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

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ABSTRACT

Cancer is a major global health challenge, contributing significantly to morbidity, mortality, and economic burden worldwide. It arises from disruptions in normal cellular processes, leading to uncontrolled cell proliferation, tumor formation, and metastasis. While advancements in conventional cancer therapies have improved outcomes, they are often associated with significant side effects and limitations, highlighting the need for alternative approaches. Medicinal plants and their bioactive compounds have emerged as promising candidates in cancer prevention and management due to their antioxidant, anti-inflammatory, and antiproliferative properties.

Millets, a group of drought-resistant cereal crops, have gained attention for their exceptional nutritional and therapeutic potential. Finger millet (ragi) and Kodo millet are rich in polyphenols, tannins, flavonoids, and phenolic acids, compounds known for their potent antioxidative properties. These bioactive substances play a crucial role in neutralizing oxidative stress, modulating signaling pathways, and inducing apoptosis in cancer cells. Epidemiological studies indicate that regular consumption of millets may reduce the risk of chronic diseases, including cancer, cardiovascular disorders, and diabetes. Among these, Kodo millet exhibits superior antioxidant capacity, attributed to its high content of phenolics and tannins.

This study highlights the unique phytochemical profiles and anticancer potential of millets, emphasizing their role as a cost-effective and sustainable dietary intervention for cancer prevention. By exploring the nutritional and therapeutic benefits of millets, this work aims to contribute to the growing body of evidence supporting the integration of functional foods into cancer management strategies. Further research is warranted to elucidate the mechanisms underlying the anticancer properties of millets and to optimize their utilization in global health initiatives.

Keywords: *Cancer prevention, Millets, Phytochemicals, Antioxidants, Functional foods*

1. Introduction

Cancer is one of the leading causes of death worldwide, posing a significant burden on healthcare systems and individuals. Characterized by uncontrolled cell growth and the potential to invade nearby tissues or metastasize to distant organs, cancer arises from a combination of genetic, environmental, and lifestyle factors[1]. While advancements in conventional therapies such as surgery, chemotherapy, and radiation have improved survival rates, these treatments often come with

considerable side effects and limited efficacy for advanced stages of the disease[2]. This has driven growing interest in complementary approaches, particularly the use of natural products and plant-based diets, which have shown promise in cancer prevention and management due to their bioactive compounds[3].

Millets, a group of ancient, drought-resistant cereal crops, have gained attention for their nutritional and therapeutic potential. Among them, **finger millet (Eleusine coracana)** and **Kodo millet (Paspalum scrobiculatum)** stand out for their rich phytochemical profiles and health-promoting properties[4]. Finger millet, commonly known as ragi, is a staple food in many developing regions, particularly in India and Africa. It is rich in polyphenols, tannins, and phenolic acids, which exhibit potent antioxidant activity[5]. These bioactive compounds protect cells from oxidative stress, a major factor in cancer development, and contribute to the modulation of signaling pathways involved in tumor growth[6]. Additionally, the high dietary fiber content of finger millet supports overall metabolic health, reducing the risk factors associated with chronic diseases, including cancer and diabetes[7].

Kodo millet, indigenous to tropical regions of Africa and India, is another nutritionally dense grain with significant therapeutic potential. Known for its robust antioxidant properties, Kodo millet contains high levels of phenolic compounds, tannins, and flavonoids, which help combat oxidative damage and inflammation—key processes in cancer progression[8]. Unlike many other cereals, Kodo millet is particularly effective in managing metabolic disorders such as diabetes, which often coexist with cancer[9]. Its unique phytochemical composition not only supports oxidative stability but also interferes with cancer cell signaling pathways, inducing apoptosis and inhibiting tumor growth[10].

This study focuses on the anticancer potential of finger millet and Kodo millet, highlighting their phytochemical composition, antioxidant properties, and role in reducing cancer risk. By emphasizing these two underutilized grains, this work aims to shed light on their relevance as functional foods and their integration into dietary strategies for cancer prevention[11].

2. Materials and Methodology

2.1. Collection and Preparation of Millet Samples

Finger millet (*Eleusine coracana*) and Kodo millet (*Paspalum scrobiculatum*) grains were procured from certified local agricultural suppliers. The grains were cleaned thoroughly to remove dirt, debris, and impurities, and then air-dried at room temperature for 48 hours. The dried grains were milled into a fine powder using a mechanical grinder and stored in airtight containers at 4°C until further use[12].

