

Antioxidant and Anti-diabetic Potential of Nutraceutical Rich *Amaranthus caudatus*

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ABSTRACT

Wild medicinal herbs are currently being viewed as alternative food sources with potential health benefits, and are mostly used as vegetables in several parts of the world. One such ignored wild medicinal herb *Amaranthus caudatus*, an underutilized vegetable from Kashmir region is believed to be highly nutritional and quite health beneficial, but is fully unexplored. Therefore, the aim of the current investigation was to investigate the phytochemical composition, antioxidant activity and antidiabetic potential of this wild vegetable under *in vitro* conditions. The phytochemical composition of aqueous extracts of *Amaranthus caudatus* (AAE) revealed it to be a rich source of total phenols ($545 \pm 0.003 \text{ mg/100g}$) and total flavonoids ($127 \pm 0.02 \text{ mg/100g}$). The AAE also exhibited strong antioxidant activity ($73 \pm 0.04 \mu\text{molFe}^{2+} \text{ FRAP/g FW}$) and dose dependent *in vitro* antidiabetic activity. The antidiabetic mode action of AAE seemed to be via targeting the key enzymes of carbohydrate metabolism with mild inhibition of α -amylase, strong inhibition of α -glucosidase and moderate inhibition of invertase activities respectively. These findings of current study are quite encouraging and suggest that the aqueous extract of *Amaranthus caudatus* can be better exploited to be used as nutraceutical rich antioxidant supplement as well as to suppress post-prandial glucose rise and can thus play a role as anti hyperglycemic food based medicine for diabetic patients.

Keywords: *Amaranthus caudatus*, Nutraceutical, Antioxidant, Hyperglycemia, Diabetes.

INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by uncontrolled hyperglycemia that is usually caused due to lack of insulin secretion, its action or both (Moradi et al., 2018). In recent times, this disease has gained immense notoriety and is becoming world's

largest silent killer (Nath, 2016). As per WHO Global report on diabetes (2016), it is the fourth leading cause of death in most high-income countries and on average, every tenth second, one person dies from diabetes or its related complications.

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According to International Diabetes Federation (2017), there are 326.5 million people of working age (20-64 years) with diabetes, and 22.8 million diabetic people in the age group of 65-99 years. The number of people of working age with diabetes is expected to increase to 438.2 million, and the number of people with diabetes in the age group of 65-99 years will increase to 253.4 million in 2045. This disease is mostly prevalent in the developing nations and the countries in world, where majority of population is suffering from this disease includes USA, India, China, Japan, Indonesia, Pakistan, Brazil, Russia, Italy and Bangladesh.

As far as India is concerned, currently this has the second largest number of diabetic patients in the world, following China which has been infamously dubbed as the “Diabetic capital of the world” (Sriram et al., 2016). Currently, India represents 49% of the world’s diabetes burden, with more than 72 million cases in 2017; a figure published by Harvard Gazette (2018) and is expected to almost double to 134 million by 2025. The most important consequence of this unexpectedly increase in diabetes prevalence in India, will in turn increase the economic burden of diabetes in the next decade. As per U.S. Department of Justice (2017) although various pharmacological agents are available commercially to combat diabetes and its associated complications, however most of such drugs in one way or the other, pose undesirable side effects. Consequently, there is an urgent need to search for some more potent and effective antidiabetic agents with lesser or no side effects. In this regard, wild herbs are being used as one of the oldest practices by mankind for natural cure of several ailments and diseases including diabetes. Due to low cost and less adverse side effects, such plants are being viewed as imperative medicinal herbs and chemical constituent vessels bearing pharmaceutical potential. Such plants, in addition to their nutrients, possess a diverse treasure of numerous bioactive compounds with nutraceutical properties.

Recent research shows that consumption of such foods rich in these bioactive compounds may delay the development of diabetic complications and correct the metabolic abnormalities associated with diabetes (Aswathy & Jessykutty, 2017). Therefore, extensive research work on plants with treasure of diverse biologically active compounds will be quite fruitful. One important category of such medicinal plants is represented by ignored wild vegetables which have vastly been used as traditional food source in the recent past but presently their knowledge is confined to only tribal and ethnic communities, living in close conformity to the nature. They have a great potential to increase the per capita availability of foods and are gaining attention due to their hidden medicinal properties (Kour., 2018). As far as the Jammu and Kashmir is concerned, the state is bestowed with a huge variety of such underutilized herbs. One such health beneficial herb commonly known as “Love lies bleeding” is *Amaranthus caudatus*, is highly unexplored in the region and has been reported to possess antidiabetic potential in various traditional system of medicines (Alicia et al., 2020). Therefore, the current investigation was carried out to investigate the bioactive composition, antioxidant activity and *in vitro* antidiabetic potential of *Amaranthus caudatus* growing in Kashmir region.

