Indian Journal of Plant Sciences ISSN: 2319–3824
Online, International Journal, Available at http://www.cibtech.org/jps.htm
2026 Vol.15, pp.159-168/Renu et al.
Research Article (Open Access)

SPROUTING-MEDIATED MODULATION OF NUTRITIONAL, ANTINUTRITIONAL, ANTIOXIDANT AND CYTOTOXIC PROPERTIES OF FINGER MILLET (ELEUSINE CORACANA (L.) GAERTN.) FROM SEED TO MICROGREEN STAGE

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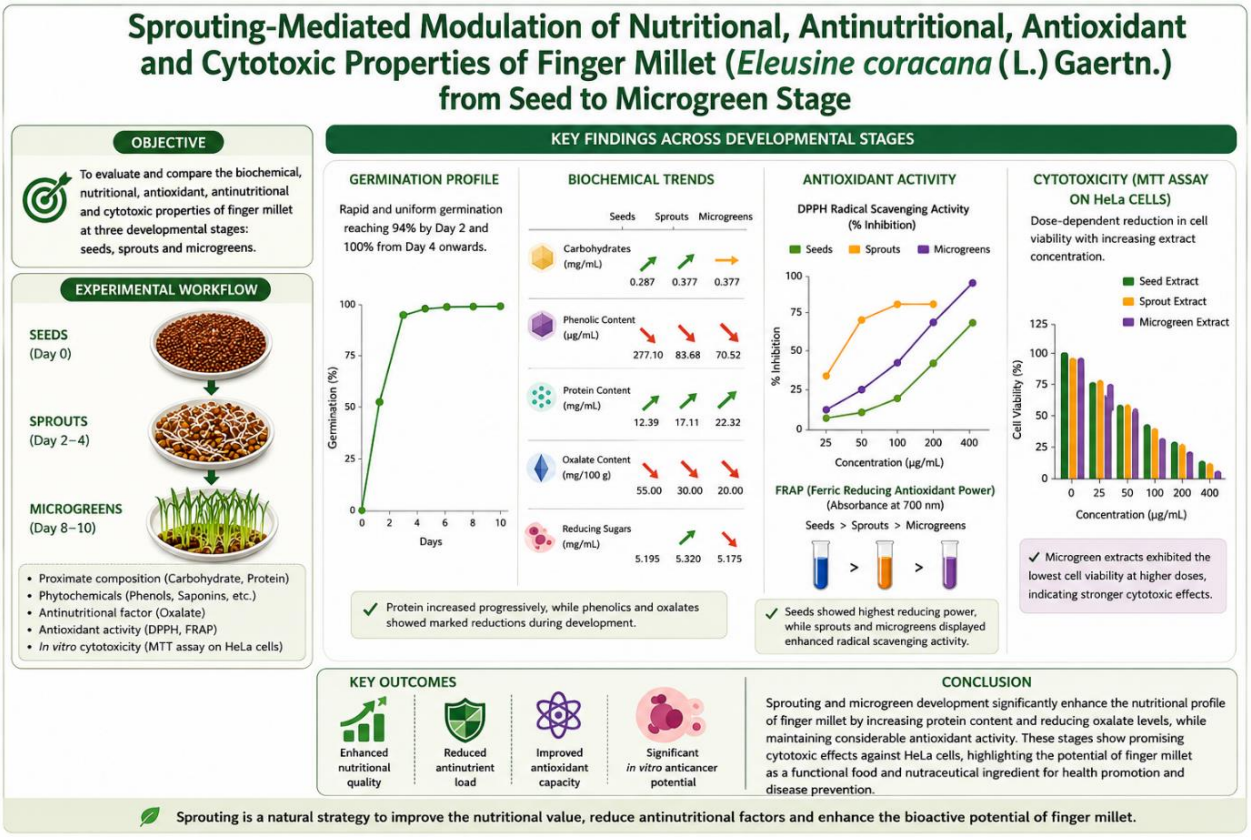
ABSTRACT

Finger millet (Eleusine coracana (L.) Gaertn.) is a nutritionally important millet recognized for its high mineral content, dietary fibre, and bioactive phytochemicals. The present study evaluated biochemical, nutritional, antioxidant, antinutritional, and cytotoxic changes occurring during the transition from seed to sprout and microgreen stages. Germination progressed rapidly, achieving 100% germination by the fourth day under controlled conditions. Quantitative analyses revealed significant developmental changes in metabolite composition. Total carbohydrate content increased from 0.287 mg mL−1 in seeds to 0.377 mg mL−1 in sprouts and remained stable in microgreens. Protein content increased progressively from 12.39 to 22.32 mg mL−1 across developmental stages, whereas phenolic content declined from 277.10 to 70.52 µg mL−1.



