



Comparative Phytochemical Analysis of Uttarakhand's Finger Millet (*Eleusine coracana* L.) Varieties

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Abstract: Background: The hardy pseudocereal finger millet (*Eleusine coracana* L.) is widely grown in Asia and Africa and is prized for its numerous nutritional and medicinal uses. Although its proximate composition has been the subject of numerous studies, little is known about the comparative phytochemical profiles of various Indian cultivars. This research quantitatively evaluated eight finger millet varieties (V1–V8) from Uttarakhand for key bioactive compounds such as tannins, flavonoids, steroids, saponins, and glycosides, using spectrophotometric assays with standard calibration curves. The method's reliability was guaranteed by the strong linearity of the regression equations produced from tannic acid, quercetin, diosgenin, and securidaside ($R^2 > 0.99$). Varietal differences were significant: V2 exhibited the highest flavonoid concentration (0.4411 mg/g), whereas V6 had the highest amounts of tannin (0.1829 mg/g), steroid (0.2292 mg/g), and glycoside (0.3990 mg/g). The levels of saponin remained relatively stable, with V7 showing a slight advantage at 0.1803 mg/g. These differences suggest that the buildup of secondary metabolites is shaped by both genetic background and environmental adaptation. The study provides the first comprehensive varietal comparison of phytochemicals in Uttarakhand finger millet and identifies V2 and V6 as promising candidates for nutraceutical and functional food applications. A methodological framework for varietal selection based on bioactive potential is also established by the findings.

Keywords: Spectrophotometry, phytochemical profiling, finger millet, nutraceutical potential, and varietal comparison.

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INTRODUCTION

Congenital melanocytic nevi (CMN) are pigmented Finger millet (*Eleusine coracana* L.), a pseudocereal that can withstand climate challenges, is cultivated widely across Africa and Asia, with India being one of its growing regions. In these regions, it is essential to guaranteeing food and nutritional security. Besides its excellent macronutrient composition, finger millet is abundant in phytochemicals associated with anti-inflammatory, anti-cancer, antioxidant, and antimicrobial effects, such as alkaloids, glycosides, tannins, flavonoids, and saponins (Owheru et al. 2019; Pandey et al. 2023). Previous studies have highlighted the nutritional and pharmacological benefits of finger millet (Devi et al. 2014; Kalsi and Sharma, 2023). However, there is limited knowledge regarding the quantitative differences in phytochemicals among specific Indian varieties, particularly those cultivated under Uttarakhand's unique agro-climatic conditions. The identification of cultivars with exceptional nutraceutical potential and the direction of their application in functional food formulations and herbal medicine depend on the precise quantification of these bioactive compounds. For this kind of analysis, spectrophotometric assays offer a dependable and repeatable method that makes it possible to compare several compounds using common calibration curves (Rani et al, 2024).

Therefore, comparing and quantifying the phytochemical makeup of eight finger millet cultivars from Uttarakhand was the specific objective of this work. In order to establish a scientific foundation for varietal selection in plant-based therapies, crop improvement initiatives, and nutraceutical development, the study will identify cultivars that possess greater concentrations of important bioactive compounds.

Material and Methods

Study area

The study was conducted at SGRR University's Department of Seed Science and Technology, which

is part of the School of Agricultural Sciences. It focused on a region located at approximately 30.305262° N, 78.030803° E in Patel Nagar, Dehradun, Uttarakhand, India (postal code: 248001).

Sample Preparation

Depending on the kind of molecule, standard solutions of phytochemicals were made at concentrations between 20 and 100 mg/μg. The following Finger millet varieties' phytochemicals were analysed:

Varieties and Phytochemicals Analysed:

S. No.	Varieties	Symbol Used
1.	PRM 1	V1
2.	PRM 2	V2
3.	VL 352	V3
4.	GPU 48	V4
5.	Nainital	V5
6.	Kanatal	V6
7.	Gopeshwar	V7
8.	Barkot	V8

Phytochemicals Analysed:

1. Tannins– Measured at 760 nm
2. Flavonoids– Measured at 510 nm
3. Steroids– Measured at 780 nm
4. Saponin – Measured at 430 nm
5. Glycosides – Measured at 495 nm

Statistical Methodology

Spectrophotometric quantification was carried out by dissolving each compound in appropriate solvent systems. Absorbance was recorded using a UV-V is spectrophotometer at respective λ max values. Plotting concentration versus absorbance allowed for the construction of calibration curves.