MATERIALS AND METHODS

Collection of Plant Material

The *Amaranthus caudatus* plants were collected from Regional cum Facilitation Centre (RCFC), Faculty of Agriculture, SKUAST-K, Kashmir, India.

Preparation of Extract

The whole plants were shade dried and coarsely powdered. Ten gram of the respective powder was mixed with 100ml of distilled water for aqueous extraction. The mixture was incubated at RT for 48 hours with occasional shaking and filtered with Whatman filter paper to obtain filtrate. For remaining residue the process was repeated once more and both the two filtrates pooled. The

Combined filtrate was dried by evaporation of water under vacuum at 50 °C. For further investigations the stock solution of aqueous extract of 1mg/ml distilled water was prepared as *Amaranthus caudatus* extract (AAE).

Estimation of total Phenol The total phenolic content of aqueous extract of *Amaranthus caudatus* was determined by modified method of Malick and Singh (1980).

Estimation of total flavonoids Total flavonoid content of aqueous extract of *Amaranthus caudatus* was determined according to modified method of Lallianrawna et al. (2013).

Estimation of total anti-oxidant activity The total antioxidant potential of aqueous extract of *Amaranthus caudatus* was determined by using the ferric reducing ability of plasma (FRAP) assay (Benzie and Strain, 1996) as a measure of its antioxidant power.

Antidiabetic Activity of *Amaranthus caudatus* extract (AAE)

In order to check the antidiabetic activity of aqueous extract of *Amaranthus caudatus* the three key enzymes of carbohydrate metabolism i.e. α -amylase α -glucosidase and invertase were targeted with different concentrations of AAE (0.01g/ml, 0.05mg/ml, 0.1mg/ml, 0.2mg/ml, 0.3mg/ml, 0.4mg/ml, 0.5mg/ml, 0.6mg/ml) under *in-vitro* conditions. The α -amylase inhibitory potential of AAE was determined by a method developed by Catherine et al. (2011). Likewise, the α -glucosidase and invertase inhibitory potential of AAE were determined by methods reported by Apostolidis et al. (2007) and Honda & Hara (1993) respectively.

Statistical analysis

The statistical analysis of data generated in the current study was carried out by means of one way analysis of variance (ANOVA) and the comprehensive statistical package SPSS (Version 20) for windows was used.

RESULTS AND DISCUSSION

Various pharmacological drugs available commercially for management of diabetes are mostly associated with several health uncertainties including psychological and economic burdens in diabetic patients and their

families. Therefore, need of developing new and safer alternatives for better management of hyperglycemia in diabetes is important. In this regard, plants used as therapy for various diseases including diabetes is an old practice and their intake may be associated with the decreased incidence of such diseases (Lin et al., 2017). Among plants *Amaranthus caudatus* due to its rich bioactive compounds such as phenolic acids, lycopene, polyphenols, unsaturated fatty acids, glucosinolates, proteins, soluble peptides, flavonoids, squalene and beta-carotene could offer novel choice to the limited therapeutic alternatives (Jimoh et al., 2019). Therefore, in the current investigation the phytochemical composition, antioxidant activity and anti-diabetic potential of unexplored *Amaranthus caudatus* from Kashmir region was investigated.

Research investigations suggests that in medicinal herbs the presence of phenolic compounds mostly bestow them the potential to curb various metabolic and oxidative stress related disorders including diabetes (Laila et al, 2017). Recent reports (Karamac et al, 2019) indicate that not only seeds but the leaves and other aerial parts of *Amaranthus* are also important sources of phenolic compounds. In this context in the current investigation, the total phenolic content of AAE was determined and found to be 545 ± 0.003 mg gallic acid equivalent /100g (Table 1). A wide range of phenol content with as low as 139mg/100g and 320mg/100g to 518mg/100g and 550 mg/100g in *Amaranthus* species have been reported by Islam et al. (2016) and Dulce et al. (2017) respectively. However, the result of current study related the phenol content of AAE to be in higher range from values reported by Islam et al. (2016) as well as 298 mg/100g reported by by Kanmoni et al. (2015) in *Amaranthus caudatus*. Thus, exploitation of such herbs from regions like Kashmir will be quite beneficial to be used as nutraceutical rich medicinal food.