Oxalate concentration showed a substantial reduction from 55 mg 100 g−1 in seeds to 20 mg 100 g−1 in microgreens. Antioxidant assays demonstrated stage-dependent variation, with seeds exhibiting higher ferric reducing antioxidant power, while sprouts and microgreens displayed enhanced radical scavenging

activity under selected concentrations. Cytotoxicity assessment using HeLa cells revealed a concentration-dependent reduction in cell viability. The findings demonstrate that germination and microgreen production improve the nutritional profile of finger millet and support its utilization in functional foods and nutraceutical formulations.

Keywords: *Antioxidant activity; Antinutrients; Cytotoxicity; Eleusine coracana; Functional foods; Germination; Microgreens; Nutritional quality; Oxalates; Phenolic compounds; Protein content; Sprouting*

INTRODUCTION

Millets, particularly *Eleusine coracana* (finger millet or ragi), have gained renewed global attention as sustainable, nutrient-rich crops capable of addressing contemporary challenges in nutrition and food security. Long valued across regions of Africa and South Asia, finger millet is characterized by its exceptional content of dietary fibre, calcium, iron, essential amino acids, polyphenols, vitamins, and bioactive antioxidants (Jena *et al.,* 2023; Srilekha *et al.,* 2019). Traditionally consumed in rural and tribal communities, the crop has now emerged as a key candidate for combating micronutrient deficiencies, lifestyle disorders, and climate-linked agricultural vulnerabilities in developing nations such as India (Kaur *et al.,* 2024; Vetriventhan *et al.,* 2021). Its outstanding drought tolerance, resilience to marginal soils, and ability to withstand climatic fluctuations further highlight its role in fostering climate-smart agricultural systems (Maharajan *et al.,* 2022).

In parallel, there has been a significant rise in the popularity and scientific interest in microgreens, which are tender seedlings harvested shortly after the emergence of the first true leaves. Microgreens of several cereals, vegetables, and herbs are recognized for their enhanced nutrient density, short growth cycle, minimal input requirements, and considerable potential for urban and home-scale cultivation (Janovská *et al.,* 2010; Kumar *et al.,* 2016). Owing to their high concentrations of vitamins, minerals, phytonutrients, and bioactive compounds, they have been promoted as functional foods capable of contributing to improved dietary quality and reduced risk of chronic diseases. Recent studies highlight their ability to offer superior antioxidant activity and therapeutic potential compared to their mature counterparts (Bhaswant *et al.,* 2023). Despite this progress, scientific information on millet microgreens remains limited, particularly regarding the biochemical transitions that occur during the shift from dry seed to sprout and microgreen stages. Such transitions involve enzymatic activation, reduction of antinutrients, mobilization of carbohydrate reserves, synthesis of phenolic compounds, and enhancement of antioxidant activity—processes that significantly influence the nutritional and functional attributes of the edible product. Moreover, understanding changes in oxalate content, an antinutrient of clinical significance for individuals with renal complications, is important for assessing dietary safety. These knowledge gaps underscore the need for comprehensive studies that examine the nutritional, phytochemical, antinutritional, and therapeutic properties of finger millet at different developmental stages.

Despite increasing interest in finger millet as a functional food crop, comparative information describing biochemical, nutritional, antioxidant, and antinutritional changes across seed, sprout, and microgreen stages remains limited. Most previous investigations have focused either on grain composition or on the effects of germination alone, while the microgreen stage has received comparatively little attention. Therefore, the present study aimed to evaluate stage-specific changes in phytochemical composition, nutritional quality, antioxidant activity, oxalate content, and cytotoxic potential of finger millet from seed to microgreen stage. Special emphasis is placed on assessing oxalate deposition, total phenols, reducing sugars, proteins, and carbohydrates, alongside evaluating the anticancer potential of finger millet microgreens through in vitro analysis. By generating comparative data across developmental phases, this study aims to bridge the existing gap in millet microgreen research and contribute to the growing knowledge base on functional foods that support sustainable nutrition, therapeutic applications, and future food security.

