



Nutritional and Anti-nutritional Evaluation of Millets from Attappady's Model Millet Village, Kerala

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Millets, Nutritional composition, Antinutritional factors, pigments, Phenolics, PCA, Attappady, functional foods.

Abstract

Millets are increasingly recognized for their nutritional value and resilience, making them an important crop for improving food and nutritional security. This study evaluated the nutritional and anti-nutritional characteristics of five millet varieties collected from Attappady's Model Millet Village, Kerala. Proximate composition, bioactive pigments and anti-nutritional factors were quantified, followed by correlation analysis and Principal Component Analysis (PCA) to identify major patterns of variation. The results revealed substantial variation among the millets in protein, carbohydrate, phenolic content and pigment profiles. Strong positive correlations were observed among anthocyanins, chlorophylls and carotenoids, indicating co-expression of bioactive compounds. Tannins and phenols were also highly correlated, reflecting their shared biochemical pathways. Protein content showed negative associations with phytate and oxalate, suggesting improved mineral bioavailability in high-protein varieties. PCA further differentiated the millets based on their nutritional and antinutritional traits, identifying Kodo and Finger millet as distinct in their biochemical composition. The findings highlight the potential of these traditional millets as nutrient-dense grains suitable for functional foods, biofortification and sustainable dietary applications.

Keywords

Millets, nutritional composition, anti-nutritional factors, pigments, phenolics, PCA, Attappady, functional foods.

1. Introduction

Millets are a diverse group of small-seeded, climate-resilient cereal grains belonging to the family Poaceae. Cultivated for thousands of years, they have historically served as an integral component of traditional diets across Asia and Africa. Known for their rich nutritional profile encompassing proteins, dietary fibre, essential minerals, vitamins, and bioactive compounds. Millets are increasingly

recognized for their potential role in enhancing global food and nutritional security under changing climatic conditions. However, despite their benefits, millet consumption and cultivation declined sharply in India following the Green Revolution, during which high-yielding rice and wheat varieties replaced many indigenous crops. Millets, once contributing nearly 40% of India's cultivated grains, have now reduced to about 20% (Press Information Bureau, 2021), prompting

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concerns regarding dietary diversity, nutritional adequacy, and agricultural sustainability.

Climate change projections by CGIAR estimate significant declines in the global productivity of major cereals such as wheat, rice, and maize in the coming decades (Gowri & Shivakumar, 2020). At the same time, India continues to grapple with widespread malnutrition, micronutrient deficiencies, and a rising burden of lifestyle diseases. Over 55% of Indian women are anaemic, and the country's Human Development Index has also shown a declining trend. In this context, millets have emerged as promising smart foods - nutrient-dense, climate-resilient, and ecologically sustainable, capable of addressing interlinked challenges of malnutrition, climate vulnerability, and rural livelihood insecurity (Lokesh *et al.*, 2022).

Millets possess several agronomic advantages, including tolerance to drought, resistance to pests and diseases, short growing periods, and the ability to flourish in marginal soils (Devi *et al.*, 2014). Nutritionally, they offer 60–70% carbohydrates, 7–11% protein, 1.5–5% fat, and 2–7% crude fibre, along with substantial quantities of minerals such as calcium, iron, magnesium, phosphorus, and manganese. They also supply sulphur-containing essential amino acids, including methionine and cysteine, which are limited in many major cereals (Obilana & Manyasa, 2002). Rich in antioxidants, phenolics, and dietary fibre, millets are associated with multiple health benefits, including improved glycemic control, reduced cardiovascular risk, enhanced digestive health, and protection against oxidative stress and chronic diseases (Shobana *et al.*, 2009; Chandrasekara & Shahidi, 2013).

Despite their proven advantages, the utilization of millets has been hindered by limited public awareness, poor cooking qualities, and underdeveloped processing technologies. Nevertheless, recent national and international initiatives have renewed interest in millet production and

consumption. India reclassified millets as "Nutri-Cereals," celebrated 2018 as the National Year of Millets, and successfully championed the United Nations resolution declaring 2023 as the International Year of Millets.

In Kerala, millet cultivation was once central to the traditional food systems of tribal communities had declined due to agricultural modernization and a dietary shift toward polished rice. Attappady, a tribal-majority region in Palakkad district, is currently at the forefront of a millet revival movement. The "Millet Village" project launched in 2017 by the Kerala Agricultural Department and the Scheduled Tribes Development Department has reintroduced traditional crops such as finger millet, little millet, and sorghum across 192 tribal hamlets, promoting food security, nutritional well-being, and sustainable agriculture. Today, these crops are cultivated using organic, rainfed practices across more than 2,000 hectares in the region (MSSRF, 2023).

Given the resurgence of millet cultivation in Attappady, comprehensive evaluation of their nutritional and antinutritional properties is essential for promoting their wider dietary adoption and understanding their contribution to community nutrition. Nutritional profiling provides insights into their macro- and micronutrient composition, while analysis of antinutritional factors such as phytates, tannins, and oxalates is crucial for assessing bioavailability and formulating strategies to improve nutrient absorption. The present study aims to characterize the nutritional and antinutritional composition of millet varieties cultivated in Attappady's Model Millet Village, thereby generating scientifically validated information that supports their nutritional significance, consumer acceptance, and potential integration into mainstream food systems.



