

INVITRO EVALUATION OF ANTIOXIDANT POTENTIAL OF *PANICUM MILIACEUM*
MICROGREENS: A NOVEL FUNCTIONAL FOOD

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ABSTRACT

Background: Proso millet (*Panicum miliaceum*) is a nutritionally important minor millet known for its health-promoting properties; however, limited information is available on the bioactive potential of its microgreens. Microgreens represent an early growth stage of plants and are reported to contain higher levels of phytochemicals compared to mature grains. The present study aimed to investigate the antioxidant potential of proso millet microgreens and to evaluate whether microgreens exhibit superior antioxidant activity when compared to edible grains. Proso millet microgreens were cultivated under controlled conditions, harvested at the appropriate growth stage, and subjected to phytochemical extraction. The antioxidant activity was assessed using standard in vitro assays, including radical scavenging and reducing power methods. The results of DPPH Radical scavenging assay and ABTS assay of sample demonstrated that proso millet microgreens possessed significantly enhanced antioxidant activity, which may be attributed to the increased accumulation of phenolic compounds and other bioactive constituents during early plant development. These findings indicate that proso millet microgreens have greater antioxidant potential than edible grains and may serve as a promising functional food with health-promoting benefits.

KEYWORDS: Panicum miliaceum, Antioxidant properties, Radical scavenging assay.

INTRODUCTION

Proso millet has nutritional and health benefits and its incorporation in the daily food diet may overcome nutrient deficiencies. Proso millet is rich in protein, phytochemicals, vitamins, minerals, micronutrients including iron, zinc, copper and manganese, and other non-nutritive compounds that provide protection to the plant. Proso millet has high protein content (11.6%), which is within the range with that of wheat (8–15%), although proso millet has higher content of leucine, isoleucine, and methionine.^[1]

Role of Antioxidant Potential of Plants in Human Health

Antioxidants significantly delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate. Antioxidants can be synthesized *invivo* (e.g., reduced glutathione (GSH),

superoxide dismutase (SOD), etc.) or taken as dietary antioxidants. Plants have long been a source of exogenous (i.e., dietary) antioxidants. Plants have an innate ability to biosynthesize a wide range of non-enzymatic antioxidants capable of attenuating ROS-induced oxidative damage. Oxidative stress has been identified as a major causative factor in the development and progression of several life-threatening diseases, including neurodegenerative and cardiovascular diseases. In addition, supplementation with exogenous antioxidants or boosting of endogenous antioxidant defenses of the body has been found to be a promising method of countering the undesirable effects of oxidative stress.^[2]

Nutritional Profile and Health Benefits

- Antioxidant Powerhouse: Proso millet microgreens are rich in phytochemicals, including phenolic

compounds and flavonoids, which aid in fighting oxidative stress.

- Heart and Metabolic Health: They help regulate blood pressure and increase high-density lipoprotein (HDL) cholesterol, aiding in cardiovascular health.
- Nutrient-Dense: They contain significant amounts of essential vitamins and minerals, often in higher concentrations than the mature plant.
- Digestibility: Germination (the sprouting process) increases the bio-accessibility of nutrients by reducing anti-nutritional factors.^[3]

MATERIALS AND METHODS

Plant collection

We collected the Proso millet (Panivaragu) Variety ATL - 1 (scientific name : *Panicum miliaceum* belongs to the family Poaceae from Kerala Agriculture University, College of Agriculture Vellanikkara, Department of Agronomy they were collected from TamilNadu Agricultural University.

Growing of microgreen

- Optimal amount of seeds of *Panicum milliaceum* is taken and soak in water for 1 day. After soaking (6-8 hour) it is tied in a clean cotton cloth for 2 days for germinating.
- Tray is prepared using tissue paper and spread it with water to ensure moisture in it.
- The germinated seeds are transferred to the tray in such a manner it has some space between two seeds. After spreading of seeds, it is covered with a plastic cover in order to avoid the loss of moisture and put in dark for two days.
- After 7-8 days the germination process is complete and seedlings reach a certain height, it is possible to uncover the trays and expose the sprouts to sunlight. A good level of sunlight will ensure optimal growth and a higher accumulation of antioxidants is produced by plants, primarily in response to solar radiation.
- The microgreens were ready to harvest on the 9 th day by using a clean scissor's.^[4]