MATERIALS AND METHODS

Sample Collection

Eleucine coracana seeds were purchased from local market and germinated to microgreen stage for various analytical studies performed in the Botany laboratory of Union Christian College, Aluva .

A) Germination Percentage

Germination percentage (GP) was used to assess seed viability using the formula: **GP = (Number of seeds germinated / Total seeds) × 100**. Fifty finger millet seeds were soaked for 1 h, placed on moistened filter paper in sterile Petri dishes, and incubated in the dark. Germinated seeds were counted and removed daily (Fig.1). Germination rate was evaluated by plotting GP at successive time intervals.

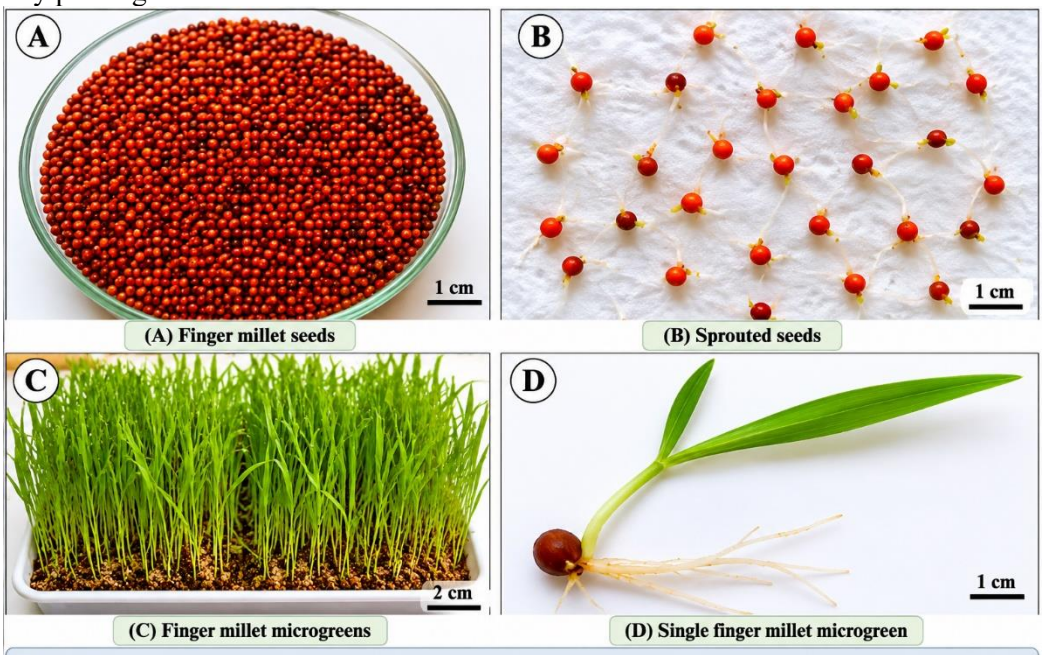


Figure 1. Different developmental stages of finger millet (*Eleusine coracana* (L.) Gaertn.) from seed to microgreen stage.

B) Qualitative Phytochemical Screening

Standard phytochemical tests were performed on plant extracts. Alkaloids (iodine test), carbohydrates (Molisch's test), reducing sugars (Fehling's test), glycosides (aqueous NaOH test), cardiac glycosides (bromine water test), proteins and amino acids (xanthoproteic test), flavonoids (alkaline reagent test), phenolics (Folin–Ciocalteu reaction), tannins (gelatin test), phlobatannins (HCl test), phytosterols (Hesse's test), saponins ($NaHCO_3$ test), terpenoids and triterpenoids (Salkowski's test), quinones (concentrated HCl test), carboxylic acids (effervescence test), coumarins (NaOH test), gums/mucilage (alcohol test), resins (turbidity test), and fixed oils/fats (spot test) were identified following standard protocols.

C) Quantitative Phytochemical Estimation

1. Total Phenol

Phenolics were quantified using the Folin–Ciocalteu method (Rekha Bora *et al.,* 2019). Ethanol extracts (0.5 g sample) were reacted with Folin–Ciocalteu reagent and sodium carbonate, heated briefly, and absorbance was measured at 650 nm.