Calibration Curve: $y = 0.0135x - 0.749$

Here:

- y = absorbance measured by the spectrophotometer

- x = concentration of the compound (mg or $\mu\text{g/mL}$, depending on the units used)
- 0.0135 = the line's slope, indicating the change in absorbance for each unit of concentration
- -0.749 = y-intercept, which indicates the absorbance value at a concentration of zero

Experimental Procedure

i. Assessment of Flavonoid content

Standards of quercetin (20–100 $\mu\text{g/mL}$) were prepared. After mixing 1 ml of the sample/standard with 1 ml of a 2% AlCl_3 solution in methanol, the mixture was incubated for 10 minutes. Absorbance measurements were taken at a wavelength of 510 nm. Subsequent to calibration, TFC was determined using the formula $(C \times V)/(m)$ and expressed in mg QE/g.

ii. Assessment of Saponin content

Standards of diosgenin (20–100 $\mu\text{g/mL}$) were made in methanol. Vanillin acetic acid reagent (8%) and perchloric acid were combined with one 2830illilitre of the sample or standard. After 15 minutes of incubation at 60 °C, the mixture was cooled and diluted with acetic acid. At 430 nm, absorbance was measured. A diosgenin calibration curve was created, and the total saponin content was measured as mg diosgenin equivalents (DE)/g extract.

iii. Assessment of Tannin content

Distilled water was used to create tannic acid standards (20–100 $\mu\text{g/mL}$). One 2830illilitre of Folin Ciocalteu reagent (diluted 1:10 with water) was combined with one 2830illilitre of sample or standard. Two 2830illilitre2830 of a 7.5% Na_2CO_3 solution were added after five minutes. The mixture's absorbance at 760 nm was measured after 30 minutes of room temperature incubation. Tannic acid was used to generate a calibration curve, and milligrams of tannic acid equivalents (TAE) per gram of extract were used to indicate the total tannin concentration.

iv. Assessment of Steroids content

Diosgenin standards (20–100 $\mu\text{g/mL}$) were prepared in methanol. One 2830illilitre of the standard or sample was mixed with one 2830illilitre of acetic anhydride and one 2830illilitre of chloroform. Carefully, 1 milliliter of strong 2830illilitre acid was added. The mixture was incubated for half an hour at room temperature. Measurements of absorbance were made at 780 nm. The total steroid concentration was expressed as mg of diosgenin equivalents (DE) per gram of extract after a calibration curve was created using diosgenin.

v. Assessment of Glycoside content

Securidaside standards (20–100 $\mu\text{g/mL}$) were created using methanol. A One 2830illilitre of the sample/standard, one 2830illilitre of glacial acetic acid, and one 2830illilitre of a 2% FeCl_3 solution were mixed together. After ten minutes of heating to 70 °C, the mixture was left to cool to room temperature. At 495 nm, the absorbance measurement was made. A calibration curve for securidaside was created, with the total glycoside content represented as mg securidaside equivalents (SE)/g extract.

2.7 Graph preparation formula

A calibration curve was created using standards, and the total phytochemical content was reported as mg equivalents (DE/TAE/SE)/g extract.

Formula:

Flavonoids/Saponin/tannin/Steroids/Glycosides (mg/g) = $\frac{C \times V}{M}$

M

Where:

- C = concentration as determined from the calibration curve ($\mu\text{g/mL}$)
- V = extract volume (in mL)
- M = mass of the sample (g)

Results & Discussion:

The current study offers a thorough quantitative analysis of several seed samples' main phytochemicals, including tannins, flavonoids, steroids, saponins, and glycosides. Using spectrophotometric techniques and standard calibration curves, we determined the content of these bioactive compounds, which are well-known for their antibacterial, antioxidant, and therapeutic properties.

Tannin:

Finger millet genotypes' tannin content was calculated using spectrophotometry at 760 nm. The method's dependability for quantification was confirmed by the calibration curve, which showed a linear relationship between concentration and absorbance using standard tannic acid concentrations (20–100 $\mu\text{g/mL}$) (Table 2; Fig. 1).

