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Phytochemical components and antioxidant properties of traditional Sudanese pearl millet non-alcoholic drink

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ABSTRACT

Pearl millet is widely recognized for its exceptional nutritional benefits and adaptability to harsh environmental conditions. Due to these valuable traits, it has become a staple food crop in various regions across the globe. This study investigates the impact of traditional processing methods on the phytochemical composition and antioxidant properties of *Hulu-mur*, a popular non-alcoholic beverage derived from Sudanese pearl millet. *Hulu-mur* processing, comprising malting, fermentation, and baking, significantly alters the biochemical makeup of the final product. Moreover, the study highlights that different processing stages using three distinct pearl millet landraces (Dembi Red, Dembi White, and Dembi Yellow) result in varied effects on the phytochemical profile and antioxidant activities. Notably, significant variations were observed in total flavonoid content (TFC), total phenolic content (TPC), carotenoids, tannins, and gamma-aminobutyric acid (GABA) among the landraces and throughout the processing stages. Accordingly, *Hulu-mur* flakes from Dembi Yellow exhibited the highest GABA content, whereas Dembi Red showed superior results in terms of TPC, carotenoids, and tannins. These findings suggest that traditional processing techniques preserve and potentially enhance the health-promoting properties of pearl millet, positioning *Hulu-mur* as a valuable dietary addition with significant nutritional benefits.

1. Introduction

Pearl millet (*Pennisetum glaucum* L. R.Br.) can become a global staple food crop in many regions that are affected by climate change (Embashu & Nantanga, 2019). It is well adapted and flourishes in hot and arid regions where most other cereal crops can hardly grow and produce grains. Despite scientific advancements, malnutrition and poverty persist, attributed to the neglect of traditional millet cultivation and food practices that once ensured a balanced lifestyle, prompting a reevaluation of millets for their health, environmental, and agricultural benefits while addressing challenges posed by advertisements, household dynamics, market forces, and economic factors (Patil et al., 2023). In Sudan, pearl millet, locally known as *Dukhun*, is an important cereal crop, coming second after sorghum in both areas (2.5–3.5 million hectares) and total production (0.35–0.5 million tons). Generally, the pearl millet production zone in Sudan extends from the western border with Chad and Central Africa to the eastern border with Ethiopia and Eritrea, covering a potential area of 51,747,360 ha with diverse soil types and climatic zones (Bashir et al., 2014). In Sudan, pearl millet is the preferred staple food crop for most inhabitants of western Sudan in the Kordofan and Darfur states, where the grain is usually processed by malting, fermentation, and milling. Grain products are consumed as foods mainly in the form of stiff porridge "*Acceda*," thin gruel "*Nasha*," thin-leavened pancakes "*Kisra*," and non-alcoholic beverages ('*Abreh*' or '*huluHu Mur*'). *Hulu-mur* (which means "sweet and sour" and is also referred to as *Abreh*) is a traditional Sudanese non-alcoholic beverage made from thin flakes of a fermented composite of unmalted and malted sorghum flour (Abdelhalim et al., 2023). *Hulu-mur* is widely consumed as a refreshing, thirst-quenching drink during the Muslims' holy Ramadan fasting month. *Hulu-mur* is ordinarily made from a fermented Sudanese sorghum *feterita* breeding pool (pigmented testa with condensed tannin contents), which affords the characteristic red color of the final product (Baidab et al., 2016). To prepare *Hulu-mur*, sorghum grain is first malted by germination for about 4 days, locally called

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"*Zurriaa*", sun-dried, and ground into flour. Subsequently, a separate batch of ungerminated sorghum grain is milled into flour and cooked into a thin porridge. The malted flour is added to the porridge to make a dough, which is permitted to ferment in a dry place at room temperature for 24–48 h. The ensuing red-colored sweet–the sour fermented dough is diluted with water to a batter consistency, spread on a hot ceramic pan locally called "*Saj*", and baked into sheets, which are then dried in the shade for about 2 days (Ibnouf, 2012). The baked sheets crumble easily into flakes, which are dissolved in water and left to soak for about several hours, and the dark reddish-brown supernatant is decanted and sweetened with sugar before serving cold (Baidab et al., 2016; Ibnouf, 2012). A recent study by Goudar et al. (2023) found that finger millet contained the most significant amount of total anthocyanins of all the varieties studied and a higher percentage of total phenolics (TPC). In addition, the bound fractions of all millet types were primarily composed of phenolic acids, particularly ferulic acid.

Moreover, the molecular docking studies demonstrated high chlorogenic acid affinity and docking scores with key metabolic enzymes like amylase, glucosidase, and leptin receptors, suggesting potential metabolic health benefits. On the other hand, pearl millet has been the subject of extensive research exploring its antioxidant properties. Significant findings have emerged across various aspects of its bioactive composition. Agrawal et al. (2016) reported that anti-oxidative peptides isolated from pearl millet demonstrated substantial antioxidant activity, indicating their potential utility in enhancing health benefits, and Mawouma et al. (2022) investigated the nutritional and antioxidant profiles of pearl millet grains cultivated in Cameroon, revealing high antioxidant levels. Furthermore, Salar et al. (2017) demonstrated that fermentation processes can further enhance the antioxidant properties of pearl millet, suggesting the development of functional foods based on fermented pearl millet. According to Abdelhalim et al. (2023), *Hulu-mur* flakes from the two Sudanese sorghum landraces are found to possess high total phenolics, carotenoids, and antioxidant compounds, namely DPPH, TRP, and FRAP compared to the corresponding ungerminated flour. The significant improvement of these phytochemical health-promoting compounds during the *Hulu-mur* processing steps could be attributed mainly to the significant reduction in antinutritional factors of the grains during the malting and fermentation.

Consequently, fermented foods and beverages are becoming fashionable in Western diets, emphasizing artisanal food processing (Marco et al., 2017). Thus, the occurrence of health-promoting components and their bioactivity make Sudanese cereal-based fermented foods and beverages worthy of being recommended for regular consumption worldwide (Abdelhalim et al., 2023; Baidab et al., 2016; Mohamed et al., 2022). In Western Sudan, *Hulu-mur* can also be made from millet flour produced at the household level from spontaneously fermented sourdough. While *Hulu-mur* from pearl millet remains a house of culinary art or a small-scale industry, information about it is still difficult to obtain. However, there is no documentation on the *Hulu-mur* bioactive compounds, processes, and characteristics of pearl millet, a popular fermented local non-alcoholic beverage in Western Sudan. Therefore, the objectives of this study were to 1) document the traditional techniques involved in the processing of *Hulu-mur* from pearl millet in Sudan and 2) investigate the bioactive compounds and antioxidant activities changes occurring in pearl millet dough during malting and fermentation in manufactured *Hulu-mur*.

