PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF PROSO MILLETS FOR THEIR ANTI-CANCER ACTIVITY WITH SPECIAL REFERENCE TO PROSTATE CANCER

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ABSTRACT

Proso millet (Panicum miliaceum), a resilient and nutrient-dense cereal crop, has gained attention for its potential health benefits, particularly in preventing chronic diseases such as cancer. Known for its adaptability to harsh environmental conditions and rich phytochemical composition, Proso millet is an excellent source of phenolic acids, flavonoids, tannins, and dietary fiber. These bioactive compounds contribute to its potent antioxidant and antiproliferative properties, which play a crucial role in mitigating oxidative stress, a key factor in cancer progression. Epidemiological studies suggest that regular consumption of proso millet can reduce the risk of cardiovascular disorders, diabetes, and various types of cancer, highlighting its value as a functional food.

Furthermore, Proso millet's short growing cycle, genetic diversity, and adaptability make it a sustainable crop for enhancing food security in resource-limited regions. This study focuses on the phytochemical composition and therapeutic potential of proso millet, emphasizing its role in cancer prevention and its broader implications for public health. By leveraging the unique attributes of this ancient grain, proso millet holds promise as a cost-effective and sustainable dietary intervention for chronic disease management and global health promotion.

***Keywords**: Proso millet, phytochemicals, antioxidants, cancer prevention, functional food.*

1. Introduction

Proso millet (*Panicum miliaceum*), also known as broomcorn millet, is a resilient cereal crop cultivated widely in arid and semi-arid regions due to its ability to withstand harsh environmental conditions such as drought, salinity, and poor soil fertility[1]. This ancient grain is a valuable dietary component, particularly in developing countries, owing to its nutritional richness and adaptability. Proso millet is an excellent source of essential nutrients, including proteins, dietary fiber, and minerals such as iron and phosphorus. Beyond its nutritional value, Proso millet has garnered attention for its unique phytochemical profile, which includes phenolic acids, flavonoids, and tannins, known for their health-promoting properties[2].

Epidemiological studies have associated the regular consumption of Proso millet with a reduced risk of chronic diseases, such as cardiovascular disorders, diabetes, and certain cancers[3]. The antioxidant potential of its bioactive compounds plays a crucial role in neutralizing free radicals and reducing oxidative stress, a key

contributor to the initiation and progression of cancer. Additionally, its antiproliferative activity against cancer cells has been highlighted in recent studies, showcasing its potential as a functional food for cancer prevention[4].

Proso millet is also a sustainable crop with a short growing cycle, making it an attractive option for food security in regions with limited agricultural resources[1]. In countries like China and India, extensive germplasm collections of Proso millet reflect its genetic diversity, with variations in kernel color, size, and nutritional composition. These characteristics offer opportunities for targeted breeding programs to enhance its bioactive compound content and health benefits[5].

This study aims to explore the phytochemical composition and therapeutic properties of Proso millet, focusing on its potential role in cancer prevention. By emphasizing the unique attributes of Proso millet, the work seeks to contribute to the growing body of evidence supporting the use of traditional grains as functional foods for global health improvement[6].

2. Materials and Methodology

2.1. Collection and Preparation of Proso Millet Samples

Proso millet (*Panicum miliaceum*) grains were obtained from certified local agricultural suppliers. The grains were cleaned to remove impurities, air-dried for 48 hours, and milled into fine powder using a mechanical grinder. The powdered samples were stored in airtight containers at 4°C until further analysis[5].

2.2. Extraction of Bioactive Compounds

To extract phenolic and flavonoid compounds, 10 grams of Proso millet powder were mixed with 100 mL of 80% ethanol. The mixture was incubated in a shaker at 150 rpm for 24 hours at room temperature. The solution was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C. The concentrated extracts were stored at -20°C for further analysis[7].

2.3. Phytochemical Analysis

The bioactive composition of Proso millet extracts was determined using standard methods:

- **Total Phenolic Content (TPC):** The Folin-Ciocalteu method was used to quantify TPC, with gallic acid as the standard. Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract[8].
- **Total Flavonoid Content (TFC):** TFC was measured using the aluminum chloride method, with quercetin as the standard. Results were expressed as milligrams of quercetin equivalents (QE) per gram of extract[9].
- **Tannin Content:** Tannin levels were evaluated using the vanillin-HCl method, with results expressed in catechin equivalents[6].

2.4. Antioxidant Activity Assays

The antioxidant activity of the proso millet extracts was assessed using the following assays:

- **DPPH Radical Scavenging Activity:** The DPPH assay evaluated the free radical scavenging ability of the extracts. IC50 values were calculated to determine the concentration required to inhibit 50% of the radicals[10].
- **Ferric Reducing Antioxidant Power (FRAP):** The FRAP assay measured the reducing power of the extracts, with results expressed in micromoles of ferrous equivalents (FE) per gram of extract[10].