Extraction method: Maceration using Ethanol

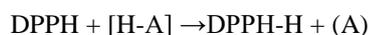
Ethanolic extraction was employed to obtain bioactive compounds from the *Panicum miliaceum* microgreens. The harvested microgreens were rinsed under cold running water to remove adhering soil and debris and were gently blotted dry using a clean towel to eliminate excess moisture. The dried microgreens were then transferred into a clean glass container, and sufficient ethanol (95%) was added to completely immerse the plant material. The container was sealed and allowed to stand for 24–48 h at room temperature with occasional agitation. After the extraction period, the mixture was filtered using a fine mesh strainer or cheesecloth to separate the plant residue. The obtained ethanolic extract was collected and stored in a cool, dark place. When required, the extract was concentrated by evaporation of ethanol either under ambient conditions or using a

temperature-controlled water bath or rotary evaporator operated below the boiling point of ethanol to prevent degradation of phenolic compounds, flavonoids, and other thermolabile constituents.^[5]

Invitro antioxidant assay of ethanolic extract of microgreens of *Panicum miliaceum* (EPPM)

1.) DPPH RADICAL SCAVENGING ASSAY: DPPH radical scavenging assay was done on the ethanolic extract of microgreens of *Panicum milliaceum* (EPPM). The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al [2001]. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used as reference.^[6]

Principle: 1,1-diphenyl-2-picryl hydrazyl is a stable free radical with pink colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,



Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.^[7]

REAGENT PREPARATION: 0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of methanol.

PROCEDURE: Different concentrations of sample such as 12.5µg/mL- 200µg/mL from stock solution were made up to a final volume of 20µl with DMSO and 1.48ml DPPH (0.1mM) solution was added. A control without the test compound, but an equivalent amount of distilled water was taken. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm (SHIMADZU(UV-1900i) UV-VIS spectrophotometer). 3ml of DPPH was taken as control.^[8,9]

CALCULATION: The radical scavenging activity (RSA) is found by,

$$\text{RSA} (\%) = [\text{Control} - \text{test} / \text{Control}] \times 100$$

RESULT

IC 50 Value: EPPM – 39.97 µg/ml (Calculated using ED 50 PLUS V1.0 Software)

Table 1: DPPH assay of Ethanolic extract of *Panicum miliaceum*(EPPM).

Concentrations (µg/ml)	Absorbance	Percentage of Inhibition (%)
Control	0.618	0.0000
Sample code-Proso Millet (EPPM)		
12.5	0.497	19.5793
25	0.400	35.28
50	0.248	59.87
100	0.182	70.55
200	0.140	77.35

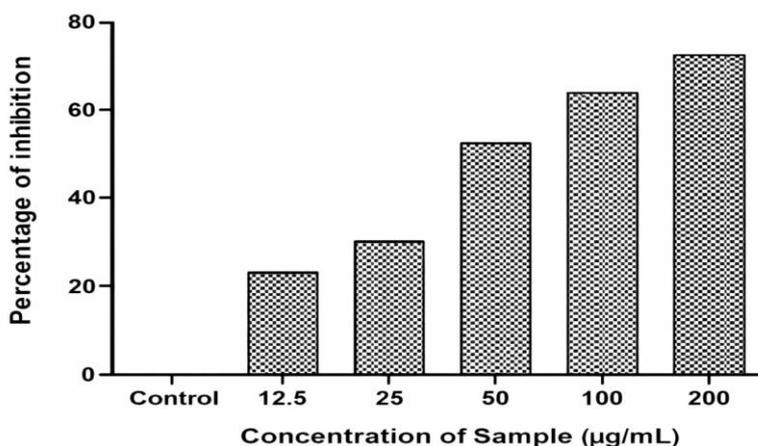


Figure 1: DPPH assay of Ethanolic extract of *Panicum miliaceum* EPPM.

2.) ABTS ASSAY

PRINCIPLE: The preformed radical monocation of 2, 2'azinobis 3 ethyl benzothiazoline 6- sulfonic acid is generated by oxidation of ABTS with potassium persulfate (a blue chromogen) and is reduced in the presence of hydrogen donating antioxidants. Ascorbic acid (10mg/mL) was used as standard.

