.05
200	21.3±0.06	28.7±0.08	34.1±0.03	27.4±0.02	34.5±0.03	51.8±0.03
300	26.4±0.03	32.9±0.04	49.4±0.03	31.5±0.01	47.7±0.01	57.7±0.01
400	34.8±0.02	43.2±0.04	55.3±0.05	39.6±0.02	57.4±0.01	71.8±0.03
500	45.3±0.03	54.8±0.03	62.4±0.01	48.7±0.06	63.7±0.02	84.7±0.02

Values are expressed in Mean ± SEM (n=3)

Table 8. Effect of Aqueous and Ethanol extracts of *Vetiveria zizanoides* L. (Nash) on Glucose Uptake by Rat Hemi-diaphragm

S.No.	Test	Glucose Uptake (mg/g tissue)
1	Control	4.36 ± 0.24
2	Positive control (Insulin)	14.54 ± 0.21
3	Aqueous extract (1mg/ml)	6.44 ± 0.36
4	Ethanol extract (1 mg/ml)	7.25 ± 0.42

Values are expressed in Mean ± SEM (n=3)

CONCLUSION

In the present study, aqueous and ethanol extracts of root *Vetiveria zizanoides* L. (Nash) was evaluated for its pharmacological properties using physicochemical standards, preliminary phytochemical screening. *In vitro* antidiabetic assays, determination of glucose adsorption, glucose diffusion, glucose uptake by yeast cells, glycosylation of hemoglobin and glucose uptake by rat hemi-diaphragm were studied. The results revealed that, the plant extracts caused reduction in the postprandial blood sugar level by inhibiting the entry of glucose into the blood by trapping it with the help of fiber content. The results of non-enzymatic glycosylation of haemoglobin reveals the anti glycation activity of selected plant, thereby it controls the development of secondary complications. Significant inhibition of glucose uptake by yeast cells and there by justifies its role in reducing the glucose absorption to blood. Enhanced uptake of glucose through rat hemi diaphragm when treated with plant extracts of *Vetiveria zizanoides* L. (Nash) revealed its potential in mobilizing the glucose into tissues and eventually resulting in reduced blood glucose level. From the results it was evidenced that ethanol extract has more potential effect compared to aqueous extract. Further investigation is required to purify the extract for the identification and isolation of bioactive compound which is responsible for its antidiabetic property.

To conclude the plant *Vetiveria zizanoides* L. (Nash) possess its antidiabetic potential by reducing the postprandial blood sugar level by inhibiting glucose diffusion, hemoglobin glycosylation and inhibit glucose uptake by yeast cell. It was also found to enhance the glucose uptake by rat diaphragm thereby might have the potency to move glucose into tissues further investigation of ethanol extract with isolation of lead molecule and *in vivo* evaluations of its antidiabetic potential is required to confirm the usage of *Vetiveria zizanoides* L. (Nash) as a potent drug for curing diabetes mellitus.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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