The eight kinds' corrected tannin values ranged from 0.1703 to 0.1829 mg/g, with very little variation (see Table 1). V3 (0.1703 mg/g) and V2 (0.1752 mg/g) had the lowest tannin concentration. On the other hand, V4 (0.1821 mg/g), V5 (0.1818 mg/g), and V6 (0.1829 mg/g) showed greater values. V7 (0.1800 mg/g), V8 (0.1799 mg/g), and V1 (0.1814 mg/g). These findings suggest that there aren't many genotypic variations in tannin accumulation because the tannin content of the varieties under study was comparatively constant with only slight variation.

Table 1. Tannin Content

Sample	Read 1	Read 2	Average Absorbance	Estimated Concentration (µg /ml)	Dilution Factor	Corrected mg/g
V1	0.5358	0.5406	0.5382	45.44	4	0.1814
V2	0.5003	0.5344	0.5174	43.85	4	0.1752
V3	0.4760	0.5254	0.5007	42.74	4	0.1703
V4	0.5395	0.5419	0.5407	45.76	4	0.1821
V5	0.5407	0.5385	0.5396	45.48	4	0.1818
V6	0.5476	0.5390	0.5433	45.74	4	0.1829
V7	0.5312	0.5357	0.5335	45.14	4	0.1800
V8	0.5220	0.5443	0.5332	45.12	4	0.1799

Table 2. Tannin Concentration

S. No	Concentration (Tannins A)	Wavelength(760nm)
1	20 µg	0.1932
2	40 µg	0.4596
3	60 µg	0.7392
4	80 µg	1.0301
5	100 µg	1.2603

Regression Line Equation:

$$Y = 0.0135X - 0.0749$$

Here:

Y = Absorbance

X = X is the tannin concentration in milligrams.

Coefficient of Determination: R² = 0.9988

Regression Equation:

$$\text{Concentration} = (\text{Absorbance} + 0.0749)/0.0135$$

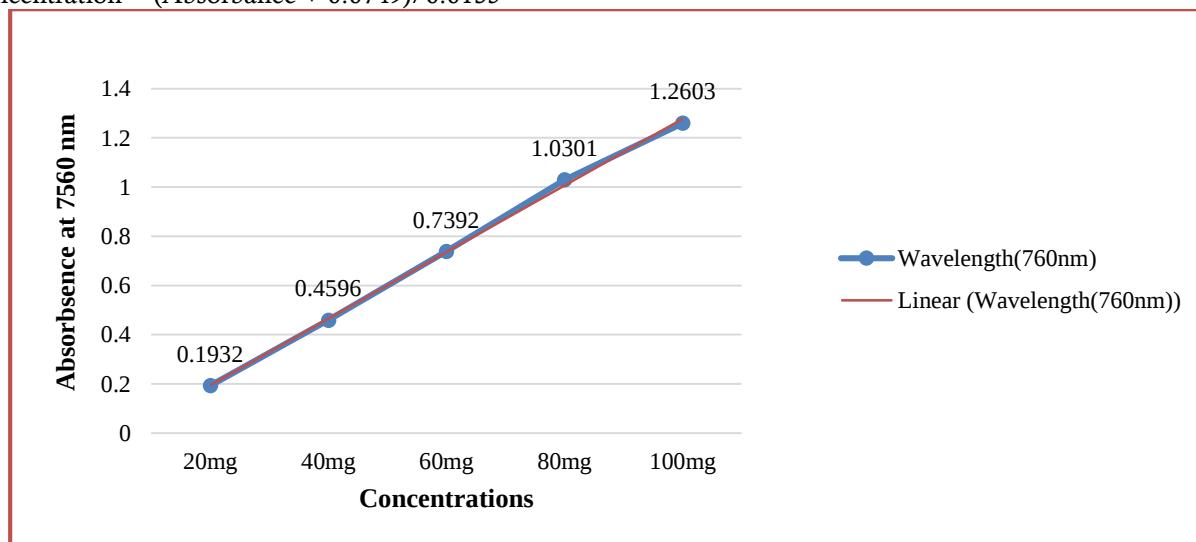


Fig 1. calibration graph of Tannin A content

Tannins are polyphenolic substances with antibacterial and antioxidant properties. The calibration curve for tannins, based on tannic acid standards, showed a high coefficient of determination ($R^2 = 0.9988$). This means there is excellent linearity between absorbance and concentration. Sample V6 had the highest corrected tannin content at 0.1829 mg/gram, indicating its potential as a rich source of tannins. These results agree with earlier studies that reported significant tannin content in different plant extracts.