2. Materials and methods

2.1. Materials and sample preparation

In this study, three widely recognized local landraces of pearl millet known as “Dembi” were acquired from the Kabkabiah local market in Northern Darfur State, Sudan. These landraces were chosen for their distinctive pericarp colors: red, white, and yellow. Each grain sample was meticulously cleaned to remove foreign substances, damaged seeds, and impurities before being stored in plastic bags. The cleaned grains were dried, ground, and passed through a 0.4 mm mesh to create raw pearl millet flour. This flour, referred to as ungerminated, was divided into two parts. One part was sealed in polypropylene bags and stored in an airtight container at 4 °C for chemical analyses. The other part was used in the subsequent stages of preparing *Hullu-murr* (Fig. 1S).

2.2. Germination process

To germinate the pearl millet grains, they were initially soaked in distilled water at room temperature for 24 h. After soaking, the grains were drained and wrapped in a damp muslin cloth to sprout for 48 to 72 h at room temperature. Once they had sprouted uniformly, the grains were sun-dried for 24 h. The dried, sprouted grains were ground and passed through a sieve with a 0.4 mm mesh to produce germinated (malted) pearl millet flour. This germinated flour was then split into two batches. One batch was sealed in polypropylene bags and stored in an airtight container at 4 °C for chemical analysis. The other batch was used in further steps to prepare fermented dough.

2.3. Fermentation process

Fermented pearl millet dough, known locally as *Ageen*, was made following a traditional Sudanese method outlined by Dirar. Initially, flour made from ungerminated pearl millet was used to cook a thin type of porridge called Medida. This porridge was then transferred from the cooking vessel to another container to ferment. At this stage, flour from germinated (malted) millet was incorporated into the hot Medida, liquefied, and stirred thoroughly with a wooden spoon. This liquefied mix was left in a warm room to ferment for 24 h. Following fermentation, the dough was divided into two parts. One part was air-dried for 24 h, ground, and sieved to create a powder for chemical analysis. The other part was used to make *Hulu-murr* flakes in subsequent steps.

2.4. Hulu-mur preparation

Hulu-mur flakes were made using a traditional Sudanese technique as described by Dirar (1993). During baking, the *Hulu-mur* batter or dough was diluted with water. About 150–250 mL of the batter was deposited on the distant end of a Saj, a heated flat plate. A tool known as Gergeriba, which is made of durable rubber or cardboard, was then used to evenly spread the batter towards the closer edge of the plate. As the Gergeriba neared the edge of the *Saj*, any remaining batter was folded back over the baking sheet’s surface. Once baked, the *Hulu-mur* sheets or flakes were allowed to cool, then they were ground and stored in a refrigerator at 4 °C for subsequent chemical analysis.

2.5. Preparation of extracts

Bioactive compounds and antioxidants were extracted from pearl millet samples and *Hulu-mur* processing ingredients followed the method described by Talhaoui et al. (2014). In summary, 2 g of flour were mixed with 50 mL of methanol, maintaining a solid-to-liquid ratio of 1:25 (w/v), and the mixture was agitated at 25 °C for a full day. Afterward, the mixture was filtered through the Whatman No.4 filter paper. This extraction step was performed twice more on the remaining residue. The final extracts were then concentrated under reduced pressure using a rotary evaporator and stored for subsequent analysis.

2.6. Estimation of total phenolic content

The total phenolic content of the ungerminated, malted, and fermented dough and *Hulu-mur* flour samples was determined using the Folin-Ciocalteu method, as A. Sharma et al. (2023) with modification. Briefly, a 20 µl sample of the dried methanolic extract (1 mg/mL) was

Table 1
Total flavonoid (TFC), total phenolic (TPC), total carotenoid, tannin, and Gamma-Aminobutyric Acid (GABA) grain contents as influenced by pearl millet landraces, *Hulu-mur* processing stages, and their interaction

	Pearl millet metabolites profiles				
Landraces	TFC (CE)/g DW)	TPC (GAE/g DW)	Total Carotenoid (μg βcarotene /g)	Tannin (CE)/g DW)	GABA (mg/g)
Dembi red	23.3 (± 5.4)a	65.7 (± 8.4)b	10.8 (± 2.4)a	4.7 (± 1.6)a	5.2 (± 1.5)b
Dembi white	23.7 (± 6.6)a	69.4 (± 9.4)a	6.6 (± 2.0)c	4.4 (± 1.7)b	3.2 (± 1.2)c
Dembi yellow	20.3 (± 5.5)b	70.3 (± 7.4)a	10.4 (± 2.7)b	3.1 (± 1.5)c	6.0 (± 1.9)a
Hulu-mur processing stages					
Ungerminated flour	14.8 (± 1.3)c	40.3 (± 4.3)c	2.2 (± 0.6)d	1.1 (± 0.5)d	0.9 (± 0.4)d
Malted flour	36.0 (± 3.4)a	62.2 (± 8.5)b	6.7 (± 1.9)c	7.4 (± 1.2)a	1.7 (± 0.4)
Fermented dough	18.0 (± 4.0)bc	59.4 (± 7.5)b	13.1 (± 2.6)b	3.4 (± 0.5)c	6.7 (± 0.3)b
Hulu-mur flakes	20.9 (± 3.6)b	112.1 (± 9.2)a	14.9 (± 3.2)a	4.5 (± 1.0)b	9.8 (± 1.3)a
Two-Way ANOVA					
Cultivars, C	16.5***	24.6**	3429.4***	2244.3***	1352.2***
Hulu-mur processing, HP	295.4***	2890.9***	1648.2***	15617.9***	8819.5***
C×HP	11.4**	258.3***	3688.6***	317.8**	1788.6***
SE±	1.5	4.9	0.9	0.4	0.76
CV%	7.3	2.5	1.4	1.5	2.8