2. Materials and methods

Millet samples were collected from Agali Panchayat in Attappady, Kerala, a major tribal region and core area of the state's Millet Village programme. Five traditionally cultivated species such as *Eleusine coracana*, *Setaria italica*, *Panicum sumatrense*, *Panicum miliaceum* and *Paspalum scrobiculatum* were sampled. Grains were cleaned, shade-dried at room temperature, finely milled, and stored in airtight containers until analysis.

Nutritional and antinutritional attributes were quantified using standard spectrophotometric and titrimetric procedures. Total carbohydrates were determined by the Anthrone method, while protein content was quantified using the method of Lowry *et al.* (1951). Carotenoids were extracted in 80:20 acetone/Tris buffer as described by Sims and Gamon (2002), and quantified using absorbance values at 470, 537, 647, and 663 nm. Total phenolics and tannins were estimated using Folin-Ciocalteu reagent following Sadasivam and Manickam (1996). Phytates and oxalates were quantified by titration using FeCl_3 and KMnO_4 , respectively, based on Halder and Khaled (2021). All analyses were performed in triplicate, and concentrations were calculated from appropriate standard curves or conversion factors.

Statistical analyses, including ANOVA, correlation analysis, box-plot visualization, and principal component analysis (PCA), were conducted using the GRAPES 1.0.0 statistical platform (Gopinath *et al.*, 2021).

3. Results and Discussion

A total of five millet species traditionally cultivated in the Millet Village of Attappady were selected for comprehensive nutritional and antinutritional profiling. These included Finger millet (*Eleusine coracana*), Foxtail millet (*Setaria italica*), Little millet (*Panicum sumatrense*), Proso millet (*Panicum miliaceum*) and Kodo millet

(*Paspalum scrobiculatum*). The analysed biochemical parameters and comparative trends are presented in Table 1.

Among the evaluated biochemical traits, carbohydrate content showed notable variation across the millet species. Proso millet exhibited the highest carbohydrate concentration (30.679%), followed by Foxtail millet (26.799%), while Little millet recorded the lowest value (17.840%). This variation highlights differences in energy-yielding potential, with Proso millet emerging as the most energy-rich. These results align with earlier observations that millets serve as excellent sources of slow-digesting complex carbohydrates with low glycaemic index benefits, making them particularly suitable for individuals with diabetes (Verma & Patel, 2022).

Protein content differed considerably among the species. Foxtail millet contained the highest protein level (0.807%), whereas Kodo millet showed the lowest (0.088%). Finger millet, Little millet, and Proso millet exhibited intermediate protein concentrations (0.318–0.588%). Higher protein content in Foxtail millet reinforces its dietary significance as a plant-based protein source, especially in regions where cereal-based diets dominate (Kumari *et al.*, 2011).

Anthocyanin levels, known for their antioxidant and health-protective functions, showed a distinct pattern. Finger millet recorded the highest anthocyanin content (242 mg/kg DW), followed by Kodo millet (136.867 mg/kg DW), whereas Foxtail millet and Proso millet contained much lower amounts (24–25.8 mg/kg DW). This variation is consistent with previous findings linking anthocyanins to the mitigation of oxidative stress and prevention of chronic diseases such as cardiovascular and metabolic disorders (Rao *et al.*, 2021). The high value in Finger millet highlights its functional food potential.

Chlorophyll pigments also varied significantly, with Finger millet showing the highest chlorophyll a (2.330 mg/kg DW) and chlorophyll b (2.6 mg/kg DW)



levels. The lowest chlorophyll a was observed in Proso millet (0.270 mg/kg DW), while Little millet had the lowest chlorophyll b (0.600 mg/kg DW). Chlorophylls are linked to detoxifying, antioxidant, and anti-inflammatory benefits (Chaudhary et al., 2023). The observed variability may reflect underlying metabolic and genetic differences among species.

Carotenoid content was highest in Finger millet (34.000 mg/kg DW), followed by Foxtail millet (4.200 mg/kg DW), while Little millet exhibited the lowest concentration (0.30 mg/kg DW). Carotenoids, which serve as precursors of vitamin A, contribute significantly to visual and immune health. Increased dietary carotenoid intake has been associated with a reduced risk of macular degeneration and improved immunity (Gupta & Sharma, 2022). The dominance of Finger millet in carotenoid concentration further enhances its nutritional value.

Phenolic compounds ranged between 0.050% (Proso millet) and 0.141% (Kodo millet). Phenolics are well-known for their antioxidant, anti-inflammatory, and antimicrobial properties (Yadav et al., 2023). Higher phenolic content in Kodo millet suggests a greater potential for combating oxidative damage compared to other species.