2. Total Carbohydrates

Carbohydrates were estimated by the Anthrone method (Igwe *et al.,* 2013). Hydrolyzed samples reacted with anthrone reagent were heated in a boiling water bath, cooled, and absorbance recorded at 630 nm.

Glucose served as the standard.

3. Total Protein

Protein content was determined by the Lowry method (Ali *et al.,* 2017). Extracts prepared in phosphate buffer were reacted with alkaline copper reagent and Folin–Ciocalteu reagent, incubated, and read at 660 nm using bovine serum albumin as standard.

4. Reducing Sugars

Reducing sugars were quantified using the DNS method (Akter, 2013). Extracts reacted with DNS reagent were heated for 1 min, cooled, and absorbance was recorded at 540 nm. Glucose was used as standard.

D) Estimation of Total Oxalate

Total oxalate was quantified by permanganometric titration. Oxalate was extracted by boiling 1 g of plant tissue in 0.5 N H_2SO_4, filtering, and titrating aliquots with standard 0.05 N $KMnO_4$ until a persistent pink endpoint appeared. Microscopic examination of oxalate crystals was also performed using hand-sectioned tissues, stained following Pizzolato or silver nitrate–rubeanic acid procedures. Crystal area was quantified using IMAGE-J software (Rajan & Nayagam, 2024).

E) Antioxidant Activity Assays

1. DPPH Radical Scavenging Assay

DPPH radical scavenging activity was measured following Baliyan et al. (2022). Different concentrations (50–150 µg/mL) of plant extracts were mixed with DPPH solution and incubated in the dark for 30 min. Absorbance was read at 517 nm and scavenging activity calculated as: **%RSA = [(Ac – As) / Ac] × 100**, where *Ac* = control absorbance and *As* = sample absorbance.

2. Ferric Reducing Antioxidant Power (FRAP)

Reducing ability was measured by mixing extracts with phosphate buffer and potassium ferricyanide, incubating at 50°C for 20 min, precipitating proteins with TCA, and reacting the supernatant with $FeCl_3$. Absorbance was recorded at 700 nm (Jayanthi & Lalitha, 2011).

F) Anticancer Activity (MTT Assay)

Phytochemical Extraction

Plant samples were air-dried, powdered, and extracted by cold maceration in suitable solvents with intermittent agitation.

MTT Cytotoxicity Assay

Cytotoxic activity was assessed using the MTT assay. Cells (10,000 cells/well) were cultured in 96-well plates and treated with extract concentrations ranging from 6.25–100 µg/mL for 24 h. After incubation, MTT solution (0.5 mg/mL) was added for 2 h to allow formazan formation. Crystals were dissolved in DMSO and absorbance measured at 570 nm. Cell viability was calculated as: **Cell viability (%) = (Absorbance of treated cells / Absorbance of control cells) × 100**.

RESULTS

Qualitative phytochemical screening revealed that carbohydrates, reducing sugars, proteins and amino acids, phenolic compounds, phytosterols, saponins, and resins were consistently present in seeds, sprouted seeds, and microgreens, indicating their stability across developmental stages (Table.1). Alkaloids were detected in seeds and sprouts but were absent in microgreens, suggesting their degradation or transformation during growth. In contrast, glycosides, cardiac glycosides, terpenoids, triterpenoids, quinones, carboxylic acids, coumarins, gums and mucilages, and fixed oils and fats were absent in all stages, indicating either lack of synthesis or presence below detectable levels.

Table 1. Qualitative phytochemical screening of finger millet (*Eleusine coracana*) at different developmental stages.

Phytochemical tests	Seeds	Sprouted seeds	Microgreens
Bioactive compounds			
1. Alkaloids (Iodine test)	Present	Present	Absent
2. Carbohydrates (Molisch test)	Present	Present	Present
3. Reducing sugars (Fehling's test)	Present	Present	Present
4. Proteins & Amino acids (Biuret test/Ninhydrin test)	Present	Present	Present
5. Phenolic compounds (Ferric chloride test)	Present	Present	Present
6. Phytosterols (Salkowski test)	Present	Present	Present
7. Saponins (Froth test)	Present	Present	Present
8. Resins (Acetone test)	Present	Present	Present
Non-detected/absent compounds			
9. Glycosides (NaOH test)	Absent	Absent	Absent
10. Cardiac glycosides (Keller–Killiani test)	Absent	Absent	Absent
11. Terpenoids (Salkowski test)	Absent	Absent	Absent
12. Triterpenoids (Liebermann–Burchard test)	Absent	Absent	Absent
13. Quinones (Bornträger's test)	Absent	Absent	Absent
14. Carboxylic acids (Sodium bicarbonate test)	Absent	Absent	Absent
15. Coumarins (NaOH test)	Absent	Absent	Absent
16. Gums & Mucilages (Ruthenium red test)	Absent	Absent	Absent
17. Fixed oils & Fats (Spot test)	Absent	Absent	Absent
18. Flavonoids (Lead acetate test)	Absent	Absent	Absent