Data were evaluated via two-way ANOVA, factors: three pearl millet landraces, *Hulu-mur* processing stages. Identical letters indicate that values do not differ significantly at $p < 0.05$ according to Tukey HSD. Asterisks (*) indicate significantly influential factors as follows: ns, not significant.
** significant at $p \leq 0.01$.
*** significant at $p \leq 0.001$ level. Each value represents the average of 3 replications. Data in parenthesis are standard deviations (±).

combined with 1.58 mL of water and 100 μl of Folin-Ciocalteu reagent. Subsequently, 300 μl of sodium carbonate was added to the mixture and thoroughly mixed using a Vortex mixer for 10 min. The mixture was left to rest in a dark environment at 20 °C for 2 h. A calibration curve was created using gallic acid in pure methanol ($R^2 = 0.9974$). Absorbance readings were taken at 765 nm using a UV-Vis spectrophotometer (UV–VIS PD-303 UV) compared against a blank solution. Results were expressed in milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

2.7. Determination of total flavonoid content

The total flavonoid content of the various *Hulu-mur* preparation steps was measured using a method adapted from Kim et al. (2003). Initially, 1 mL of aqueous extract from each sample was combined with 300 μL of a 5 % NaNO2 solution and 300 μL of a 10 % aluminum chloride solution, then incubated at 25 °C for 5 min. After incubation, 2 mL of 1 M NaOH was added to each mixture, diluted to a final volume of 10 mL with water, and mixed vigorously using a vortex mixer. The absorbance of these solutions was measured at 510 nm with an ultraviolet-visible spectrophotometer (UV–VIS PD-303 UV). A calibration curve was established using various concentrations of catechin, achieving an R^2 value of 0.99316. The results for total flavonoid content were expressed as milligrams of catechin equivalents per gram of sample (mg CE/g DW).

2.8. Determination of tannin content

The tannin content in the pearl millet samples was determined using a spectrophotometric method that employs vanillin-HCl, as detailed by Price et al. (1978), with slight modifications. In this procedure, 1 mL of methanol containing 1 % HCl was combined with 5 mL of vanillin reagent (4 % HCl in methanol and 0.5 mL of vanillin in methanol). The mixture was then thoroughly mixed and incubated at 30 °C. Absorbance was measured at 500 nm after 20 min using an ultraviolet-visible spectrophotometer (UV–VIS PD-303 UV). A standard curve was created using various concentrations of catechin, and the tannin levels

Table 2
Free radical scavenging DPPH (mg/g dry weight), total reducing power (AAE/g sample), ferric reducing antioxidant power FRAP (mg/g dry weight), and ABTS radical scavenging activity (milligrams of Trolox equivalents (TE) per gram) grains contents as influenced by pearl millet landraces, Hulu-mur processing stages and their interaction

	In vitro antioxidant activity			
Landraces	DPPH as Trolox (TE/100 g sample)	TRPA as ascorbic acid (AAE/100 g sample)	FRAP as Trolox (TE/100 g sample)	ABTS (milligrams of Trolox (TE) per gram sample)
Dembi red	0.47 (± 0.07)a	10.9 (± 1.5)a	6.6 (± 1.4)a	19.9 (± 2.6)c
Dembi white	0.38 (± 0.09)c	10.8 (± 1.9)b	5.4 (± 1.3)b	21.8 (± 2.0)b
Dembi yellow	0.40 (± 0.10)b	10.0 (± 1.9)c	5.3 (± 1.5)b	24.6 (± 1.5)a
Hulu-mur processing stages				
Un-germinated flour	0.30 (± 0.05)d	5.4 (± 0.3)d	2.0 (± 0.9)d	24.7 (± 1.3)a
Malted flour	0.41 (± 0.07)c	10.5 (± 0.4)c	4.6 (± 1.0)c	23.4 (± 1.2)a
Fermented dough	0.46 (± 0.04)b	12.4 (± 1.0)b	5.8 (± 0.8)b	21.6 (± 1.8)b
Hulu-mur flakes	0.50 (± 0.03)a	14.0 (± 1.0)a	10.6 (± 1.4)a	18.6 (± 1.5)c
Two-Way ANOVA				
Cultivars, C	200.4***	1922.2***	507.6***	140.5***
Hulu-mur preparation, HP	612.1***	4129.0***	993.88***	128.6***
C×HP	37.9**	111.5**	123.5***	26.0**
SE±	0.02	0.56	0.54	0.57
CV%	2.6	1.4	1.9	3.2

Data were evaluated via two-way ANOVA, factors: three pearl millet landraces, *Hulu-mur* processing stages. Identical letters indicate that values do not differ significantly at $p < 0.05$ according to Tukey HSD. Asterisks (*) indicate significantly influential factors as follows: ns, not significant.
** significant at $p \leq 0.01$.
*** significant at $p \leq 0.001$ level. Each value represents the average of 3 replications. Data in parenthesis are standard deviation of means.