Tannins were present in moderate quantities, with the highest value recorded in Kodo millet (0.056%) and the lowest in Foxtail millet (0.010%). Although tannins are considered anti-nutritional for their ability to bind proteins, at moderate levels they can contribute to antioxidant defence and antimicrobial activity (Mehta et al., 2023). The values recorded in this study fall within safe consumption limits.

Phytate content ranged from 0.186% (Finger millet) to 0.278% (Little millet). Phytates can chelate essential minerals such as iron, calcium, and zinc, potentially reducing their bioavailability. However, the observed concentrations in all species were significantly below the critical threshold (>1%) known to impair

mineral absorption (Singh et al., 2021). Traditional processing techniques such as soaking, fermentation, and germination can further reduce phytate content.

Oxalate concentrations varied between 0.209% in Kodo millet and 0.483% in Finger millet. Although oxalates are associated with kidney stone formation when consumed excessively, the levels observed here are well within safe limits for human consumption (<2–5%) (Sharma & Rao, 2022). Cooking and co-consumption with calcium-rich foods can minimize oxalate absorption.

Overall comparison with Recommended Dietary Allowance (RDA) and established safe intake levels indicates that all the evaluated anti-nutritional factors such as phenols (0.050–0.141%), tannins (0.010–0.056%), phytates (0.186–0.278%), and oxalates (0.209–0.483%) remain well within acceptable limits and do not pose health risks. Phenolic content remained within beneficial antioxidant ranges (<1% of diet) (Kumar et al., 2022), tannins were below the threshold known to interfere with protein absorption (0.5% of dietary intake), phytates were well below the mineral chelation risk limit (Singh et al., 2021), and oxalates were substantially lower than harmful dietary levels (Sharma & Yadav, 2024).

Collectively, the data demonstrate significant interspecific variation in nutritional and anti-nutritional attributes among the Attappady millets. Finger millet emerged as the most nutritionally rich species based on its high carbohydrate, anthocyanin, chlorophyll, and carotenoid content. Foxtail millet ranked highest in protein content, while Proso millet was identified as the most energy-dense grain. Kodo millet showed the highest phenolic and tannin content, contributing to a strong antioxidant profile. Little millet, despite showing lower values in several traits, still exhibited nutrient and antinutrient levels within safe dietary limits. The biochemical diversity observed among these species highlights their dietary relevance and potential applications in

functional food development and nutritional security programmes.

Box Plot Analysis

Box plot analysis (Fig. 1-10) revealed clear variations in the biochemical composition of the selected millets, demonstrating the influence of inherent varietal properties and treatment-associated factors on nutrient retention. Carbohydrate contents showed a wide distribution, ranging from 15% in Kodo millet to 35.4% in Finger millet. This variation highlights the differential capacity of each millet to accumulate carbohydrates, which serve as the primary energy source in cereal grains. Such differences may arise from genotype-specific metabolic attributes, environmental influences during grain development, and post-harvest handling conditions (Saleh *et al.*, 2013).

Protein levels also varied substantially across the treatments, with Finger millet recording the highest concentration (0.75%). As proteins contribute significantly to the nutritional value of millets providing essential amino acids and functioning in enzymatic and physiological pathways factors such as soil fertility, climatic variability, and genetic makeup likely contributed to the observed distribution (Kumar *et al.*, 2018).

A pronounced declining trend was observed in the anthocyanin content, decreasing from 2507 mg/kg DW in Finger millet to 100 mg/kg DW in Kodo millet. Anthocyanins, known for their antioxidant and anti-inflammatory properties, are highly sensitive to oxidative stress, temperature, and processing conditions, which may explain their marked reduction across treatments (Chandrasekara & Shahidi, 2010). Similar trends occurred in chlorophyll pigments, where chlorophyll-a and chlorophyll-b concentrations declined in the later treatments. These decreases likely reflect variations in light exposure, physiological maturity, and stress

responses affecting pigment biosynthesis.

Carotenoid content also declined progressively, with the highest concentration recorded in Finger millet (3.07 mg/kg DW). Carotenoids, vital for vision, immune regulation, and antioxidant activity, are susceptible to thermal degradation and varietal differences, factors that may have influenced their distribution in the present study (Sharma & Niranjan, 2018).