Present: Detected; Absent: Not detected.

The comparative biochemical analysis of seeds, sprouts, and microgreens revealed significant variation in metabolite composition across developmental stages. The data clearly indicated dynamic metabolic reprogramming during germination and early plant growth, influencing both nutritional quality and antinutritional factors.

A marked decline in total phenolic content was observed from seeds to microgreens. Seeds exhibited the highest phenol concentration (277.1 µg/ml), which decreased substantially in sprouts (83.68 µg/ml) and further in microgreens (70.52 µg/ml) (Graph 1). This represents an approximate 69.8% reduction during sprouting and a total decrease of about 74.5% by the microgreen stage. The sharp decline suggests active utilization or transformation of phenolic compounds during germination, possibly due to their involvement in antioxidative defence and metabolic activation processes.

Total carbohydrate content showed a moderate increase during germination. Seeds contained 0.287 mg/ml, which increased to 0.377 mg/ml in sprouts and remained constant in microgreens (0.377 mg/ml). This represents a ~31.4% increase from seeds to sprouts. The stabilization of carbohydrate levels in microgreens suggests a balance between carbohydrate synthesis (via photosynthetic initiation) and utilization for growth and respiration.

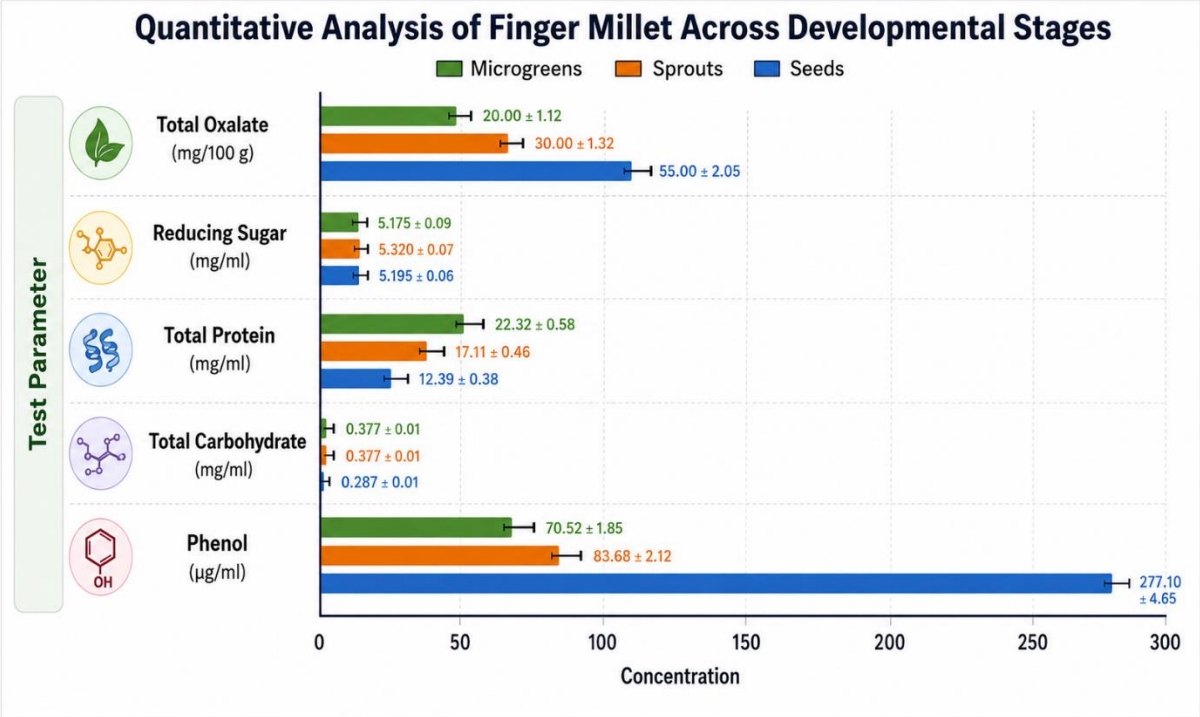
Protein content demonstrated a progressive increase across the developmental stages. Seeds contained 12.39 mg/ml protein, which increased to 17.11 mg/ml in sprouts and reached the highest level in microgreens (22.32 mg/ml). This corresponds to an increase of approximately 38.1% from seeds to sprouts and an overall increase of 80.1% from seeds to microgreens. The observed rise reflects enhanced enzymatic synthesis and mobilization of storage proteins into functional proteins required for growth and metabolic activities.