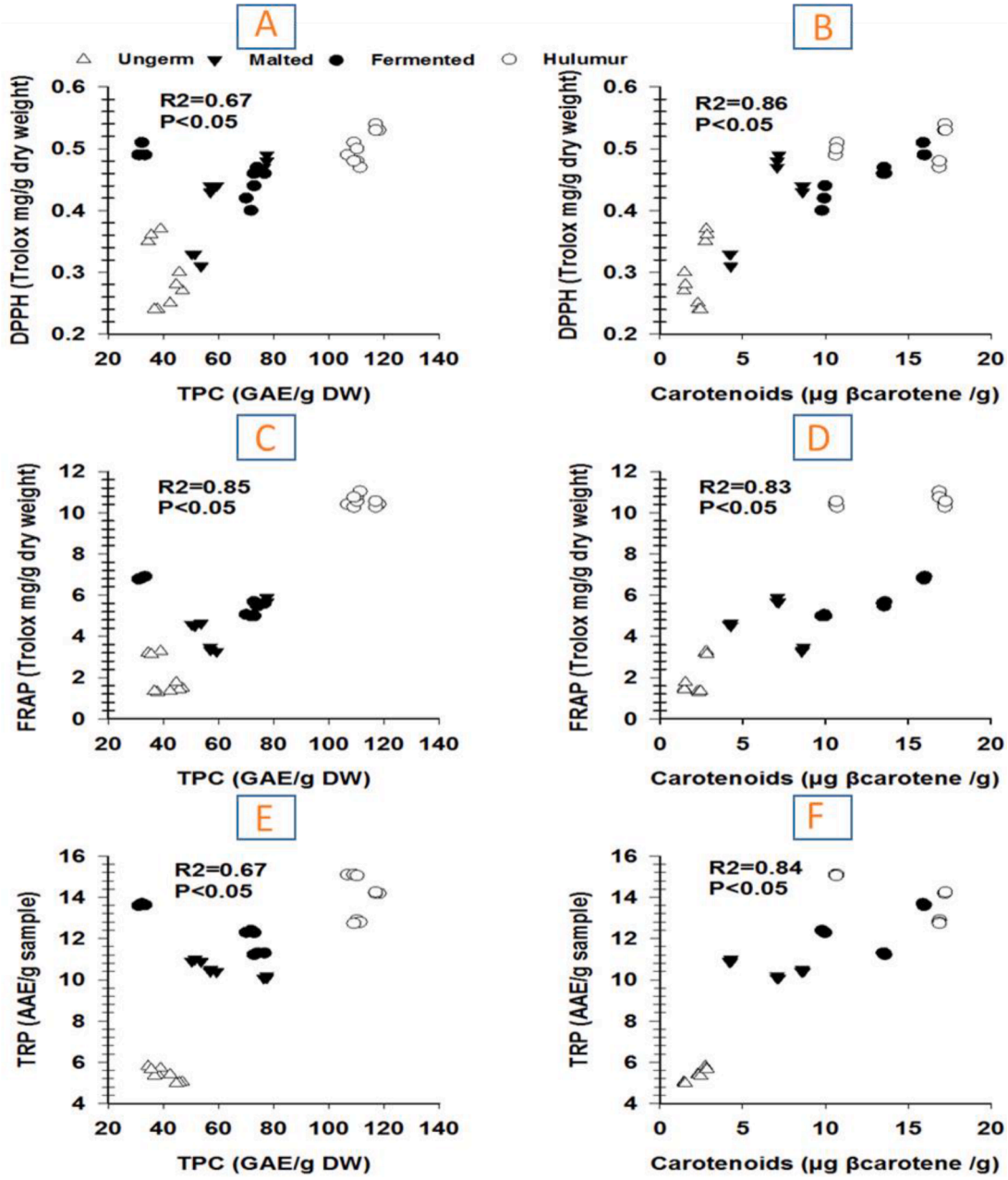

Fig. 1. Relationships between total phenolic contents and free radical scavenging DPPH (A), total phenolic contents versus ferric reducing antioxidant power (C), total phenolic contents versus total reducing power (C), and total carotenoids contents versus free radical scavenging DPPH (B), total carotenoids versus ferric reducing antioxidant power (D) and total carotenoids versus total reducing power (F).

were reported as catechin equivalents (mg CE/100 g).

2.9. Carotenoids determination

Carotenoids were isolated using a method adapted from Jacques et al. (2009). Initially, 2 g of pearl millet powder from each *Hulu-mur* preparation step was mixed with 25 mL of cold acetone. The mixture was then agitated for 10 min at 20 °C before filtering through Whatman No. 1 filter paper. The resulting supernatant was transferred into a separating funnel to perform a liquid-liquid extraction with 20 mL of petroleum ether. Excess acetone was removed by washing with 100 mL of distilled water, and the aqueous phase was discarded. This extraction process was repeated twice. The petroleum ether phase was then passed through Whatman No. 1 filter paper topped with 5 g of anhydrous sodium sulfate to eliminate any remaining water. The absorbance of the extracts was measured at 450 nm. The total carotenoid content was calculated in micrograms of β-carotene per gram of dry matter using the formula:

$$Total\ carotenoids\left(\mu g\beta - caroteneg^{-1}\right) = \left(\frac{A \times V \times 10^4}{E_{1\%cm} \times P}\right)$$

Where A is the absorbance at 450 nm, V is the total extract volume, P is the sample weight, and $E_{1\%cm}$ is the extinction coefficient of β-carotene in petroleum ether, valued at 2592.

2.10. γ-aminobutyric acid (GABA) content determination

The GABA content in pearl millet samples was determined by adapting a method originally described by Sansenya et al. (2017). For this analysis, 2 g of the dried sample from each *Hulu-mur* preparation

step was placed in a 15 mL test tube, combined with 5 mL of distilled water, and extracted for one hour. The mixture was then centrifuged at 4000 rpm for 15 min. The clear supernatant was filtered using filter paper and a 0.45 μm syringe filter. Following filtration, 0.5 mL of the sample was reacted with 0.2 mL of 0.2 M borate buffer (pH 9.0), 1 mL of 6 % w/v phenol reagent, and 0.4 mL of 9 % w/v sodium hypochlorite. This mixture was heated in a water bath for 10 min, then cooled in a bath for 30 min or until it turned blue. The absorbance was measured at 645 nm using a spectrophotometer (UV–VIS PD-303 UV). The GABA content was quantified by comparing the absorbance values to a calibration curve for standard GABA, defined by the equation

$y = 0.616x + 0.1273$ with an R^2 value of 0.9923.

2.11. DPPH radical scavenging activity

The scavenging activity of diphenyl-2-picrylhydrazyl (DPPH) radicals in pearl millet extracts was assessed using a method modified by Sharma et al. (2023). In brief, a mixture containing 0.9 mL of 50 mM Tris-HCl buffer (pH 7.4) and 0.1 mL of extracts or deionized water (as a control) was incubated at 20 °C for 30 min. Following incubation, the absorbance of the mixture was measured at 517 nm with an ultraviolet-visible spectrophotometer (UV–VIS PD-303 UV). The results of the DPPH scavenging activity were quantified and reported as milligrams of Trolox equivalents per gram (mg Trolox/g).