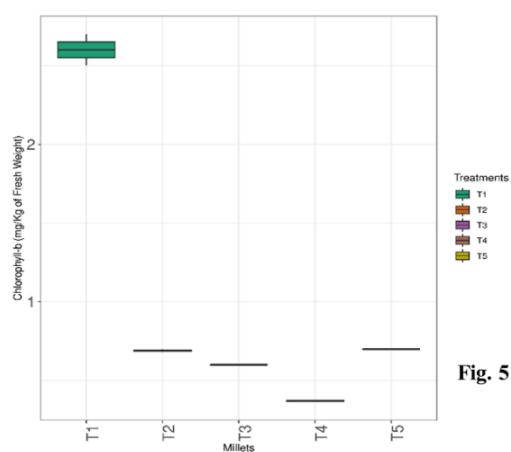
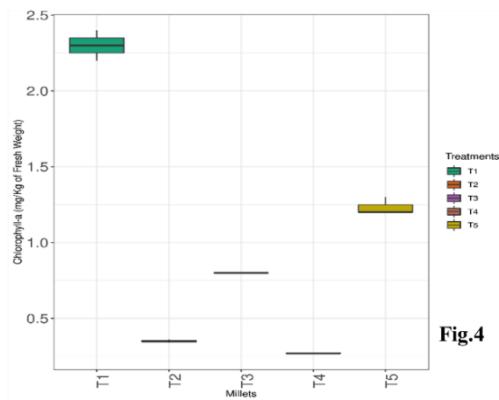
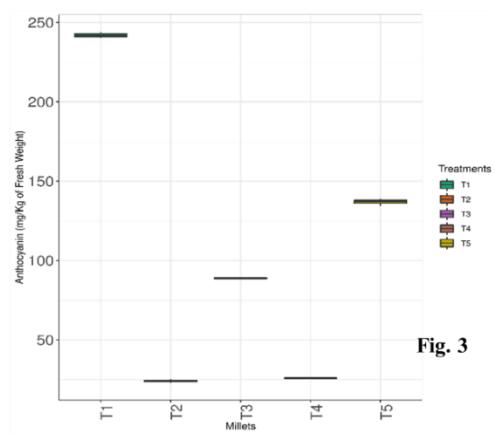
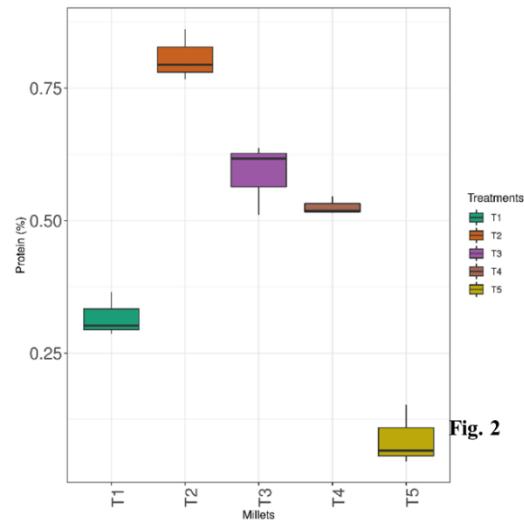
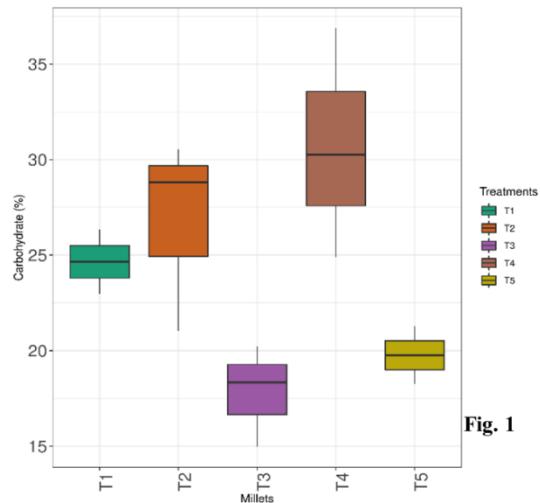
Among the anti-nutritional factors, oxalate content ranged from 0.51% in Finger millet to 0.27% in Kodo millet. Lower oxalate levels in certain treatments are desirable, as high oxalate intake can interfere with calcium absorption and contribute to the formation of kidney stones (Devi *et al.*, 2014). Phenolic content showed a decreasing pattern from Finger millet (0.15%) to Kodo millet (0.05%). Phenolic compounds contribute to antioxidant capacity and play protective roles against oxidative damage; thus, their reduction may be attributed to varietal differences, processing losses, or enzymatic degradation (Verma & Patel, 2013).

Phytate levels followed a similar trend, ranging from 0.275% in Finger millet to 0.175% in Kodo millet. Although phytates act as natural antioxidants, they also reduce mineral bioavailability by chelating iron, zinc, and calcium (Obilana & Manyasa, 2002). Therefore, the reduction observed in later treatments may improve the nutritional accessibility of micronutrients. Tannin content showed a comparable decline, with values ranging from 12.5% in Finger millet to 5.0% in Kodo millet. While tannins may inhibit protein digestibility, they also contribute to antioxidant activity; hence, their variation likely reflects interactions between genotype, processing, and environmental exposure (Taylor & Emmambux, 2008).

Overall, the box plot analysis demonstrates that the biochemical composition of millets is significantly influenced by both intrinsic (genetic) factors and extrinsic factors such as processing, storage, and environmental

conditions. Reductions in anti-nutritional factors including oxalates, phytates, and tannins suggest potential improvements in nutrient bioavailability. However, concurrent decreases in beneficial bioactive compounds such as anthocyanins, phenols, and carotenoids highlight the need for optimized

processing methods that minimize nutrient losses (Udeh & Duodu, 2021; Wang *et al.*, 2020). These findings underscore the importance of developing millet-specific post-harvest and processing strategies to maximize their nutritional and functional properties.



