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ORIGINAL ARTICLE

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In Vitro Antidiabetic Activity of Ethanolic Extract of *Amaranthus viridis* Linn.

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Objective: To investigate the antidiabetic potential of ethanoilc extract of *Amaranthus viridis* Linn. *in vitro* assays.
Methods: Ethanolic extract of aerial parts of *Amaranthus viridis* Linn. was extracted and tested for presence of phytochemical constituents, α-amylase inhibitory activity, determination of glucose adsorption and inhibition of glucose diffusion using dialysis membrane done by plant extracts.
Results: Presence of Flavonoids, alkaloids, sugars, quinones, tannins, terpenes, coumarin and phenols.were identified in the extract. The results of *in vitro* antidiabetic assays showed moderate activity of α-amylase inhibitory activity with increase in the percentage inhibition of alpha amylase enzyme in a dose dependent manner. The IC_{50} value was found to be 155.60 µg. The adsorption of glucose by the plant extract was increased remarkably with an increase in glucose concentration. The rate of glucose diffusion was found to increase with time from 30 – 180 min, and the plant extract demonstrated significant inhibitory effects on movement of glucose into external solution across the dialysis membrane and compared to control.
Conclusion: The antidiabetic effect of aqueous extract of *Amaranthus viridis* Linn. is mediated by increasing glucose adsorption, decreasing glucose diffusion rate and moderately inhibiting the alpha amylase enzyme thereby exhibiting the potential antidiabetic effect revealed by *in vitro* models. These findings suggest that the plant may be a potential source for the development of new oral hypoglycemic agent.

Key words: Diabetes mellitus, Alpha amylase, glucose adsorption, GDRI

Diabetes mellitus is a class of metabolic disorders marked by persistently high blood sugar levels, which can be caused by abnormalities in the secretion of insulin, the action of insulin, or both. A higher thirst threshold, increased urine production, ketonemia, and ketonuria are typical signs of diabetes mellitus. Diabetes, a metabolic disease affecting protein, fat, and carbohydrates, affects a lot of people worldwide **(Pareek *et al*., 2009)**. India had an estimated 22 million people with diabetes in 1990, 28 million in 1995, and 33 million in 2000. It is the most prevalent metabolic disease worldwide. Nearly 90% of people with diabetes worldwide have non-insulin-dependent diabetic DM (NIDDM), though the percentage varies by location **(Singh, 2011)**.

Management of Diabetes Mellitus is a global problem. Successful treatment is very important for preventing or at least delaying the onset of long-term complications. Drug therapies have been used to treat diabetes in recent years. Standard synthetic drugs used to treat diabetes include glinides, biguanides, α-glucosidase inhibitors, and sulfonylureas. Side effects from these medications frequently include head pain, nausea, vomiting, diarrhea, abdominal pain, low blood sugar, dark urine, fluid retention, and unusual weight gain. Moreover, using them while pregnant is not safe **(Anbu *et al*., 2012).** As a result, managing diabetes without experiencing any negative side effects remains difficult. Alternative medications are constantly being sought after **(Amin, 2011).** The need for new diabetes therapies is anticipated to increase significantly over the next ten years due to the global diabetes epidemic, the lack of efficacy and numerous side effects of currently available medications, and several other factors. Thus, it is imperative to develop affordable, safe, and effective medications for the management of diabetes immediately due to the side effects connected with the current allopathic treatments for the disease. Medicinal plants, which have been utilized by people since the beginning of civilization to treat or prevent illnesses like diabetes, can be used to make these medications. The presence of active ingredients such as alkaloids, flavonoids, terpenoids, phenol, and others in these plant-based herbal medicines is thought to make them safe, effective, and inexpensive for the general public in underdeveloped and developing nations of the world **(Shobana *et al*., 2018)**.