Flavonoids

The flavonoid content in finger millet varieties was measured at 510 nm. A robust linear connection between concentration and absorbance was shown by the calibration curve made from standard flavonoid concentrations (20–100 µg/ml) (Table 4, Fig. 2). This confirmed the reliability of the estimation. Among the genotypes, the flavonoid content varied more widely than that of tannins, ranging from 0.2163 to 0.4411 mg/g (Table 3). The highest flavonoid concentration was found in V2 at 0.4411 mg/g, which was almost double the lowest value seen in V1 at 0.2163 mg/g. V5 (0.2537 mg/g), V7 (0.2489 mg/g), V8 (0.2460 mg/g), and V6 (0.2396 mg/g) showed moderate levels, whereas V3 (0.2295 mg/g) and V4 (0.2283 mg/g) had lower concentrations in comparison. Overall, the results show that flavonoid content had more variation between genotypes. V2 stood out as the variety with the highest flavonoid levels among those tested.

Table 3. Flavonoids Content

SAMPLE	READ 1	READ 2	AVERAGE ABSORBANCE	FLAVONOID CONCENTRATION (µg/ml)	DILUTION FACTOR	CORRECTED mg/g
V1	0.5005	0.5774	0.5389	43.26	5	0.2163
V2	0.9455	0.9972	0.9714	88.22	5	0.4411
V3	0.5589	0.5689	0.5639	45.89	5	0.2295
V4	0.5585	0.5610	0.5598	45.65	5	0.2283
V5	0.5849	0.6337	0.6093	50.74	5	0.2537
V6	0.5680	0.5968	0.5824	47.91	5	0.2396
V7	0.5762	0.6248	0.6005	49.77	5	0.2489
V8	0.5756	0.6148	0.5952	49.20	5	0.2460

Table: 4 Flavonoids Concentration

S.NO	CONCENTRATION	WAVELENGTH (510nm)
1	20 µg	0.2997
2	40 µg	0.4469
3	60 µg	0.6248
4	80 µg	0.8818
5	100 µg	1.0155