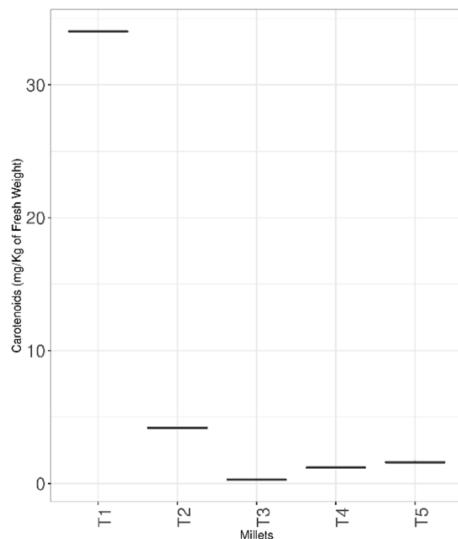


Fig. 6

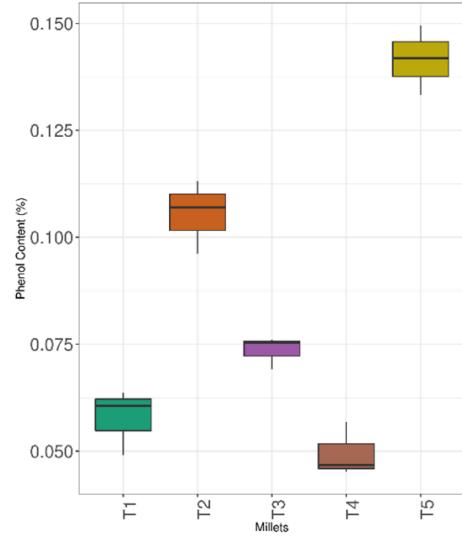


Fig. 7

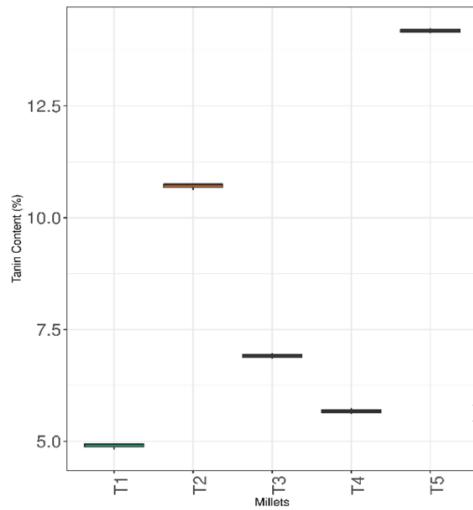


Fig. 8

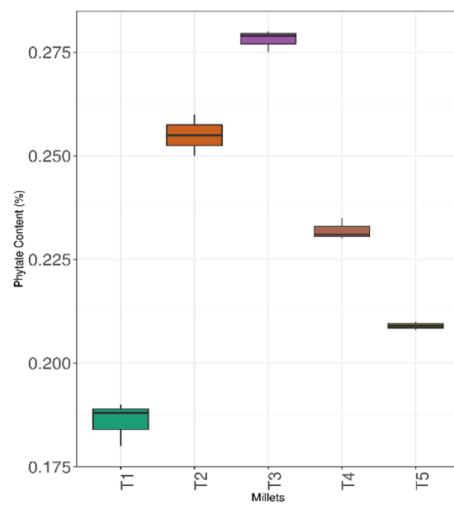


Fig. 9

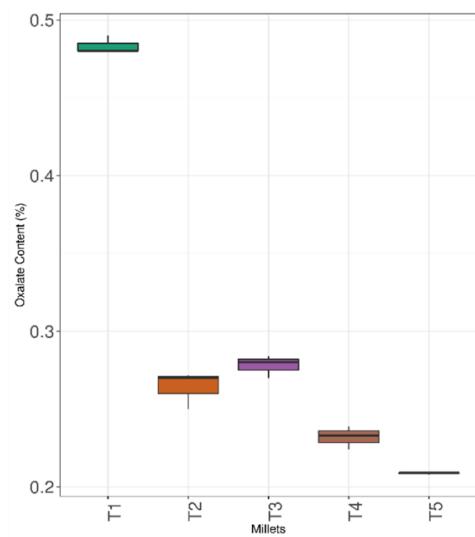


Fig. 10

Fig 1-10. Box plot analysis between the parameters (1 - carbohydrates, 2 – proteins, 3 – anthocyanin, 4 – Chl a, 5 – Chl b, 6 – carotenoids, 7 – phenol, 8 – tannin, 9 – phytate, 10 – oxalate).



Correlations between the parameters

The correlation matrix revealed several significant positive and negative associations among the nutritional and antinutritional parameters of the millets analyzed (Fig. 11). Positive correlations indicated that an increase in one biochemical constituent was associated with a corresponding increase in another, whereas negative correlations reflected inverse relationships, suggesting potential metabolic or physiological trade-offs.