Amaranthus viridis Linn.(Tamil: Kuppai keerai) is a cosmopolitan annual herb.It is belonging to the family of *Amaranthaceae.* The plant is usually known as green amaranth or slender amaranth. Possible origin is South America, although widely distributed in tropical weed, foreign to hot-temperate regions and distributed in the tropical and subtropical regions of the world. The *A. viridis* is a good source of vitamins B and C, taken as vegetables **(Sayeed *et al.,* 1997).** Leaves and seeds are also edible. Previous experiments ascertained it to be a superior source of protein **(Macharla *et al*., 2011).** Traditionally it is used to cure eczema, psoriasis and rashes including antinociceptive and antipyretic properties, reported by Kumar **(Kumar *et al*., 2009).** Besides these, it is reported that *A. Viridis* has anti-inflammatory, antihyperglycemic, hypolipidemic activity as well as acne and skin cleansing property **(Krishnamurthy *et al*., 2011)**. It has a wide application over diuresis, for snake bites, scorpion stings, dysentery, constipation, eczema, bronchitis, anemia, leprosy and stomach problems like many incidences **(Pandhare *et al*., 2012)**. Based on the literature review, *Amaranthus viridis* Linn. was selected under study and it was evaluated to carry out the *in vitro* antidiabetic activity using ethanol extract.

MATERIALS AND METHODS

Identification and Authentication

Plant ***Amaranthus viridis* Linn.** selected for the present study was collected from in and around Trichy, identified with the help of Flora of Presidency of Madras **(Gamble, 1997)** and authenticated with the specimen deposited at **RAPINAT Herbarium**, Department of Botany, St. Joseph's college, Trichy.

Preparation of ethanolic plant Extract

Fresh aerial part of plant material was shade dried and powdered coarsely using electric blender. Ethanol extract was prepared by soaking 250 g of dried material in ethanol for 48 hours. The solution was filtered and

evaporated to dryness. The residue was dissolved in isotonic saline and used for the study.

Preliminary Phytochemical Screening of Various Extracts

Preliminary phytochemical screenings of various extracts and drug powder were carried out as per the standard textual procedure**. (Brindha *et al.,* 1981)**

***IN VITRO* ANTIDIABETIC STUDIES**

In vitro antidiabetic evaluation was performed based on the assays of α-amylase enzyme inhibition (**Narkhede *et al.*, 2011**), determination of glucose adsorption capacity (**Ou *et al.,* 2011**) and *In vitro* glucose diffusion using dialysis membrane (**Ou *et al.,* 2011**).

RESULTS AND DISCUSSION

The preliminary phytochemical screening analysis of aqueous extract of *Amaranthus viridis* Linn. showed the presence of Flavonoids, alkaloids, quinones, tannins, terpenes, coumarin and phenols. The anti-diabetic activities of the plants were due to the presence of flavonoids and phenols **(Sharma *et al.,* 2011).**

Table 2 shows the inhibition of alpha amylase enzyme by the ethanol extract of *Amaranthus viridis* Linn. There was a dose dependant increase in percentage inhibition of alpha amylase enzyme. At a concentration 50 µg of extract showed a percentage inhibition of 15.27% and for 250 µg it was 89.30%. The IC_{50} value was found to be 155.60 µg.

α-Amylase enzyme hydrolyses alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. It is the major form of amylase found in humans and other mammals. These α- amylase inhibitors are also called as starch blockers as they prevent or slows the absorption of starch into the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltriose and other simple sugars **(Dineshkumar *et al*., 2010).** The inhibitions of α amylase reduce the high post prandial blood glucose peaks in diabetes. The α-amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrate **(Narkhede *et al.,* 2011).** The α amylase inhibitory activity in ethanol extract is most likely to be due to polar compounds. The present study showed promising result in α-amylase inhibition assay, suggesting that ethanol extract of *Amaranthus viridis* Linn. might be effective in slowing down hydrolysis of starch to minimized glucose availability.

The glucose adsorption capacity of *Amaranthus viridis* Linn. is shown in **Table 3** and **Figure 2.** The adsorption capacity of ethanolic extract of *Amaranthus viridis* Linn. was found to be directly proportional to the molar concentration of glucose and higher amount of glucose was bound with the plant extract with increase in its concentration. The results also revealed that the plant extract under study could bind glucose even at lower concentrations of glucose (5 mM/L) thereby reducing the amount of glucose available for transport across the intestinal lumen, consequently blunting the postprandial hyperglycemia. This may be due to their both insoluble and soluble constituents and fibers present in the plant and different sources are reported to adsorb glucose **(Chau *et al.,* 2004)**.