2.12. Ferric reducing antioxidant power (FRAP)

The FRAP (ferric reducing antioxidant power) assay for pearl millet extracts was conducted using the method established by Sharma et al. (2023). In this procedure, a FRAP reagent was freshly prepared by combining acetate buffer (25 mL, 300 mmol/L, pH 3.6), TPTZ solution (2.5 mL, 10 mmol/L in 40 mmol/L HCl), and FeCl3 solution (2.5 mL, 20 mmol/L) in a ratio of 10:1:1 (v/v). This mixture was warmed in a water bath at 37 °C for 10 min. Subsequently, 0.5 mL of a tenfold diluted pearl millet extract was mixed with 2 mL of the FRAP reagent in a 10 mL test tube, further diluted with distilled water to 10 mL, and allowed to stand in the dark for 20 min. The absorbance of the resulting solution was measured at 593 nm using a UV/visible spectrophotometer (UV–VIS PD-303 UV), with a blank for reference. Trolox was used as a standard to construct the calibration curves, and the results were expressed as micromoles of Trolox equivalents (TE) per gram of dry weight (DW).

2.13. Total reducing power assay

The total reducing power (TRP) of pearl millet samples was evaluated using a method adapted from Gülçin et al. (2002). Initially, 2.5 mL of 1 % potassium ferricyanide and 2.5 mL of phosphate buffer (0.2 M, pH 6.6) were combined with the methanolic extract prepared at 1 mg/mL. This mixture was incubated at 50 °C for 20 min, followed by adding 2.5 mL of 10 % trichloroacetic acid. The mixture was then centrifuged at 1038 × g for 10 min. Subsequently, 2.5 mL of the resulting supernatant was mixed with 2.5 mL water and 0.5 mL of 0.1 % ferric chloride. The absorbance of this final mixture was measured at 700 nm using an ultraviolet-visible spectrophotometer (UV–VIS PD-303 UV). The results for the total reducing power were expressed in milligrams of ascorbic acid equivalents (AAE) per gram of sample (dry weight, DW).

2.14. ABTS radical scavenging activity

The ABTS cation radical scavenging activity of Hulu-mur preparation ingredient samples was assessed using a modified protocol from Sharma et al. (2023). The ABTS solution was prepared by combining 7 mM ABTS with 2.45 mM potassium persulfate in equal volumes and allowing the mixture to stand in the dark at 20 °C for 12–16 h. Before use, the ABTS cation radical solution was diluted with methanol to achieve an absorbance of (0.70 ± 0.05) at 734 nm and stabilized at 30 °C. For the assay, 1 mL of the diluted ABTS cation radical solution was added to 50 mL of the extracted samples from pearl millet grains. The mixture was then allowed to react at room temperature for two hours in a dark environment. The absorbance of the mixture was measured at 734 nm using an ultraviolet-visible spectrophotometer (UV–VIS PD-303 UV). The scavenging activity was quantified and expressed as Trolox equivalent antioxidant capacity (TEAC) in milligrams of Trolox equivalents (TE) per gram of extract.

2.15. Statistical analysis

Data were evaluated for uniformity of variances and normality using Shapiro-Wilk's and Levene's tests, respectively, with a significance level set at $P < 0.05$. The influence of pearl millet landraces, *Hulu-mur* processing, and their interactive effects were analyzed using a two-way ANOVA in the SAS 9.1 software package (SAS Institute, Cary, NC, United States). The Tukey HSD post hoc test was applied for treatments exhibiting significant differences for further analysis. The multivariate analysis utilized HJ-Biplot PCA algorithms implemented in XLSTAT software (Vidal et al., 2020). Additionally, a Partial Least Squares Regression (PLS) test was conducted to explore the impact of *Hulu-mur* processing on the phytochemical and antioxidant activity profiles of three pearl millet landraces, using XLSTAT (Tenenhaus et al., 2005).

3. Results

3.1. Impact on phytochemicals

Across the pearl millet landraces, Dembi red (DR) was found to possess the highest total carotenoids and tannin contents, while the highest TFC content was detected in Dembi white (DW) (Table 1). Meanwhile, the landraces DW and Dembi yellow (DY) had almost similar TPC contents and were significantly ($P < 0.001$) higher compared to the DR. The highest (6.0 mg/g) GABA content was recorded in DY, followed by DR (6.0 mg/g), and was significantly ($P < 0.001$) higher than that of the DW. Among the pearl millet landraces, DR showed the highest DPPH, TRP, and FRAP contents, significantly ($P < 0.001$) different from the remaining two landraces (Table 2). However, the situation was reversed in the case of the ABTS, as the lowest ABTS content was measured in the grains of DR, whereas the highest ABTS contents were detected in DY. However, FRAP contents were insignificant ($P < 0.001$) between the DW and DY.

3.2. Influence of processing stages

Among *Hulu-mur* processing stages, *Hulu-mur* flakes maintained the highest TPC, total carotenoids, and GABA contents, significantly ($P < 0.001$) different from the other *Hulu-mur* ingredients (Table 1) . At the same time, the highest TFC contents (36.0 CE/g DW) were recorded in the malted flour, followed by the *Hulu-mur* flakes (20.9 CE/g DW) and were significantly ($P < 0.001$) different compared to the ungerminated flour (control). The lowest (3.4 CE g DW) tannin contents were obtained in the fermented dough. Moreover, across *Hulu-mur* processing steps, *Hulu-mur* flakes maintained the highest DPPH, TRP, and FRAP contents and were significantly different from the other *Hulu-mur* ingredients (Table 2). Meanwhile, the lowest antioxidant activity was encountered in the ungerminated flour. A similar reverse trend was observed for the ABTS contents, as the highest (24.7 mg/g dry weight) ABTS was measured in the ungerminated flour (control), followed by the malted flour (23.4 mg/g dry weight), while the lowest ABTS (19.9 mg/g dry weight) content was detected in the *Hulu-mur flakes*.