2.2. Extraction of Bioactive Compounds

For the extraction of polyphenols and flavonoids, 10 grams of the powdered samples were subjected to solvent extraction. Each sample was mixed with 100 mL of 70% ethanol and incubated in a shaker at 150 rpm for 24 hours at room temperature. The mixture was then filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C. The concentrated extracts were stored at -20°C for subsequent analysis[13].

2.3. Phytochemical Analysis

The presence of polyphenols, tannins, flavonoids, and phenolic acids in the extracts was assessed using standard qualitative and quantitative methods.

- **Total Phenolic Content (TPC):** TPC was determined using the Folin-Ciocalteu assay, with gallic acid as the standard. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract[14].
- **Total Flavonoid Content (TFC):** TFC was measured using the aluminum chloride colorimetric method, with quercetin as the standard. Results were expressed as milligrams of quercetin equivalents (QE) per gram of extract.
- **Tannin Content:** Tannin content was quantified using the vanillin-HCl assay, with catechin as the standard[15].

2.4. Antioxidant Activity

The antioxidant activity of the millet extracts was evaluated using two assays:

- **DPPH Radical Scavenging Assay:** The scavenging ability of the extracts against DPPH radicals was measured spectrophotometrically, and the results were expressed as IC50 values (concentration required to inhibit 50% of DPPH radicals)[16].
- **Ferric Reducing Antioxidant Power (FRAP):** The reducing power of the extracts was determined using the FRAP assay, with results expressed as micromoles of ferrous equivalents (FE) per gram of extract[16].

2.5. Antiproliferative Activity

The antiproliferative potential of the extracts was tested against selected human cancer cell lines (e.g., HeLa for cervical cancer and PC-3 for prostate cancer) using the MTT assay. Briefly, cancer cells were cultured in 96-well plates and treated with varying concentrations of the millet extracts (10, 50, and 100 µg/mL) for 24 and 48 hours. Cell viability was assessed by adding MTT reagent, and absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability and IC50 values were calculated for each extract[17].

2.6. Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean ± standard deviation. Statistical analysis was conducted using one-way ANOVA followed by Tukey's post-hoc test to determine significant differences between groups ($p < 0.05$). Statistical software (e.g.,

SPSS or GraphPad Prism) was used for all analyses[18].

3. Results

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.

Comparative Analysis

A comparative analysis of millet varieties indicated variations in their bioactive compound composition & anti-cancer

efficacy. Finger millet consistently emerged as most effective owing to its higher antioxidant capacity & bioactive content. However other millets foxtail & pearl millet also exhibited noteworthy activities supporting potential of a multi-grain approach in dietary & therapeutic applications.

PARAMETERS TO BE EVALUATED

MTT Assay

1. Finger millet

MTT assay results demonstrated the cytotoxic effects of both the standard and the test sample on the evaluated cell lines, with a dose-dependent increase in cell inhibition and a corresponding decrease in cell viability. For the standard, at a concentration of 20 µg/mL percentage of inhibition was 22.34% with 77.66% cell viability, which progressively increased to 86.58% inhibition and 13.42% viability at 120 µg/mL. The IC_{50} value for the standard was calculated as 45.984 µg/mL, indicating its potency in inhibiting 50% of cell viability at a relatively.

Sample code	Conc.	OD	OD	OD	Mean	% of inhibition	% of viability	IC50
		1	2	3				
Control			1.549					
Standard	20	1.203	1.205	1.201	1.203	22.34%	77.66%	
	40	0.798	0.799	0.797	0.798	48.49%	51.51%	
	60	0.499	0.498	0.497	0.498	67.86%	32.14%	45.984
	80	0.326	0.323	0.325	0.324	79.09%	20.91%	
	100	0.219	0.219	0.218	0.218	86%	14.07%	
	120	0.207	0.208	0.211	0.208	86.58%	13.42%	
Sample	20	1.318	1.316	1.317	1.317	14.98%	85.02%	
	40	1.242	1.244	1.246	1.244	19.70%	80.30%	
	60	1.063	1.065	1.062	1.063	31.38%	68.62%	
	80	0.815	0.812	0.816	0.814	47.45%	52.55%	86.896
	100	0.618	0.616	0.621	0.618	60.11%	39.89%	
	120	0.476	0.474	0.478	0.476	69.28%	30.72%	