The effect of the plant extracts on retarding glucose diffusion across the dialysis membrane is shown in Table 4. The rate of glucose diffusion was found to increase with 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 ethanolic extract of selected plant demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane compared to control.

The glucose dialysis retardation index (GDRI) is a useful *in vitro* metric for assessing the gastrointestinal tract's absorption of glucose **(Lopez *et al*., 1996)**. Greater glucose retardation index by the sample is indicated by a higher GDRI. In order to gradually control the release of glucose from the starch, α-amylase inhibition is a crucial step in the extracts under study's glucose diffusion retardation process **(Ou *et al*., 1999).** Numerous factors have been identified in previous reports as potentially causing GDRI, including fiber concentration, inhibitors on fibers, encapsulation of starch/enzyme by sample fibers, which lowers starch accessibility to the enzyme, and direct adsorption of the enzyme on fibers, which lowers amylase activity

(Bhutkar and Bhise, 2012). It has also been suggested that these factors could be the cause of GDRI.

From the results obtained, it is clear that the ethanoilc extract of *Amaranthus viridis* Linn. possess hypoglycemic potential by inhibiting alpha amylase enzyme and also inhibiting the entry of glucose thereby controlling the post prandial blood glucose and was found to be a potential drug for the management of diabetic mellitus.

Table 1. Preliminary phytochemical screening of *Amaranthus viridis* Linn.

S.No	Test For	Drug powder	Aqueous extract	Ethanol extract
1	Saponin	-	-	-
2	Tannin	+	+	+
3	Sterol	-	-	-
4	Terpene	-	-	+
5	Flavonoid	-	+	+
6	Coumarin	-	+	+
7	Quinone	+	-	-
8	Lignin	-	-	-
9	Alkaloid	+	+	+
10	Phenol	+	+	+

+ indicates Presence - indicates Absence

Table 2. Inhibition of alpha amylase by the ethanolic extract of *Amaranthus viridis* Linn.

S. No	Concentraion (µg)	% Inhibition
1	50	15.27 ± 0.99
2	100	33.33 ± 2.27
3	150	48.20 ± 2.62
4	200	66.57 ± 2.71
5	250	89.30 ± 1.16

IC_{50} = 155.60 µg

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

Table 3. Glucose adsorption capacity of ethanolic extract of *Amaranthus viridis* Linn.

	Glucose Concentration mM/L				
	5mM	10mM	20mM	50mM	100mM
Glucose adsorption mM/L	12.10 ± 0.31	24.33 ± 0.30	47.90 ± 0.73	60.67 ± 0.63	76.93 ± 0.68

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

Table 4. Effect of ethanolic extract of *Amaranthus viridis* Linn. on glucose diffusion and GDRI

Sample	Glucose content in dialysate (mM/L)			
	30 minutes	60minutes	120 minutes	180 minutes
Plant extract	2.55 ± 0.12	3.07 ± 0.10	3.81 ± 0.14	4.75 ± 0.27
Control	1.41 ± 0.11	2.45 ± 0.14	3.36 ± 0.20	3.61± 0.15
GDRI	63.2%	58.7%	44.9%	32.3%

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

GDRI – Glucose Dialysis Retardation Index

CONCLUSION

The present investigation revealed that the diabetic potentials of ethanolic extract of *Amaranthus viridis* Linn. by *in vitro* assays. Preliminary phytochemical screening of the plant extract was done and the results showed that the presence of Flavonoids, alkaloids, sugars, quinones, tannins, terpenes, coumarin and phenols which could be responsible for the pharmacological properties of this plant. *In vitro* antidiabetic assays such as inhibitory activity of α – amylase, determination of Glucose adsorption and Glucose diffusion were studied in the ethanolic extract of *Amaranthus viridis* Linn. The results obtained revealed that the plant extracts showed significant inhibitory activity on carbohydrate metabolizing enzymes to reduce the postprandial blood sugar level by inhibiting the entry of glucose into the blood by trapping it with the help of fiber content. Further indepth studies may be carried out to identify the lead molecules present in this plant and carried out for antidiabetic activity in *in vivo.*

CONFLICTS OF INTEREST

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

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