A strong positive correlation ($r > 0.85$) was observed between anthocyanins and chlorophyll pigments, including chlorophyll a ($r = 0.88$), carotenoids ($r = 0.81$), and chlorophyll b ($r = 0.91$). This pattern suggests that millet varieties with elevated anthocyanin concentrations also tend to accumulate higher levels of chlorophylls and carotenoids. The co-expression of these pigments is consistent with previous findings indicating that bioactive compounds involved in pigmentation often share common biosynthetic pathways and regulatory mechanisms (Sharma & Niranjan, 2018). Furthermore, chlorophyll a and chlorophyll b exhibited an exceptionally strong positive correlation ($r = 0.99$), reflecting their tightly coordinated biosynthesis during grain development. Similar relationships have been reported in pigmented cereals, where enhanced pigment levels correspond to increased antioxidant potential (Udeh & Duodu, 2021).

Among the antinutritional constituents, tannins and phenols showed a very strong positive correlation ($r = 0.97$), underscoring the fact that phenolic compounds form the structural basis of tannins in many cereals. This supports previous reports that phenolic-rich millets exhibit higher tannin content, contributing both to their antioxidant profiles and their anti-nutritional properties by binding proteins and essential minerals (Devi *et al.*, 2014; Wang *et al.*, 2020). Phytate and oxalate also showed a strong positive correlation

($r = 0.96$), indicating that these anti-nutrients may accumulate concurrently in millet grains and could jointly influence mineral bioavailability (Saleh *et al.*, 2013).

Protein content demonstrated significant negative correlations with several bioactive pigments, including anthocyanins ($r = -0.69$), chlorophyll a ($r = -0.65$), and chlorophyll b ($r = -0.35$). These inverse relationships suggest that, in some millet varieties, increased protein accumulation may occur at the expense of pigment biosynthesis, possibly due to genetic or metabolic allocation trade-offs during grain filling (Kumar *et al.*, 2018). Additionally, protein exhibited a negative correlation with phytate ($r = -0.72$), indicating that protein-rich millets tend to have lower phytate concentrations, which may contribute to improved mineral bioavailability. Similar observations have been reported in studies on biofortified and improved cultivars that show reduced antinutrient levels alongside elevated protein content (Verma & Patel, 2013).

Antinutritional factors such as tannins, phytates, and oxalates showed consistent negative correlations with beneficial nutritional components. Phytate showed a moderate negative correlation with carbohydrates ($r = -0.22$) and a stronger negative correlation with carotenoids ($r = -0.68$), suggesting that higher phytate levels could diminish the availability of provitamin A compounds (Obilana & Manyasa, 2002). Oxalate exhibited negative correlations with both protein ($r = -0.14$) and phenols ($r = -0.35$), supporting earlier findings that elevated levels of oxalate may interfere with nutrient assimilation and antioxidant activity (Chandrasekara & Shahidi, 2010).

Overall, the correlations highlight the intricate balance between nutritional and anti-nutritional factors in millets. While higher phenolic levels enhance antioxidant properties, excessive tannins and phytates can adversely impact nutrient digestibility and mineral bioavailability (Taylor & Emmambux,

2008). The strong relationship between phytate and oxalate suggests that breeding or processing interventions that reduce one may simultaneously reduce the other, thereby improving overall nutritional quality. Moreover, varieties rich in anthocyanins, chlorophylls, and carotenoids hold promise for functional food applications due to their superior antioxidant profiles (Chandrasekara & Shahidi, 2010; Sharma & Niranjan, 2018).

These findings are valuable for breeding programs aimed at enhancing

the nutritional value of millets while minimizing antinutritional drawbacks. Processing techniques such as fermentation, soaking, germination, and hydrothermal treatments have been shown to effectively reduce phytate, tannin, and oxalate levels while helping preserve beneficial bioactive compounds (Devi *et al.*, 2014; Udeh & Duodu, 2021), and may be integrated with varietal improvement strategies to develop nutritionally superior millet-based foods.