Regression Line Equation

$$Y = 0.0095 X + 0.1273$$

Y = Absorbance

X = Concentration of Flavonoid in µg

Coefficient of Determination

R² = 0.9960 Regression equation

Concentration = (Absorbance - 0.1273)/0.0095

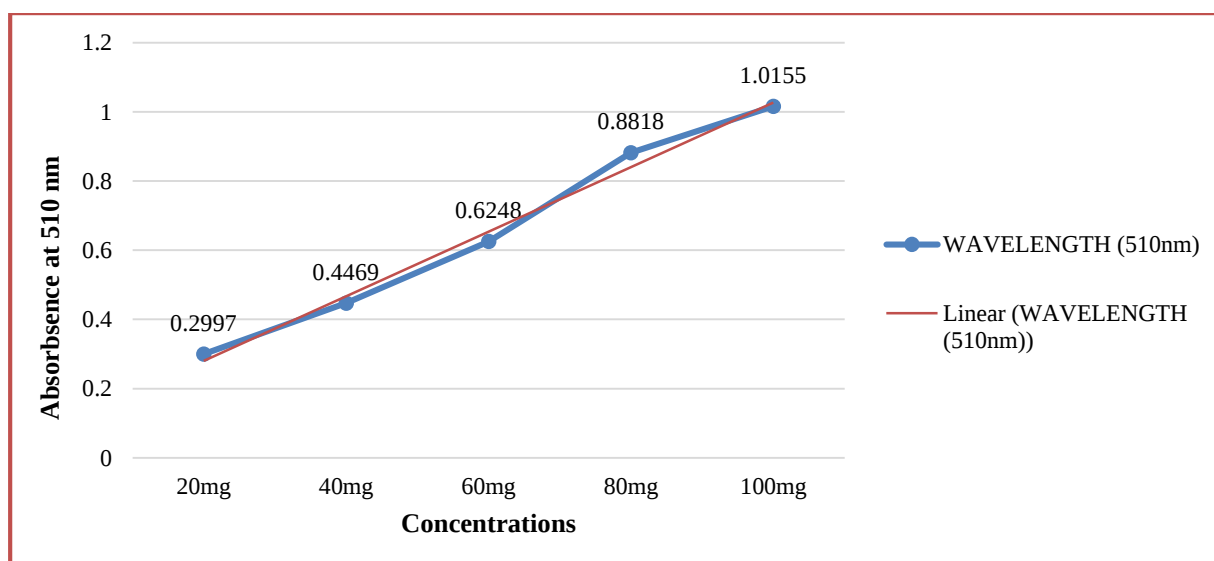


Fig 2. Content calibration graph of Flavonoids A

Flavonoids, recognized for their antioxidant and anti-inflammatory qualities, were measured using a calibration curve based on quercetin standards. The equation $Y = 0.0095X + 0.1273$ gave an R^2 of 0.9960, which shows the method is reliable. Sample V2 had the highest corrected flavonoid content at 0.4411 mg/gram. This matches findings by Hayat et al. (2020), who noted different flavonoid concentrations in various plant extracts.

Steroids

Using a spectrophotometer set to 780 nm, the steroid levels in different varieties of finger millet were assessed. With steroid concentrations ranging from 20 to 100 µg/ml, the standard calibration curve showed a clear increase in absorbance with concentration (Table 6; Fig. 3). This confirms the reliability of the quantification. Among the genotypes, the corrected steroid content varied significantly, ranging from 0.1026 to 0.2292 mg/g (Table 5). The highest concentration was found in V6, at 0.2292 mg/g, closely followed by V5 at 0.2063 mg/g. V8 (0.1539 mg/g), V2 (0.1478 mg/g), V7 (0.1476 mg/g), and V3 (0.1417 mg/g) recorded moderate levels. The lowest steroid content was found in V4 at 0.1026 mg/g. V1 also showed a relatively low value at 0.1265 mg/g. These findings suggest that there is a significant difference in steroid accumulation among the genotypes. V6 and V5 are the richest sources, while V4 is the poorest of the varieties studied.

Table 5. Steroids Content

SAMPLE	READ 1	READ 2	AVERAGE ABSORBANCE	STERIOD ESTIMATED CONCENTRATION (µg/ml)	DILUTION FACTOR	CORRECTED mg/g
V1	0.5987	0.5805	0.5896	31.62	4	0.1265
V2	0.6790	0.6617	0.6704	36.96	4	0.1478
V3	0.6540	0.6402	0.6471	35.42	4	0.1417
V4	0.4991	0.5000	0.4996	25.65	4	0.1026
V5	0.9950	0.8471	0.8911	51.58	4	0.2063
V6	0.9833	0.9664	0.9773	57.29	4	0.2292
V7	0.6661	0.6729	0.6695	36.91	4	0.1476
V8	0.6813	0.7048	0.6930	38.47	4	0.1539

Table 6: Steroid Concentration

S.NO	CONCENTRATION (Steroids)	WAVELENGTH (780nm)
1	20 µg	0.3907
2	40 µg	0.7492
3	60 µg	0.9923
4	80 µg	1.357
5	100 µg	1.5943