Among the diverse group of phenolic compounds, flavonoids have been recognized as strong antioxidants with antidiabetic and anti-proliferative effects (Pulipati et al., 2017).

Flavonoids of underutilized crops including *Amaranthus caudatus* have been negligibly identified or studied. Therefore considering their diverse role, in the current investigation, the total flavonoid content of AAE was evaluated and found to be 127 ± 0.02 mg of quercetin equivalents/100g DW (Table 1). These values are much higher than (12mg/100 g DW) as reported by Sarkar et al. (2020) in green morph *Amaranthus* leafy vegetable. The results of current investigation are slightly lower than 144mg/100g as reported by Torane et al. (2017) in water extract of *Amaranthus carentus*. The results indicate that AEE possess good amount of flavonoids and can be used for medicinal purposes.

The natural antioxidants present in plants have gained a great deal of attention of consumers and researchers worldwide due to their health promoting activities (Murtaza et al., 2012). Phenolics and flavonoids from

plants are nowadays been viewed as potential group of nutraceuticals with strong antioxidant properties (Fatima et al., 2020). In the present study, the phenol and flavonoid rich aqueous extract of AAE was evaluated for its total antioxidant potential and found to possess 73 ± 0.04 mM Fe^{2+} /100g DW (Table 1). These results are slightly higher than the values (66.5 mM Fe^{2+} /100g DW) reported recently by Karamac et al. (2019) in methodic extract of *Amaranthus caudatus*. Comparatively the values of current study are much higher than the values reported in other species like *Amaranthus tristis* and *Alternanthera sessilis* with 32.56 mM/100g and 27.06 mM/100g FRAP equivalent respectively (Murugan et al., 2013). It is clear from this study that the *Amaranthus caudatus* collected from Kashmir region has a great antioxidant potential and can better be exploited to act as an ideal antioxidant dietary supplement.

Table 1: Total phenolics, Total flavonoids content and Total antioxidant activity of Aqueous extract of *Amaranthus caudatus*

Total phenolic content*	Total flavonoid content**	Total antioxidant activity (FRAP) ***
545 \pm 0.003	127 \pm 0.02	73 \pm 0.04

*mg gallic acid equivalent (GAE)/100g DW, **mg quercetin equivalent/100g DW, ***mM Fe^{2+} /100g DW Values are mean \pm SD of three replicates.

Diabetes is one of the most leading chronic medical conditions, caused due to imbalance in glucose production and glucose intake eventually leading to hyperglycemia and regulating blood glucose near to normal levels is highly vital for delaying or reversing this disease and its related complications (Aravind et al., 2015). Hyperglycemia could be controlled by decreasing the absorption of glucose through the inhibition or decreasing the effect of certain enzymes responsible for the hydrolysis of carbohydrate (Rhabasa-Lhoret et al., 2004). Among the various available therapeutic approaches adopted for hyperglycemia management, one of the important strategies involves the inhibition of

carbohydrate hydrolyzing enzymes such as α -amylase, α -glucosidase and invertase (Gajbhiye et al., 2018; Murtaza et al., 2013). The inhibition of these key enzymes with natural plant based means is gaining much attention due to their minimal side effects as compared to available therapies.

In the present investigation all the three enzymes were targeted with eight different concentrations of AAE as mentioned in methodology. As shown in figure 1 the α -amylase inhibitory property of AEE was found to be dose dependent causing 1.51%, 3.03%, 6.06%, 19.69%, 27.27%, 36.36%, 48.48%, 53.03% inhibition respectively with IC_{50} value of 0.56 ± 0.047 mg/ml (Table 2).

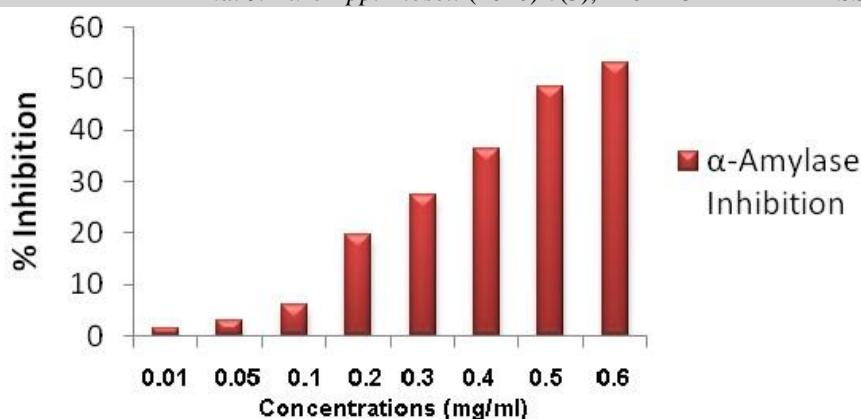


Fig. 1: Effect of aqueous extract of *Amaranthus caudatus* extract (AAE) on α -Amylase activity