Table 1: MTT ASSAY FOR FINGER MILLET

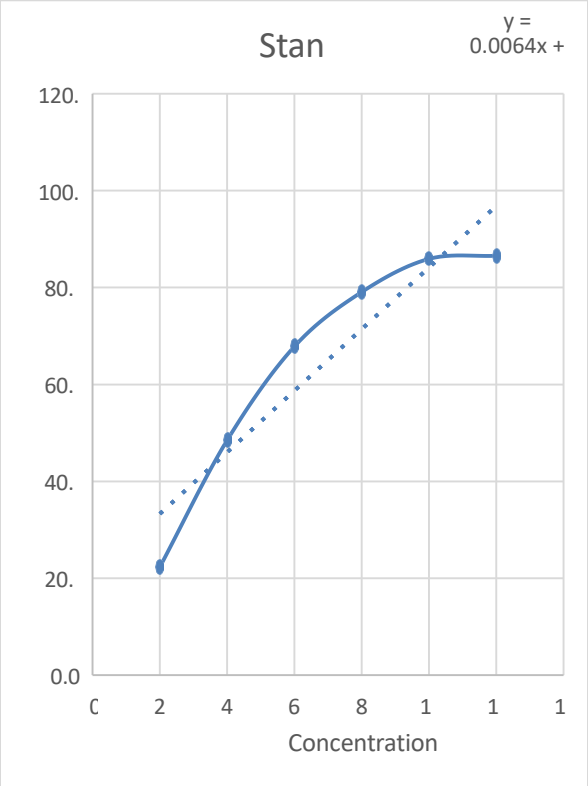


Figure 1:FINGER MILLET MTT ASSAY STANDARD GRAPH

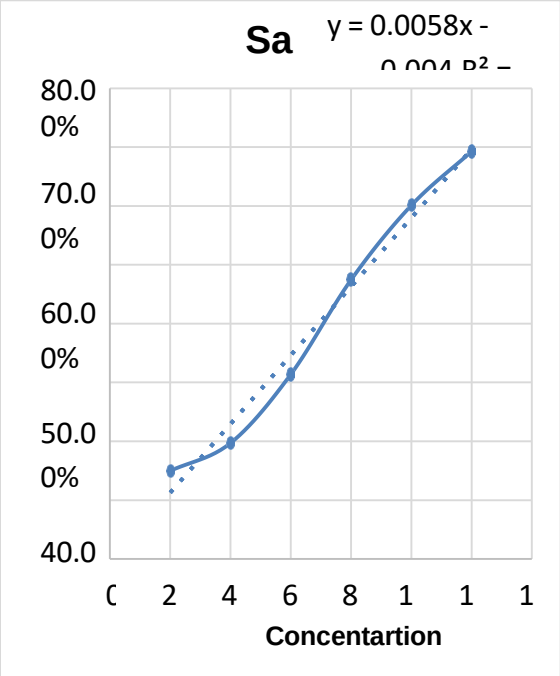


Figure 2: FINGER MILLET MTT ASSAY SAMPLE

Table 2 - Kodo millet

Sr No	Sample Code	Conc. (µg/ml)	OD			Mean	% of Inhibition	% of Viability	IC50 (µg/ml)
1	Control		1.31			-	-	-	-
2	Standard	20	0.731	0.732	0.731	0.731	44.19%	55.80%	36.51
	(5, Flurouracil)	40	0.625	0.626	0.625	0.625	52.29%	47.70%	
		60	0.554	0.553	0.554	0.554	57.70%	42.29%	
		80	0.438	0.437	0.438	0.438	66.56%	33.43%	

		100	0.276	0.277	0.276	0.276	78.39%	21.06%	
3	CB	20	1.21	1.211	1.21	1.21	7.63%	92.36%	
		40	0.989	0.989	0.99	0.989	24.50%	75.49%	
		60	0.781	0.782	0.781	0.781	40.38%	59.61%	79.41
		80	0.639	0.64	0.639	0.639	51.22%	48.77%	
		100	0.507	0.506	0.507	0.507	61.29%	38.70%	

Table 2: KODO MILLET MTT ASSAY

Control exhibited an optical density (OD) of 1.31 representing 100% cell viability. For standard a dose-dependent reduction in OD was observed with 44.19% inhibition and 55.80% viability at 20 µg/mL increasing to 78.39% inhibition and 21.06% viability at 100 µg/mL. The IC_{50} value for the standard was calculated as 36.51 µg/mL demonstrating its potency as an anti-cancer agent.