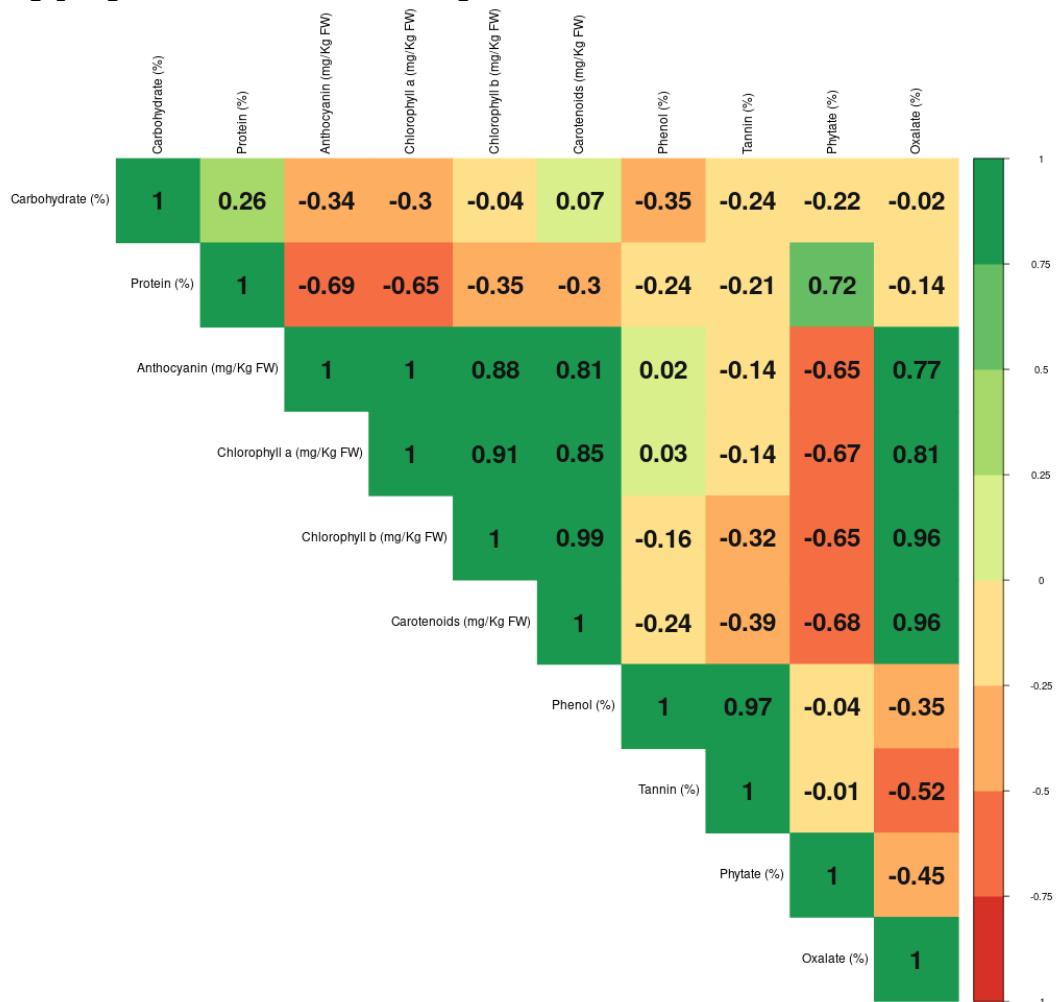


Fig 11. Correlation analysis between the parameters



Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was employed to identify major patterns among the nutritional and anti-nutritional parameters of the millets. PCA reduced the dimensionality of the dataset and enabled visualization of associations among variables, with PC1 and PC2 explaining most of the total variance (Jolliffe & Cadima, 2016). Parameters positioned closely in the biplot showed strong positive correlations, whereas those in opposite quadrants indicated negative associations (Fig. 12).

Bioactive pigments such as anthocyanins, chlorophylls, and carotenoids clustered together along PC1, reflecting their strong positive association and co-expression during grain development (Sharma & Niranjan, 2018; Udeh & Duodu, 2021). Phenols and tannins also grouped tightly, consistent with their shared biochemical origin (Wang *et al.*, 2020). Protein showed an opposite loading to phytate and oxalate, indicating that protein-rich varieties tend to have reduced anti-nutritional factors, a trend previously reported in millets (Devi *et al.*, 2014). Carbohydrates appeared independently along PC2, suggesting minimal association with pigment and anti-nutrient accumulation (Taylor & Emmambux, 2008).

PCA of the millet treatments (T1-T5) revealed clear varietal differentiation (Fig. 13). Kodo millet (T5) showed distinct positive PC1 loadings, corresponding to

elevated levels of bioactive compounds (Chandrasekara & Shahidi, 2010). Finger millet (T1) aligned with PC2, indicating variation in macronutrient composition (Devi *et al.*, 2014). Foxtail (T2) and Proso millet (T4) clustered closely, suggesting similarities in their nutritional and antinutritional profiles, possibly influenced by genetic or processing factors (Kumar *et al.*, 2018).

The scree plot confirmed that PC1 and PC2 were the only components with substantial eigenvalues, capturing the meaningful variation in the dataset (Fig. 14). The rapid decline in variance after PC2 followed the “elbow rule” (Cattell, 1966), indicating that additional components contributed little explanatory power. These results are consistent with earlier studies where PC1 typically represented macronutrients and PC2 secondary metabolites (Sharma & Niranjan, 2018).

Overall, PCA effectively differentiated millet varieties based on their biochemical profiles and highlighted key traits for breeding and functional food development. The findings support targeted selection of anthocyanin- and chlorophyll-rich varieties for antioxidant benefits and low-phytate, high-protein types for improved mineral bioavailability. Processing methods such as fermentation and germination may further enhance nutritional quality by modulating anti-nutritional factors (Udeh & Duodu, 2021).