The results of our study demonstrated that water based extracts possess much lower α -amylase inhibition activity compared to methanolic extract of *Amaranthus caudatus* as reported by Kumar et al. (2011) who found $44.01 \pm 0.12\%$, $65.56 \pm 0.18\%$ and $74.98 \pm 0.11\%$ α -amylase inhibition with $10 \mu\text{g/ml}$, $50 \mu\text{g/ml}$ and $100 \mu\text{g/ml}$ methnolic extract respectively and IC_{50} value of only $19.233 \mu\text{g/ml}$. However, the results of current investigation are more or less similar to the values reported in ethanolic extract of *Amaranthus tristis* Linn by Rajan & Aanandhi (2017) who observed 21%, 59%, 65%, 81%, and 97% inhibition of α -amylase activity at 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1mg/ml respectively. Though lesser, but overall α -amylase inhibition activity of AAE water extracts are encouraging compared to extracts made in organic solvents that are

comparatively inappropriate for human consumption.

As far as α -glucosidase is concerned, this enzyme results in breakdown of di- and oligosaccharides into glucose residues and its subsequent absorption in the small intestines. The α -glucosidase inhibitors, decreases the rate of glucose absorption in the intestines through competitive and reversible inhibition of intestinal α -glucosidase enzyme, thereby, reducing the absorption of glucose after a meal and thus play a beneficial effect in controlling the postprandial blood sugar levels (Chan et al., 2018). As shown in figure 2 in the current study the α -glucosidase inhibitory property of AEE was also found to be dose dependent causing 1.92%, 2.56%, 3.59%, 7.95%, 11.55%, 25.28%, 39.40%, 43.64% respectively (Fig. 2) with predicted IC_{50} value of $0.68.74 \pm 0.13 \text{mg/ml}$ (Table 2).

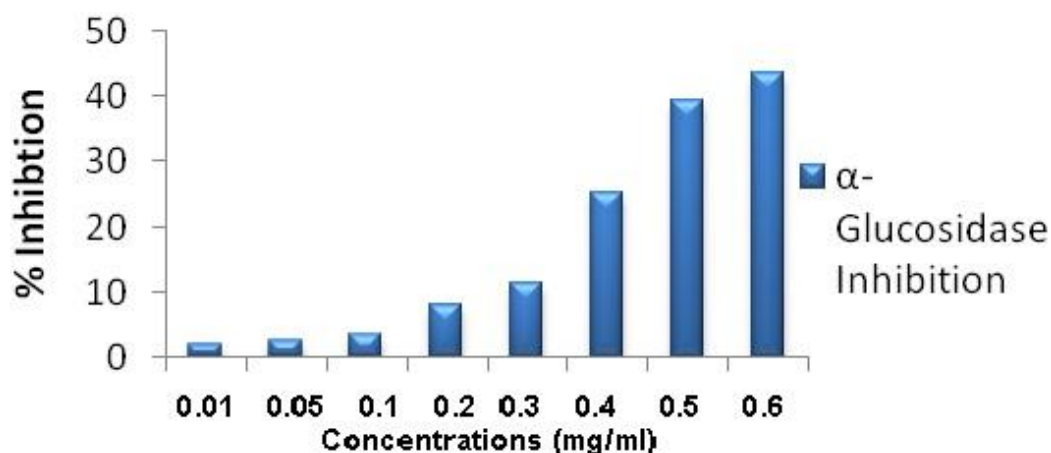


Fig. 2: Effect of aqueous extract of *Amaranthus caudatus* (AAE) on α -glucosidase activity

Similar kind of observations were also reported by Oboh et al. (2013) while using ethnolic extracts of *Amaranthus cruentus* leaves against α -glucosidase that caused 13%, 30%, 35%, 42% inhibition of α -glucosidase activity with IC_{50} value of 0.19 ± 0.01 mg/ml at 0.05 mg/ml, 0.1 mg/ml, 0.15 mg/ml and 0.2 mg/ml concentrations of each extract. As α -glucosidase is an important chemotherapeutic target involved in diabetic therapy, therefore further exploitation of AAE like inhibitors of this key enzyme from plant sources can play an important role for managing hyperglycemia in diabetic patients.