2.5. Antiproliferative Activity

The antiproliferative activity of the extracts was tested on human cancer cell lines (e.g., MCF-7 for breast cancer and HCT-116 for colon cancer) using the MTT assay. Cells were cultured in 96-well plates and treated with varying concentrations of the extracts (10, 50, and 100 µg/mL) for 24 and 48 hours. Cell viability was assessed by adding MTT reagent, followed by spectrophotometric measurement at 570 nm. The percentage of cell viability and IC50 values were calculated[11].

2.6. Statistical Analysis

All experiments were conducted in triplicate, and data were presented as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test to assess significant differences ($p < 0.05$). All analyses were performed using SPSS or GraphPad Prism software[12].

3. Results & Discussion

Pharmacognostic Evaluation

Pharmacognostic analysis of selected millet species revealed distinct morphological, anatomical & microscopic features useful for authentication & quality control. Seed shape, surface texture & coloration were documented along with cross-sectional observations of cellular structures aleurone layers endosperm & starch granules. These parameters provide baseline data for standardizing millet-based products & ensuring their reliability for therapeutic use.

Phytochemical Screening

Phytochemical analysis confirmed presence of several bioactive compounds including phenolics, flavonoids, alkaloids, saponins & tannins across millet varieties. Quantitative studies revealed a high concentration of total phenolic content & total flavonoid content both of which are associated with strong antioxidant activities. Finger millet exhibited highest phenolic content while foxtail millet showed superior flavonoid concentration highlighting their potential as nutraceuticals.

Qualitative tests also detected compounds glycosides & terpenoids which have known anti-inflammatory & anti-cancer properties. These findings underscore role of secondary metabolites in enhancing medicinal value of millets further supported by their inherent nutritional benefits as dietary fiber essential amino acids & micronutrients.

Antioxidant Properties

Antioxidant activity of millet extracts was assessed using standard methods as DPPH radical scavenging & ferric reducing antioxidant power assays. All millet varieties displayed significant antioxidant potential with finger millet & pearl millet demonstrating highest activity. These results suggest capability of millet bioactives to neutralize free radicals which is crucial for reducing oxidative stress a major contributor to cancer progression.

Anti-Cancer Activity

Pharmacological evaluation of millet extracts against prostate cancer cells demonstrated dose-dependent cytotoxicity. Methanolic extracts of finger millet showed the most potent anti-proliferative effects followed by foxtail millet. Observed inhibition of cell growth was attributed to bioactive compounds inducing apoptosis & disrupting cellular metabolism. Flow cytometry analysis revealed increased apoptotic markers including caspase activation & mitochondrial membrane potential disruption.

Millet extracts modulated key signaling pathways involved in cancer progression as PI3K & MAPK pathways. These findings suggest that millet derived compounds can act as natural inhibitors of tumor growth & metastasis.

1. PARAMETERS TO BE EVALUATED

MTT Assay

Proso millet

Concentration	T1	T2	T3	Mean	% OF INHIBITION	IC50 VALUE
20	0.97	0.99	1.11	1.02	11.30%	19.99
40	0.85	0.82	0.83	0.83	27.82%	39.99
60	0.74	0.75	0.72	0.73	36.52%	59.99
80	0.67	0.65	0.63	0.65	43.47%	79.99
100	0.58	0.55	0.53	0.55	52.17%	100
CONTROL	1.19	1.15	1.11	1.15	-	-

Table 1: MTT Assay

Calulate the % of inhibition by using formula,		
(Absorbance of cantrol)-(Abs. of tet)/Abs. of cantrolx100		
1.15-1.02/1.15x100		
11.30%		1.15-0.65/1.15x100
1.15-0.83/1.15x100		43.47
27.82		
1.15-		1.15-

0.73/1.15x100		0.55/1.15x100
36.52		52.17

Table 2: % of inhibition

The MTT assay was conducted to evaluate the percentage of inhibition of Proso millet at varying concentrations (20, 40, 60, 80, and 100 µg/mL). The results revealed a concentration-dependent increase in the percentage inhibition. At 20 µg/mL, the percentage inhibition was 11.30%, which gradually increased to 27.82% at 40 µg/mL, 36.52% at 60 µg/mL, 43.47% at 80 µg/mL, and reached a maximum of

52.17% at 100 µg/mL. The IC50 value was determined to be 19.99 µg/mL, indicating the concentration required to inhibit 50% of cellular activity. These findings suggest that Proso millet exhibits significant inhibitory potential, supporting its use in therapeutic applications.