3.3. Correlations and multivariate analysis

Significant positive correlations were encountered between TPC and DPPH contents (R2 = 0.67; $P < 0.05$) (Fig. 1A), TPC and FRAP (R2 =

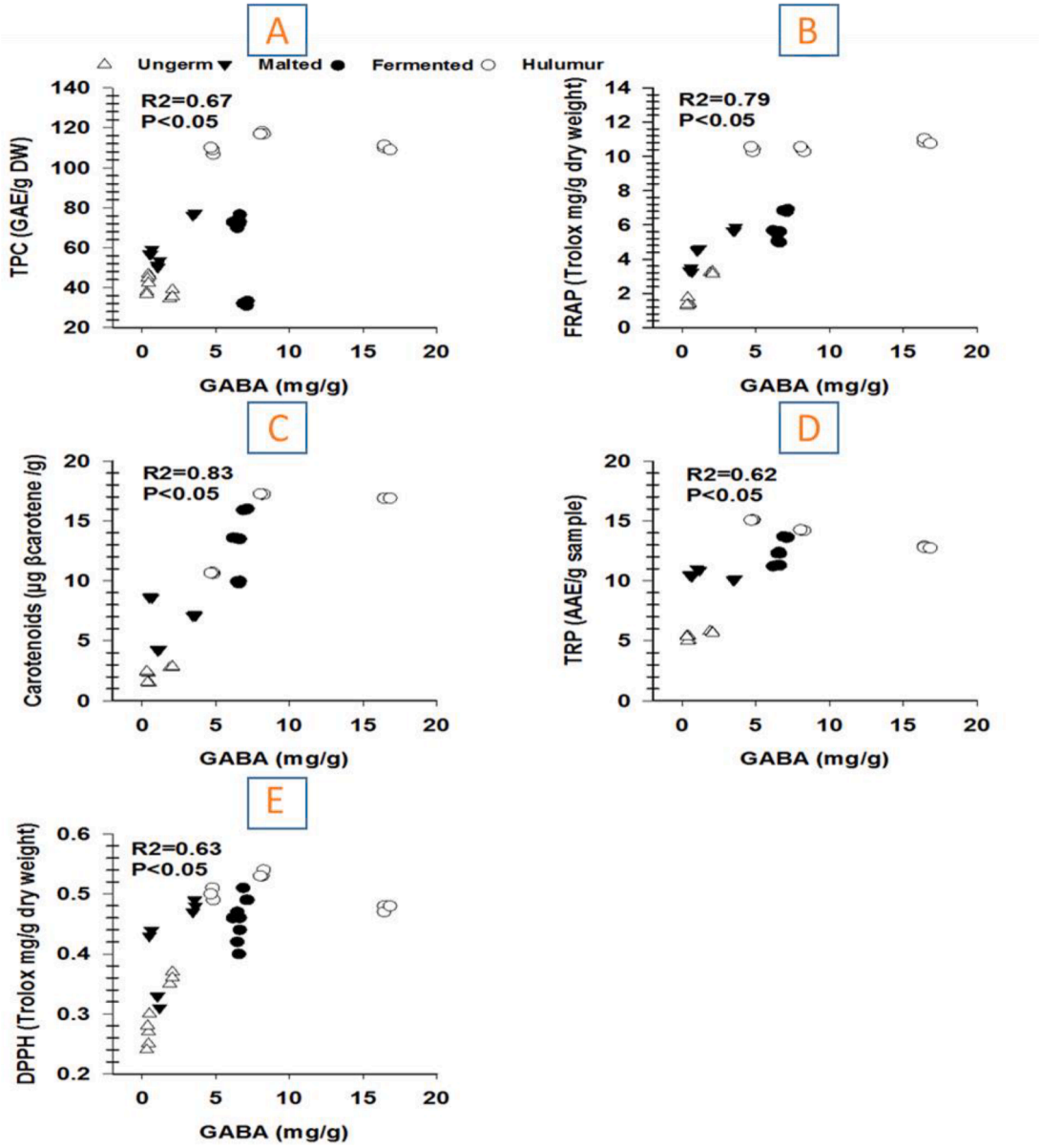

Fig. 2. Relationships between Gamma-Aminobutyric Acid (GABA) and total phenolic contents (A), GABA versus ferric reducing antioxidant power (B), GABA versus total carotenoids (C), GABA versus total reducing power (D) and GABA versus free radical scavenging DPPH (E).

0.85; $P < 0.05$) (Fig. 1C), and between TPC and TRP (R2 = 0.6; $P < 0.05$) (Fig. 1E). Similarly, total carotenoids were found to be positively correlated with DPPH contents (R2 = 0.86; $P < 0.05$) (Fig. 1B), FRAP (R2 = 0.83; $P < 0.05$) (Fig. 1D), and TRP (R2 = 0.84; $P < 0.05$) (Fig. 4F).

GABA contents were found to be positively correlated with TPC (R2 = 0.67; $P < 0.05$) (Fig. 2A) and total carotenoids (R2 = 0.83; $P < 0.05$) (Fig. 2C). Additionally, there were positive correlations between GABA contents and DPPH (R2 = 0.63; $P < 0.05$) (Fig. 2E), GABA contents and FRAP (R2 = 0.79; $P < 0.05$) (Fig. 2B), and between GABA contents and TRP (R2 = 0.63; $P < 0.05$) (Fig. 2D).

Principal Component Analysis (PCA) is a multivariate technique that transforms correlated variables into a set of linearly uncorrelated variables called principal components, effectively condensing and simplifying large datasets (Sharma et al., 2023). The principle component of analysis (PCA) biplot displayed a distinct clustering of the *Hulu-mur* preparation stages ingredients from the three pearl millet landraces (Fig. 3A). The first two principal components account for 82.85 % of the total variation. The component PC1 explained 61.04 %, and PC2 explained 21.81 % of the total variation. TPC, total carotenoids, GABA, DPPH, TRP, FRAP, and ABTS, were significantly associated with PC1. By contrast, TFC and tannin contents were highly correlated with PC2. Accordingly, the PCA factor loading correlates the FRAP, total carotenoids, and GABA with the fermented dough and *Hulu-mur* flakes. Additionally, TFC and tannin contents were positively correlated with germinated (malted) flour from the landrace Dembi red. Furthermore, the Partial least squares analysis described the interactive impacts of *Hulu-mur* preparation stages ingredients from three Sudanese pearl millet landraces (x variables) on the stated factors (y variables) of phytochemicals and antioxidant activity (Fig. 3B).