Reducing sugar content exhibited minor fluctuations across the stages. Seeds recorded 5.195 mg/ml, which slightly increased to 5.32 mg/ml in sprouts and marginally decreased to 5.175 mg/ml in microgreens. The variation was minimal (within ±2.5%), indicating a relatively stable pool of readily available sugars. This stability suggests a tightly regulated balance between carbohydrate hydrolysis and utilization during germination and early growth.

A significant reduction in total oxalate content was observed with development. Seeds contained the highest oxalate level (55 mg/100 g), which decreased to 30 mg/100 g in sprouts and further to 20 mg/100 g in microgreens. This represents a reduction of approximately 45.5% during sprouting and an overall decrease of 63.6% in microgreens compared to seeds. The decline in oxalate content is particularly noteworthy, as it indicates a reduction in antinutritional factors, enhancing the nutritional quality and bioavailability of minerals.

The ferric reducing antioxidant power (FRAP) assay of finger millet samples (seeds, sprouted seeds, and microgreens) showed a clear concentration-dependent increase in antioxidant activity across all stages at 50, 100, and 150 µg/ml. Seeds exhibited the highest FRAP values (4.714, 6.398, and 8.426), followed by sprouted seeds (3.683, 3.099, and 5.298) and microgreens (3.546, 3.786, and 4.439). Overall, seeds demonstrated superior reducing power compared to sprouts and microgreens, likely due to higher levels of antioxidant compounds such as phenolics, while the relatively lower activity in later stages may be attributed to their utilization or transformation during germination and growth.

The cytotoxic effect of the test sample on HeLa cancer cells demonstrated a clear dose-dependent reduction in cell viability across the tested concentrations (6.25–100 µg/mL). Cell viability remained high at lower concentrations, with 99.11% at 6.25 µg/mL and 97.82% at 12.5 µg/mL, but gradually decreased to 95.65% at 25 µg/mL and 92.66% at 50 µg/mL. A more pronounced decline was observed at 100 µg/mL, where viability dropped to 82.59%, indicating the highest cytotoxic effect at this concentration.



Graph: 1- Quantitative analysis of Finger millet across developmental stages.

DISCUSSION

Finger millet is well-established as a nutritionally dense cereal, particularly rich in calcium (Patel *et al.,* 2016), minerals, dietary fibre, and essential amino acids (US NRC, 1996). Its phenolic profile contributes to notable antioxidant activity (Dykes & Rooney, 2006), and its gluten-free nature supports its use in porridge and therapeutic diets. Earlier reports on nutrient composition (Antony *et al.,* 1996; Hulse *et al.,* 1980; Subbarao & Muralikrishna, 2001) align with its recognized status as a superior cereal. Protein

digestibility reported across cultivars (Ramachandra *et al.,* 1977; Mittal, 2002) also supports its nutritional significance.

In the present study, carbohydrate levels increased markedly from the seed (0.287 mg/ml) to the sprout stage (0.377 mg/ml) and remained stable in microgreens, demonstrating active conversion of stored carbohydrates into simpler sugars during germination. Reducing sugars followed a similar metabolic trend, increasing from seeds (5.195 mg/ml) to sprouts (5.320 mg/ml), followed by a slight decline in microgreens (5.175 mg/ml), likely due to utilization during early vegetative growth. These findings complement previous reports indicating that finger millet flours possess slow-digesting carbohydrates conducive to glycaemic control in diabetic individuals (Kang *et al.,* 2008).