REAGENTS

Solution I: ABTS, 20mM solution is prepared using H₂O
 Solution II: Potassium persulfate, 17Mm solution is prepared using H₂O
 0.3ml of Solution II was added to 50ml of solution I. The reaction mixture was left to stand at room temperature overnight in dark before use.

PROCEDURE

1mL of distilled water was added to 0.2mL of different concentrations of the sample such as 12.5-200µL. 0.16ml

of ABTS solution was added and made up to the final volume 1.36ml. A control without the test compound, but an equivalent amount of distilled water was taken. Absorbance was measured after 20 minutes at 734nm using a UV-Visible spectrophotometer (SHIMADZU-UV-1900i).^[10]

CALCULATION

$$\text{Percentage of inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

RESULTS

IC₅₀ Value- Proso Millet: 143.26 µg /ml (Calculated using ED50 PLUS V1.0 Software)

Table 2: ABTS assay of Ethanolic extract of *Panicum miliaceum* (EPPM).

Concentration(µg/ml)	Absorbance	Percentage of inhibition
CONTROL	0.465	0.00000
Sample code-Proso Millet (EPPM)		
12.5	0.401	13.76344
25	0.372	20.00000
50	0.315	32.25806
100	0.271	41.72043
200	0.182	60.86022



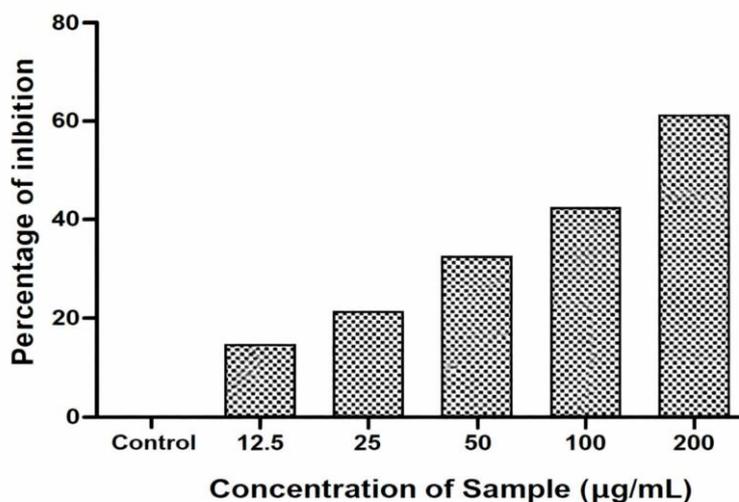


Figure 2: ABTS assay of Ethanolic extract of *Panicum miliaceum* (EPPM).

DISCUSSIONS

Antioxidant capacity of proso millet milled fractions from existing studies.^[11]

Millets	DPPH % inhibition
Whole	31.55±0.09
Dehusked	17.57±0.08
Polished	16.27±0.31

ABTS antioxidant values across proso millet samples were reported as: 3.08 – 9.55 µmol TE/g for whole proso millet flour extracts. These numbers also represent how effectively components in the grain scavenge ABTS free radicals compared with Trolox.^[12]

By comparing the existing study result, percent (%) inhibition of DPPH by the samples were 31.55±0.09% in whole, 17.57±0.08% in dehusked and 16.27±0.31% in polished proso millet grains with the microgreen of proso millet the percentage inhibition is 77.35 indicating that the microgreen have greatest antioxidant potential.

The ABTS⁺ radical scavenging activity of proso millet extract increased in a concentration-dependent manner, showing 60.86% inhibition at 200 µg/ml with an IC₅₀ value of approximately 143.26 µg/ml. Nevertheless, the results confirm that proso millet possesses appreciable natural antioxidant activity, likely due to its phenolic constituents.

CONCLUSION

Small millets, despite their name, are nutritionally rich and offer substantial health benefits. Studies indicate that cultivating small millets in the form of microgreens significantly enhances their nutritional profile, leading to improved levels of bioactive compounds and essential nutrients. The incorporation of millet microgreens into regular diets can therefore provide amplified health benefits. Translating this scientific knowledge from

research findings into everyday dietary practices is essential, as widespread adoption at the household level can contribute meaningfully to the development of a healthier society.

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