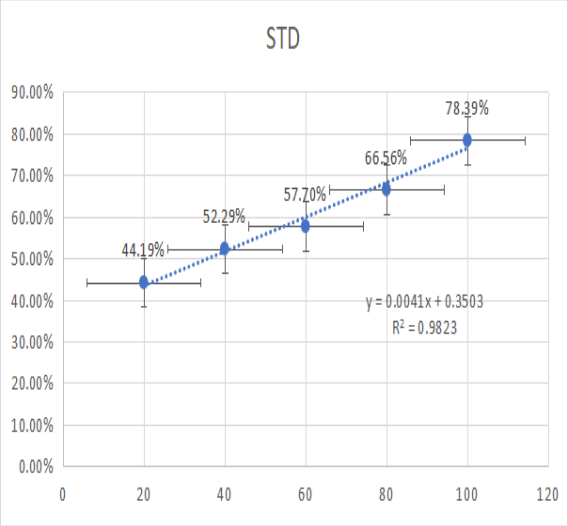


Figure 3: KODO MILLET MTT ASSAY SAMPLE GRAPPH

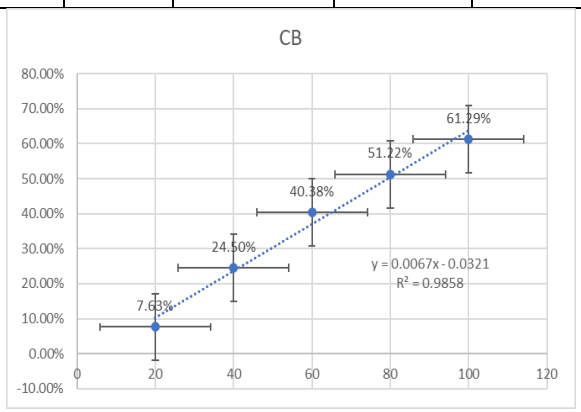


Figure 4: KODO MILLET MTT ASSAY STANDARD GRAPH

Test sample (CB) also showed a concentration-dependent increase in inhibition but with lower potency compared to standard. At 20 µg/mL inhibition was 7.63% with a viability of 92.36%. As concentration increased to100 µg/mL inhibition reached 61.29% with 38.70% cell viability. The IC_{50} value for the test sample was determined to be 79.41 µg/mL indicating that higher concentrations are required to achieve comparable effects to the standard.

1. Antioxidant Assay

(a) PM Assay Finger millet

Figure 1: KODO MILLET MTT ASSAY STANDARD GRAPH

Sample code	conc.	Absorbance at 510nm			Mean	% Inhibition	IC50
		1	2	3			
Control		1.51	1.51	1.51			
Standard	20	1.47	1.46	1.48	1.47	2.64%	
	40	1.36	1.38	1.34	1.36	9.93%	
	60	1.06	1.04	1.08	1.06	29.80%	
	80	0.91	0.92	0.93	0.92	39.07%	89.875
	100	0.61	0.63	0.59	0.61	59.60%	
Sample	20	1.46	1.48	1.44	1.46	3.31%	
	40	1.4	1.42	1.4	1.4	7.28%	
	60	1.3	1.31	1.32	1.31	13.24%	
	80	1.08	1.06	1.1	1.08	28.47%	135.39
	100	0.98	0.96	0.98	0.97	35.76%	

Table 3: FINGER MILLET PM ASSAY

PM assay results assess the cytotoxic potential of the standard and the test sample at various concentrations by measuring absorbance at 510 nm and calculating percentage inhibition. Control exhibited an absorbance of 1.51, indicating 100% viability.

For standard a dose-dependent increase in inhibition was observed with 2.64% inhibition at 20 µg/mL and a mean absorbance of 1.47. This inhibition increased significantly to 59.60% at100 µg/mL with a mean absorbance of 0.61. The IC_{50} value for the standard was calculated as 89.875 µg/mL suggesting its potency in reducing cell viability.

The test sample showed a less pronounced cytotoxic effect compared to the standard. At 20 µg/mL the sample exhibited 3.31% inhibition with a mean absorbance of 1.46. At 100 µg/mL the inhibition increased to 35.76%, with a mean absorbance of 0.97. The IC_{50} value for the test sample was determined to be 135.39 µg/mL indicating lower efficacy compared to the standard.