The phenolic content exhibited a decreasing trend across developmental stages, with the highest concentration in seeds (277.10 µg/ml), followed by sprouts (83.68 µg/ml) and microgreens (70.52 µg/ml). This reduction is consistent with the metabolic use of phenolic compounds during germination and biomass expansion. Earlier studies similarly reported high phenolic concentrations in seed coats and their role in antioxidant defence (Banerjee *et al.,* 2012; Chakraborty & Pandey, 2024).

Protein concentration increased progressively from seeds (12.39 mg/20 ml) to sprouts (17.11 mg/20 ml) and microgreens (22.32 mg/20 ml), reflecting enhanced biosynthetic activity and nutrient mobilization during early growth. This is consistent with reports that sprouting improves protein quality and digestibility in finger millet (Rao *et al.,* 1994; Ravindran *et al.,* 1992).

Oxalate content showed a substantial reduction following germination, decreasing from 55.00 mg/100 g in seeds to 30.00 mg/100 g in sprouts and 20.00 mg/100 g in microgreens. This decline aligns with earlier observations that germination and fermentation significantly reduce antinutritional factors such as phytates and oxalates (Mamiro *et al.,* 2001), thereby enhancing nutrient bioavailability.

Antioxidant activity demonstrated stage- and concentration-dependent variations. DPPH assay results indicated enhanced radical scavenging activity in sprouts at lower concentrations, while microgreens showed the highest activity at higher concentrations, suggesting accumulation of bioactive compounds during later development. Conversely, the Ferric Ion Reducing Assay (FRAP) revealed higher antioxidant capacity in seeds across all concentrations, likely reflecting concentrated antioxidant reserves prior to germination. Such contrasting trends underscore the dynamic redistribution and metabolism of antioxidant compounds during early plant growth.

The study also confirmed the anticancer potential of finger millet extracts. The MTT assay on HeLa cells revealed a concentration-dependent decline in optical density, with the highest extract concentration (100 µg/ml) producing the greatest reduction in cell viability. These findings are consistent with earlier reports on the anticancer activity of ragi-derived inhibitors and phenolic compounds (Sen & Dutta, 2012; Kuruburu *et al.,* 2022; Sawant & Tavahare, 2022). The observed cytotoxicity suggests that finger millet, particularly in its microgreen and sprouted forms, may serve as a promising candidate for nutraceutical and therapeutic applications.

Overall, the results highlight the nutritionally enhanced profile, improved digestibility, reduced antinutrient content, and significant bioactive potential of sprouted and microgreen stages of finger millet. These developmental stages demonstrated superior functional attributes, supporting their application in health-promoting foods, low-oxalate diets, and potential anticancer interventions.

CONCLUSION

Finger millet undergoes significant nutritional and biochemical transformations during germination and microgreen development. The present study demonstrated enhanced protein accumulation, reduced oxalate concentration, and stage-specific modifications in antioxidant activity across developmental stages. While seeds retained higher phenolic content and ferric reducing power, sprouts and microgreens exhibited favorable nutritional characteristics and effective radical scavenging activity. The observed concentration-dependent cytotoxic effects against HeLa cells further indicate the presence of bioactive phytochemicals

with potential biological significance. Overall, germination and microgreen production improve the functional quality of finger millet and support its utilization as a value-added ingredient in functional foods and nutraceutical applications.

Future Perspectives

In the future, finger millet (Eleusine coracana) is poised to become a key crop for addressing food and nutritional security, particularly as climate change and diet-related health issues intensify. Advancements in biofortification, including CRISPR-Cas9–based gene enhancement and genomic selection, are expected to further elevate its naturally high micronutrient levels and improve nutrient bioavailability. Processing innovations—such as optimized germination, fermentation, and enzymatic treatments—will likely become more scalable and accessible, especially for rural and small-scale industries. Research on finger millet microgreens, with their superior antioxidant profiles, is anticipated to expand, positioning them as future functional foods. Reduction of antinutritional factors through precision breeding, gene editing, and advanced processing will continue to improve the crop's nutritional quality. The development of diverse, value-added, gluten-free products is expected to strengthen its role in the health and wellness market. Increased policy attention, targeted investments, and public awareness initiatives will be crucial for promoting cultivation, processing, and global adoption. Overall, future progress will rely on integrative, interdisciplinary research linking agronomy, food science, nutrition, and genomics to fully realize the crop's potential.

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