Another important chemotherapeutic target enzyme in diabetes is invertase (also

called beta-fructofuranosidase or sucrase) that cleaves the terminal non-reducing β -fructofuranoside residues and catalyses the hydrolysis (breakdown) of sucrose (table sugar) in the intestine. Its inhibition can delay the digestion and absorption of carbohydrates, thereby, inhibiting postprandial hyperglycemia (Abdelhady et al., 2016). In this study, the AAE was evaluated for its invertase inhibitory activity at different concentrations. As shown in figure 3, the invertase inhibitory property of AAE was also found to be dose dependent causing 1.08%, 2.16%, 3.84%, 7.39%, 14.81%, 22.19%, 32.06%, 39.14% respectively with predictive IC_{50} value of $0.76.64 \pm 0.07$ mg/ml (Table 2).

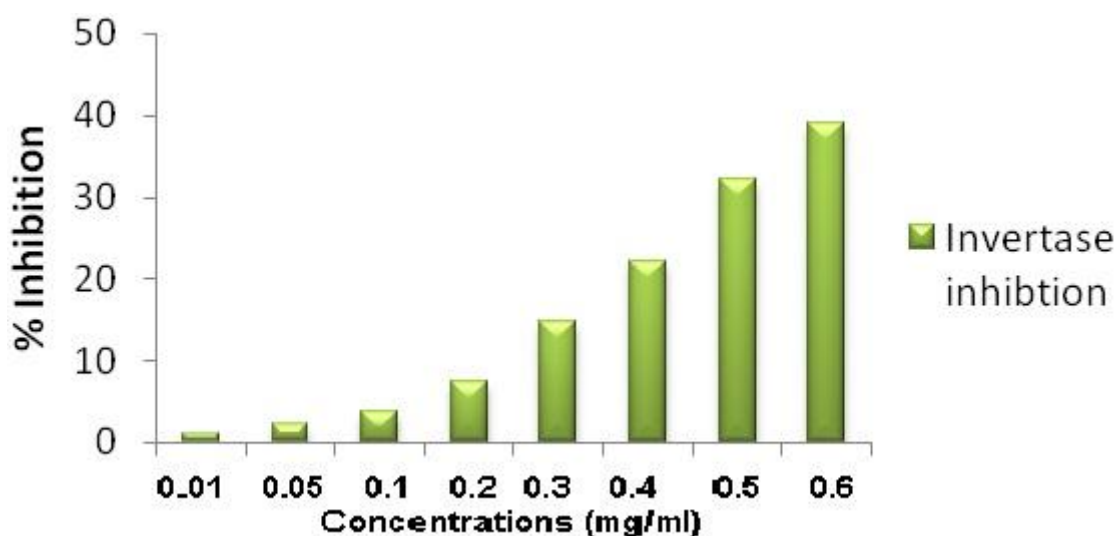


Fig. 3: Effect of aqueous extract of *Amaranthus caudatus* (AAE) on invertase activity

Similar kind of results were reported by Abdelhady et al. (2016) in ethanolic extract of *Azadirachta indica* but much higher inhibitory potential that caused $37.52 \pm 0.92\%$, $42.56 \pm 3.08\%$, $62.72 \pm 1.83\%$, and $71.35 \pm 3.21\%$

invertase inhibition at 0.025 mg/ml, 0.05 mg/ml, 0.1 mg/ml, and 0.2 mg/ml respectively with IC_{50} value of only 0.068 mg/ml.

Table 2: IC_{50} value of % α -Amylase, % α -Glucosidase and % Invertase inhibition essays

α -Amylase*	α -Glucosidase *	Invertase *
0.56 ± 0.047 mg/ml	$0.68.74 \pm 0.13$ mg/ml	$0.76.64 \pm 0.07$ mg/ml

* IC_{50} (mg/ml)

CONCLUSION

The present study was carried out to investigate the bioactive constituents and antidiabetic potential of crude aqueous extract of *Amaranthus caudatus* (AAE) from Kashmir

valley. The study clearly suggest that the aqueous extract of *Amaranthus caudatus* is rich source of nutraceutical phytochemicals and possess great antioxidant potential. Under *in vitro* conditions AAE causes appreciable

inhibition of α -amylase, α -glucosidase and invertase, the three key enzymes involved in carbohydrate metabolism and thus possess great power to reduce high post-prandial blood glucose levels associated with diabetes and various other associated health problems. Despite these encouraging results, more research in this direction is required especially extensive mechanistic studies of AAE under preclinical and clinical settings are warranted before recommending it as anti-hyperglycemic agent.

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