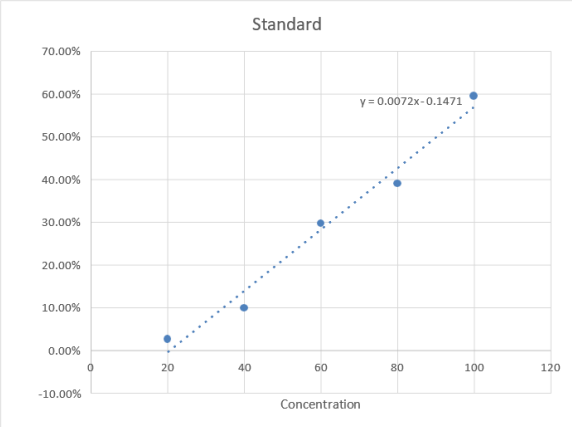


Figure 5: FINGER MILLET PM ASSAY STANDARD GRAPH

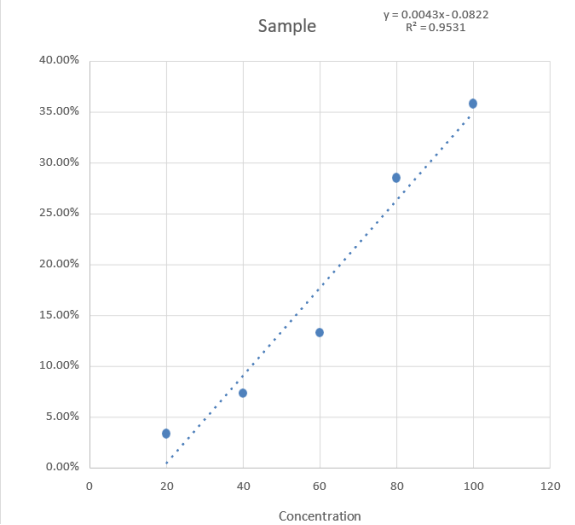


Figure 6: FINGER MILLET PM ASSAY SAMPLE GRAPH

Kodo millet

Table 4: KODO MILLET PM ASSAY

Sr. No	Sample Code	Concentration (µg/ml)	Absorbance at 695nm				% Inhibition	IC50 (µg/ ml)
			Test 1	Test 2	Test 3	Mean		
1	Control	-	1.52	1.52	1.52			
2	Standard	20	1.35	1.36	1.36	1.36	10.52%	37.54

	(Ascorbic Acid)	40	1.26	1.27	1.27	1.27	16.44%	
		60	1.07	1.05	1.09	1.07	29.60%	
		80	0.92	0.93	0.94	0.93	38.81%	
		100	0.62	0.64	0.6	0.62	59.21%	
4	CB	20	1.46	1.44	1.44	1.44	5.26%	
		40	1.36	1.35	1.36	1.36	10.52%	
		60	1.25	1.24	1.24	1.24	18.42%	50.83
		80	1.07	1.09	1.05	1.07	29.60%	
		100	0.92	0.9	0.94	0.92	39.40%	

PM assay results demonstrated the inhibitory effects of both the standard (ascorbic acid) and the test sample (CB) on cell viability, as measured by absorbance at 695 nm. The control group exhibited a stable absorbance of 1.52, representing 100% cell viability.

The standard (ascorbic acid) showed a clear dose-dependent inhibition, with 10.52% inhibition observed at 20 µg/mL and increasing to 59.21% inhibition at 100 µg/mL. The IC_{50} value for the standard was calculated as 37.54 µg/mL, indicating its high potency in reducing cell viability. These results confirm the standard's effectiveness as a reference compound for assessing cytotoxic activity.