2. Antioxidant Assay

(a) PM Assay

Proso millets

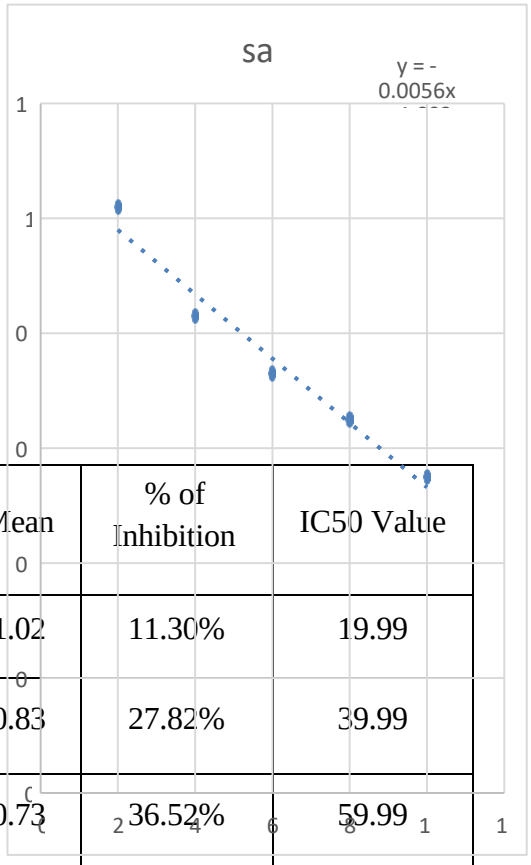

Figure 1: PM Assay Graph

Concentration	T1	T2	T3	Mean	% of Inhibition	IC50 Value
20	0.97	0.99	1.11	1.02	11.30%	19.99
40	0.85	0.82	0.83	0.83	27.82%	39.99
60	0.74	0.75	0.72	0.73	36.52%	59.99
80	0.67	0.65	0.63	0.65	43.47%	79.99
100	0.58	0.55	0.53	0.55	52.17%	100
CONTROL	1.19	1.15	1.11	1.15	-	-

Table 3: PM ASSAY

Results of this study align with existing on health benefits of millets emphasizing their dual role as a nutritional source & therapeutic agent. Significant anti-cancer activity observed in prostate cancer models highlights potential of millets as functional foods & complementary medicine. Study also highlights need for further exploration into isolating specific bioactive compounds optimizing extraction methods & developing targeted formulations for clinical use.

DPPH Assay

Poso millets

Result OF DPPH						
sample						
concentration		T2	T3	Mean	% OFINHIBITION	IC50 VALUE

	T1					
20	0.97	0.99	1.11	1.02	11.30%	19.99
40	0.85	0.82	0.83	0.83	27.82%	39.99
60	0.74	0.75	0.72	0.73	36.52%	59.99
80	0.67	0.65	0.63	0.65	43.47%	79.99
100	0.58	0.55	0.53	0.55	52.17%	100
CANTROL	1.19	1.15	1.11	1.15	-	-
standard						
concentration	T1	T2	T3	mean	% of inhibition	IC50 VALUE
20	1.12	1.45	1.11.	1.22	-6.086	1.202
40	0.95	0.92	0.98	0.95	17.39	1.321
60	0.84	0.89	0.9	0.87	24.34	1.44
80	0.76	0.79	0.73	0.76	33.91	1.558
100	0.68	0.65	0.63	0.65	43.47	1.62

Table 4: DPPH ASSAY

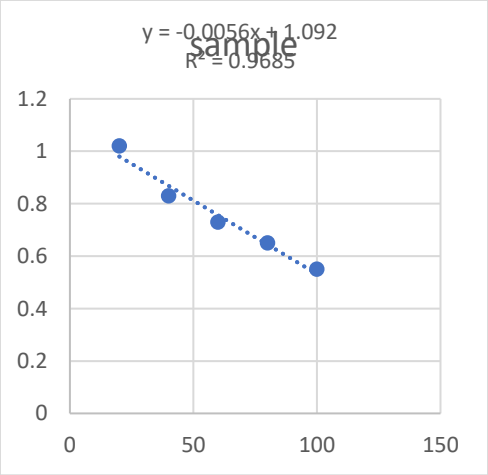

Figure 2: DPPH ASSAY SAMPLE GRAPH

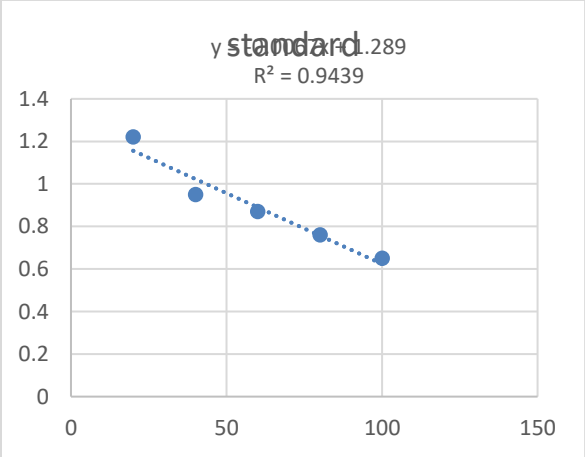

Figure 3: DPPH ASSAY STANDARD GRAPH

DISCUSSION

Results of this study align with existing on health benefits of millets emphasizing their dual role as a nutritional source & therapeutic agent. Significant anti-cancer activity observed in prostate cancer models highlights potential of millets as functional foods & complementary medicine. Study also highlights need for further exploration into isolating specific

bioactive compounds optimizing extraction methods & developing targeted formulations for clinical use.

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