Results of quantitative descriptive analysis from radar plots indicated that TFC was more pronounced in Dembi Red Landrace's malted flour than the others (Fig. 4A). However, for the TPC, *Hulu-mur* flakes from the landrace Dembi red were the highest (Fig. 4B). Surprisingly, malted flour and *Hulu-mur* flakes had the greatest tannin content than the other *Hulu-mur* preparation ingredients (Fig. 4C). Both fermented dough and *Hulu-mur* flakes, irrespective of pearl millet landraces, possessed more significant total carotenoids (Fig. 4D). Interestingly, *Hulu-mur* flakes from Dembi yellow contained the highest GABA

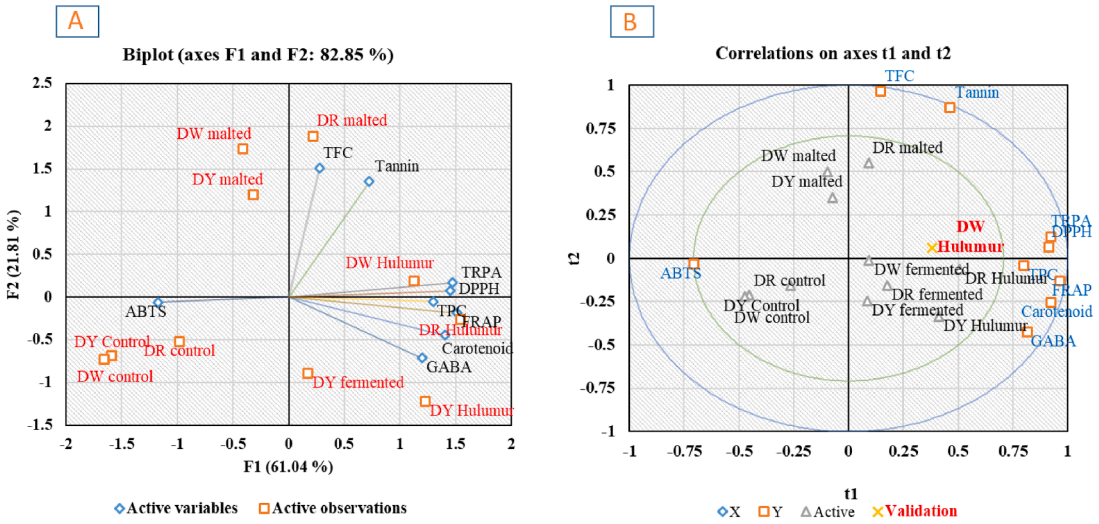

Fig. 3. (A) Principal component (PC) scores for the experimental variables determined in grains of three Sudanese pearl millet landraces and Hulu-mur processing stages. The percentage values in parentheses indicate the variation explained by each PC. The plot shows the distribution of the experimental individuals according to the PCs and grouped according to the Hulu-mur processing stages. (B) Partial Least Squares regression analysis (PLS) for the experimental variables determined in grains of three pearl millet landraces in response to Hulu-mur processing stages.

contents (Fig. 4E). Additionally, *Hulu-mur* flakes, irrespective of pearl millet landraces, maintained higher DPPH, FRAP, and TRP scavenging capacity (Fig. 4 F-H). Neither pearl millet landraces nor *Hulu-mur* processing steps influenced ABTS scavenging capacity (Fig. 4 I). Referring to the *Radar* model, *Hulu-mur* flakes from the landrace Dembi White were the most valid ones, which women in Western Sudan might consider in producing healthy and high-nutritious *Hulu-mur*.

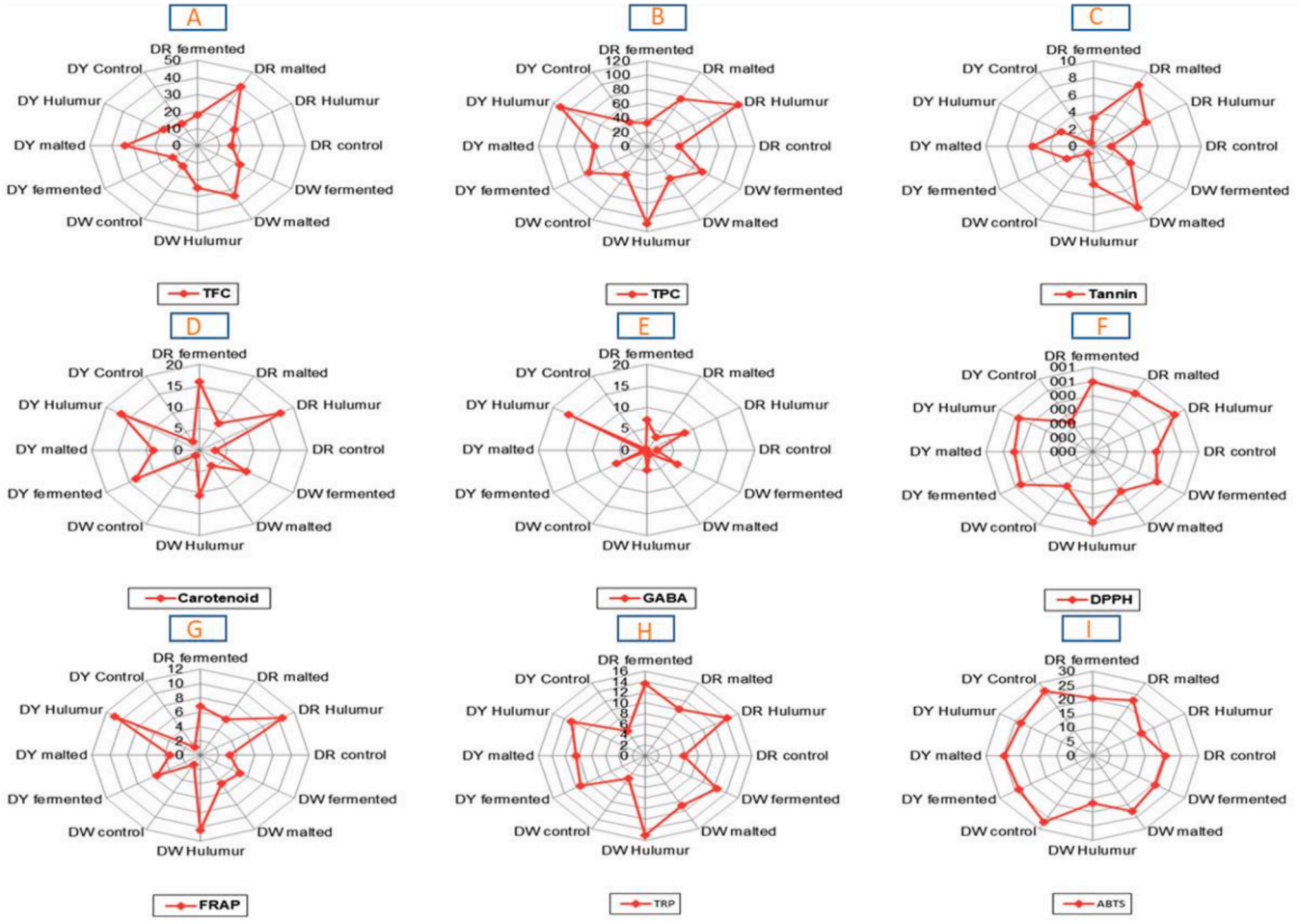

Fig. 4. Radar charts of phytochemicals and antioxidant activity as influenced by pearl millet landraces and Hulu-mur processing steps.