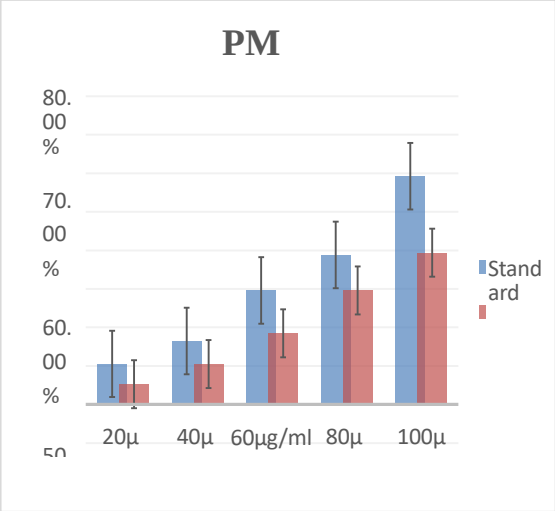


Figure 7: KODO MILLET PM ASSAY COMPARATIVE GRAPH

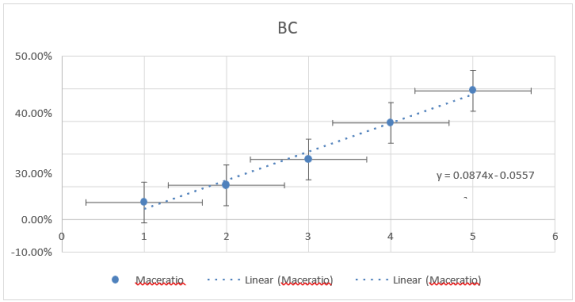


Figure 8: KODO MILLET PM ASSAY SAMPLE GRAPH

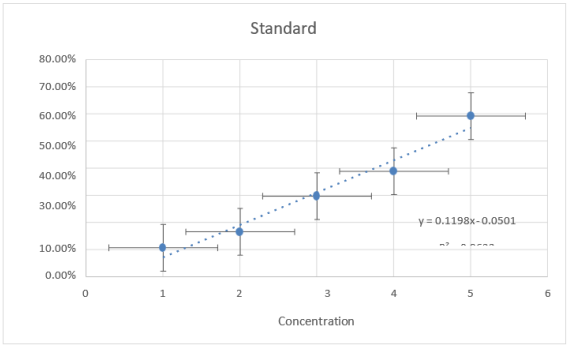
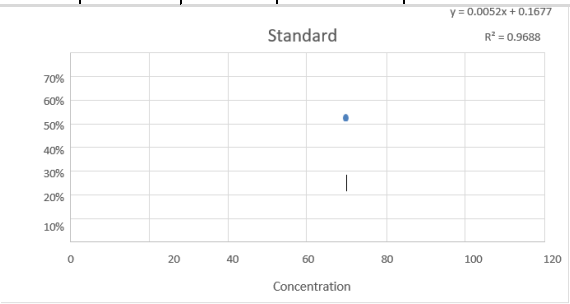


Figure 9: KODO MILLET PM ASSAY STANDARD GRAPH

Sampl e code	Conc.	sorbance at 510nm			Mean	% Inhibition	IC50	
		1	2	3				
Contro l		1.92	1.92	1.92	1.92			
Standa rd	20	1.45	1.44	1.45	1.44	25%		
	40	1.21	1.19	1.21	1.2	38%		
	60	0.9	0.91	0.9	0.9	53.12%		
	80	0.82	0.83	0.82	0.82	57.29%	63.9	
	100	0.63	0.63	0.63	0.63	67.18%		
Sampl e	20	1.62	1.63	1.64	1.63	15.10%		
	40	1.5	1.49	1.52	1.5	21.87%		
	60	1.46	1.44	1.47	1.45	24.47%	97.26	

	80	1.06	1.04	1.07	1.05	45.31%		
	100	0.9	0.91	0.93	0.91	52.60%		



DPPH Assay Finger millet

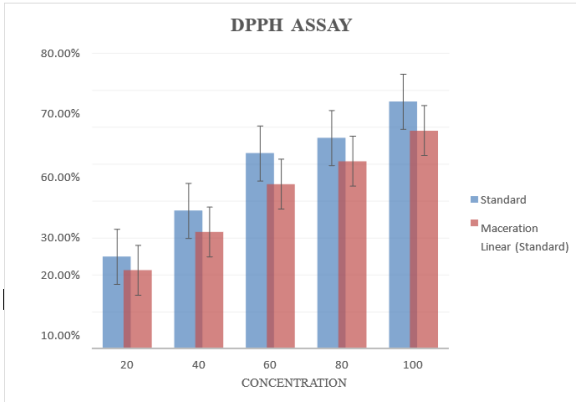


Figure 10: KODOD MILLET DPPH ASSAY COMPARATIVE GRAPH

For the standard, a dose-dependent increase in antioxidant activity was observed. At 20 µg/mL % inhibition was 25% with a mean absorbance of 1.44. As concentration increased % inhibition increased significantly reaching 67.18% at 100 µg/mL with a mean absorbance of 0.63. IC_{50} value for standard was calculated to be 63.9 µg/mL suggesting a moderate antioxidant potential.

For the test sample DPPH assay also showed dose-dependent inhibition, although it was less potent than the standard. At 20 µg/mL % inhibition was 15.10% with a mean absorbance of 1.63. % inhibition gradually increased, reaching 52.60% at 100 µg/mL with a mean absorbance of 0.91. IC_{50} value for the sample was determined to be 97.26 µg/mL, indicating that higher concentrations are required to achieve significant antioxidant activity compared to the standard.

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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