Fig 12

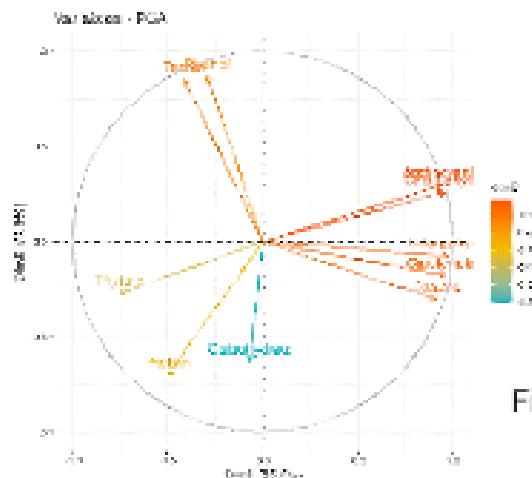


Fig 12a

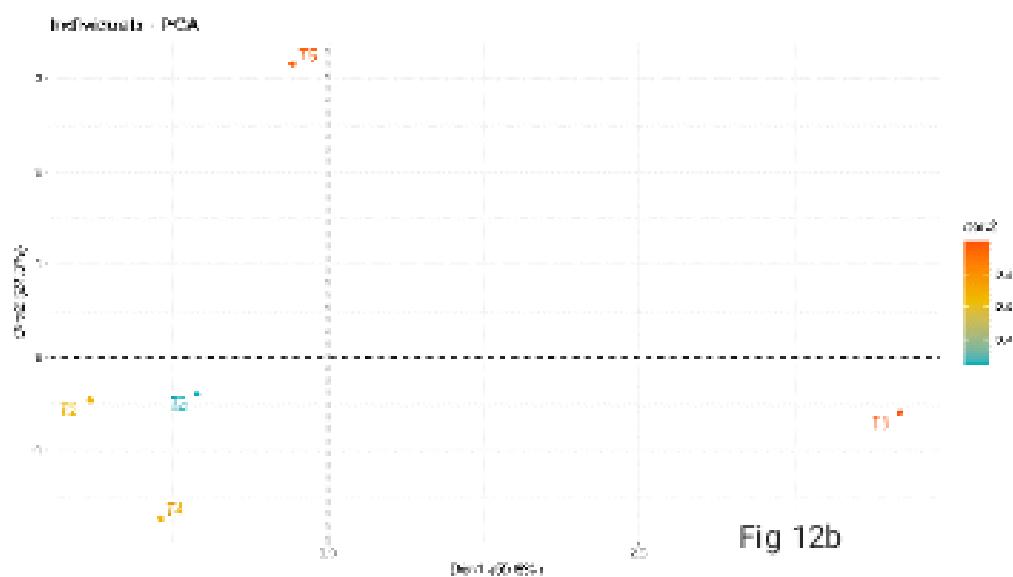


Fig 12b

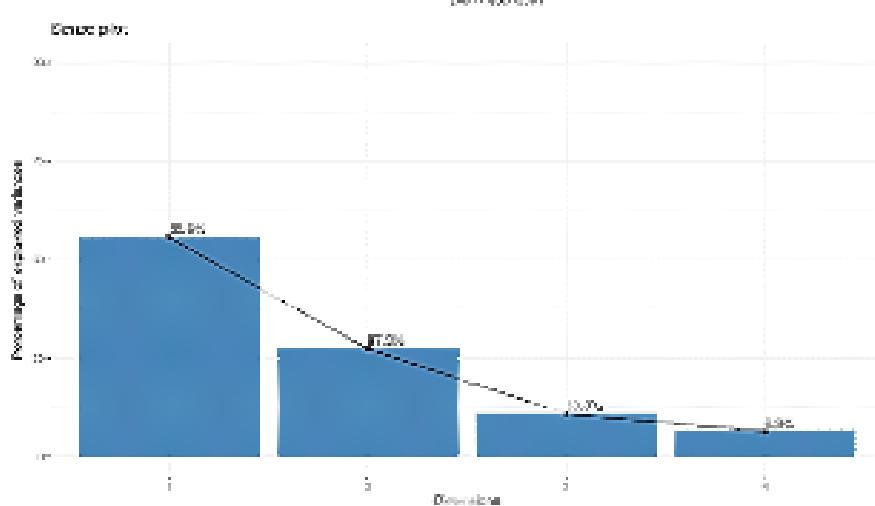


Fig 12c

Fig 12. Principal Component Analysis (PCA) of Nutritional and Antinutritional Traits in Millets (12a. PCA biplot showing the distribution of nutritional and antinutritional parameters, 12b. PCA biplot illustrating the clustering of different millet varieties under study, 12c. Scree plot depicting eigenvalues and the selection of major principal components).



4. Conclusion

The findings of this study highlight the superior nutritional profile and dietary significance of millets collected from Millet Village, Attapadi. Rich in essential nutrients, dietary fiber, and bioactive compounds, these millets demonstrate strong potential in supporting health and addressing nutritional deficiencies. Although antinutritional factors were detected, conventional and traditional processing methods were effective in reducing their levels and enhancing nutrient bioavailability. The study also underscores the value of indigenous knowledge in sustaining millet cultivation and utilization. Overall, millets emerge as a nutritionally dense, environmentally resilient, and culturally important food source. Their broader adoption through awareness initiatives, policy support, and development of value-added products can contribute meaningfully to sustainable diets and future food security.

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Fig. 26

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