4. Discussion

Hulu-mur is a popular and refreshing sorghum beverage in Sudan, consumed daily, during celebrations, or on special occasions. It is rich in sugars, minerals, and vitamins and has been reported to contain high levels of phytochemical compounds and antioxidant activity (Sulieman, 2022). However, there is no report on the nutritional value of *Hulu-mur*, which is made from pearl millet. In addition, pearl millet undergoes a processing method that includes malting, fermentation, and baking into flakes, which can impact the total phenolic and tannin content, developing unique flavors, aromas, and potential health-promoting properties, such as carotenoids, GABA, and antioxidants. Accordingly, this study aimed to examine the changes in phytochemicals and antioxidant activity during the processing of *Hulu-mur* made from three pearl millet landraces: Dembi red, Dembi white, and Dembi yellow.

The results revealed that the *Hulu-mur* flakes from the Dembi red landrace maintained the highest phenolics and tannin contents. However, the overall *Hulu-mur* processing, which involves malting, fermentation, and baking, led to significant positive changes in the metabolite profiles (TFC, TPC, total carotenoids, tannins, and GABA) and *in vitro* antioxidant activities (DPPH, TRP, and FRAP). These phytochemical and antioxidant properties improvements can be attributed to indigenous food processing knowledge, such as malting, sprouting, fermentation, and hot-plate baking, which might have enhanced the final product quality (Abdelhalim et al., 2023).

The higher total phenolic content in the *Hulu-mur* flakes from the Dembi red landrace may result from the synthesis and accumulation of pigments like anthocyanins and flavonoids, which contribute to the red, purple, or dark brown color of millet seeds (Serna-Saldivar & Espinosa-Ramírez, 2019). However, the correlation between seed color and total phenolic content in millet is not always consistent across all landraces, as the phenolic profile is genetically controlled, and environmental factors can also influence it (Hassan et al., 2020).

The malting (sprouting) process activates enzymes, resulting in changes in biochemistry and the breakdown of complex molecules into simpler ones. This process can release and synthesize phenolic compounds, increasing *in vitro* antioxidant activity measured by DPPH, TRP, and FRAP assays (Jaybhaye et al., 2014). Conversely, sprouting may introduce compounds interfering with the ABTS assay, reducing the measured antioxidant activity (Kim et al., 2014).

Germination can also significantly increase the carotenoid and GABA content in grains. The increase in carotenoids is a response to light exposure during sprouting and converting inactive forms to active formats (Saini et al., 2019). The increase in GABA is due to the enzymatic breakdown of complex proteins into amino acids, including glutamic acid, followed by the decarboxylation of glutamic acid to form GABA (Saini et al., 2019).

Fermentation can lead to oxidative and reductive reactions, affecting the phenolic content. Additionally, oxidation reactions mediated by enzymes like polyphenol oxidases can form new phenolic compounds, while reduction reactions can increase their content by transforming certain phenolic compounds into more stable forms (Dai & Mumper, 2010). Furthermore, fermentation also involves the action of various enzymes produced by microorganisms, some of which can positively impact carotenoid content by releasing and modifying the initial carotenoids (Mapelli-Brahm et al., 2020). The primary mechanism for GABA production during fermentation is the action of the enzyme glutamate decarboxylase (GAD), which converts glutamate into GABA (Yogeswara et al., 2020). Moreover, fermentation can also affect the availability of tannins by modifying the structure of compounds that interact with them (Siddiqui et al., 2022). On the other hand, Hot-plate cooking has been shown to increase the levels of beneficial compounds, such as total flavonoids, phenolics, carotenoids, and GABA, in various foods (Alide et al., 2020; Rodríguez-López et al., 2018; Tiansawang et al., 2016; Verni et al., 2019). This increase in bioactive compounds can contribute to the enhanced antioxidant activity observed in the baked *Hulu-mur* flakes (Benítez et al., 2011).

The positive correlations between total phenolics, total carotenoids, and antioxidant activity suggest a synergistic relationship where these bioactive compounds work together to provide antioxidant protection (Kelley et al., 2018). The PLS results indicate that *Hulu-mur* from the Dembi white landrace may be the most suitable for producing healthy and nutritious products. However, it is essential to note that the genetic variation in different pearl millet varieties can influence the accumulation of phytochemical compounds (Hassan et al., 2020). Additionally, genetic factors can affect the activity and regulation of antioxidant enzymes, contributing to the overall antioxidant capacity of pearl millet.

5. Conclusions

The present study is the first report on phytochemical compounds and antioxidants manufacturing traditional non-alcoholic beverages, *Hullu-mur* from Sudanese pearl millet. The processing of *Hulu-mur* from pearl millet involves a series of transformations, including malting, fermentation, and baking, which can significantly enhance the final product's phytochemical profile and antioxidant properties. The Dembi white landrace is the most suitable for producing a nutritious and health-promoting *Hulu-mur*. Further research is needed to explore the specific genetic and environmental factors that influence the accumulation of these beneficial compounds in different pearl millet varieties and landraces.

CRediT authorship contribution statement

Mohammed Elsafy: Writing – original draft, Funding acquisition. **Mazahir H. Othman:** Methodology, Data curation. **Amro B. Hassan:** Writing – review & editing. **Khitma A. Sir Elkhatim:** Methodology, Data curation. **Manhal Gobara Hamid:** Methodology, Data curation. **Mahbubjon Rahmatov:** Writing – review & editing. **Tilal Sayed Abdelhalim:** Visualization, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2024.100739.

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