Regression Line Equation

$$Y = 0.0151 X + 0.1122$$

Y = Absorbance

X = Steroid Concentration in

Coefficient of Determination

$$R^2 = 0.9951$$

Regression equation

$$\text{Concentration} = (\text{Absorbance} - 0.1122)/0.0151$$

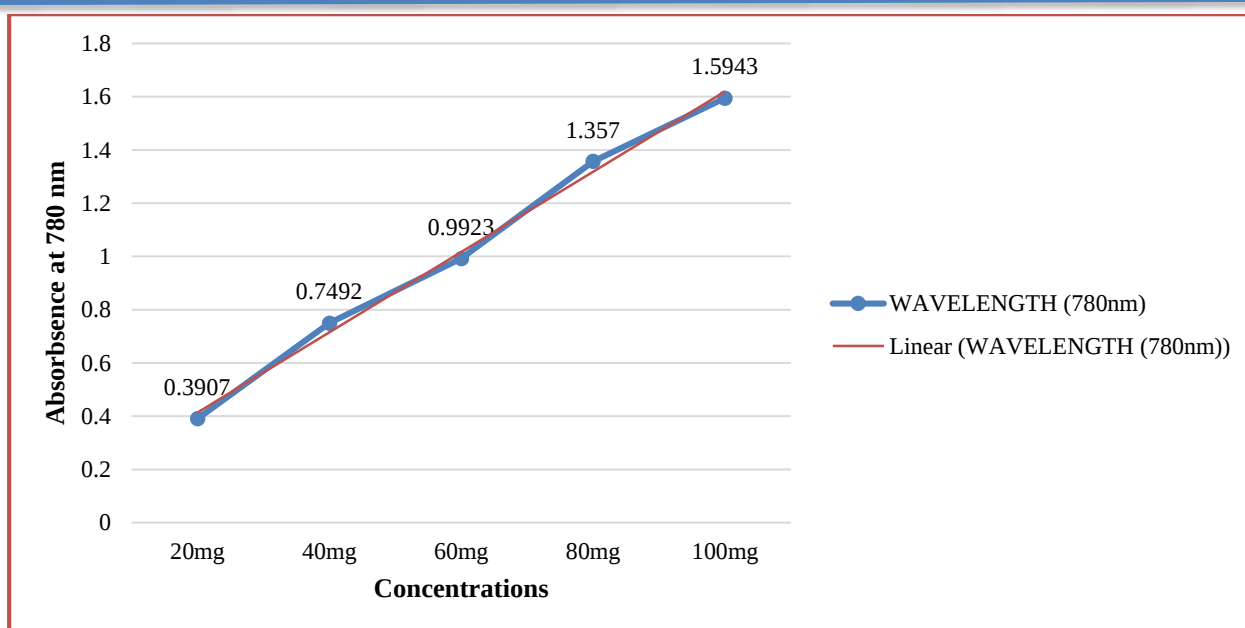


Fig 3. Content calibration graph of Steroid A

Steroids are important for many biological processes and medical uses. The calibration curve for steroids, based on diosgenin standards, showed a straight-line relationship with an R^2 of 0.9951. Sample V6 had the highest corrected steroid content at 0.2292 mg per gram. These findings match studies that report significant steroid content in plant extracts.

Saponin

Finger millet types' saponin content was tested at 430 nm. A linear relationship between concentration and absorbance was demonstrated by the standard curve made using varying saponin concentrations (20–100 μ g/ml) (Table 8; Fig. 4). This indicates that the method is reliable. The corrected saponin content varied among the genotypes, ranging from 0.1676 to 0.1803 mg/g (Table 7). V7 exhibited the highest concentration at 0.1803 mg/g, with V6 at 0.1783 mg/g and V4 at 0.1782 mg/g coming in close behind. Values of moderate magnitude were detected in V3 (0.1752 mg/g) and V8 (0.1754 mg/g). Levels detected in V2 (0.1684 mg/g), V5 (0.1683 mg/g), and V1 (0.1676 mg/g) were comparatively lower. Overall, the saponin levels exhibited limited variation among the tested varieties. There were only minor differences between the genotypes. V7 stood out as the highest in saponin levels.

Table 7. Saponin Content

SAMPLE	READ 1	READ 2	AVERAGE ABSORBANCE	SAPONINS (μ g/ml)	DILUTION FACTOR	CORRECTED mg/g
V1	0.3705	0.3680	0.3692	83.81	2	0.1676
V2	0.3661	0.3751	0.3706	84.20	2	0.1684
V3	0.3802	0.3848	0.3825	87.61	2	0.1752
V4	0.3869	0.3889	0.3879	89.14	2	0.1782
V5	0.3740	0.3671	0.3706	84.19	2	0.1683
V6	0.3914	0.3845	0.3880	89.16	2	0.1783
V7	0.3960	0.3872	0.3916	90.19	2	0.1803
V8	0.3792	0.3865	0.3829	87.70	2	0.1754

Table 8: Saponin Concentration

S.NO	Concentration	Wavelength (430nm)
1	20 μ g	0.1359
2	40 μ g	0.2278
3	60 μ g	0.2994
4	80 μ g	0.3514
5	100 μ g	0.4287

Regression Line Equation

$$Y = 0.0035 X + 0.0759$$

Y = Absorbance

X = Concentration of Saponin in ug

Coefficient of Determination

$$R^2 = 0.9920$$

Regression equation

$$\text{Concentration} = (\text{Absorbance} - 0.0759) / 0.0035$$

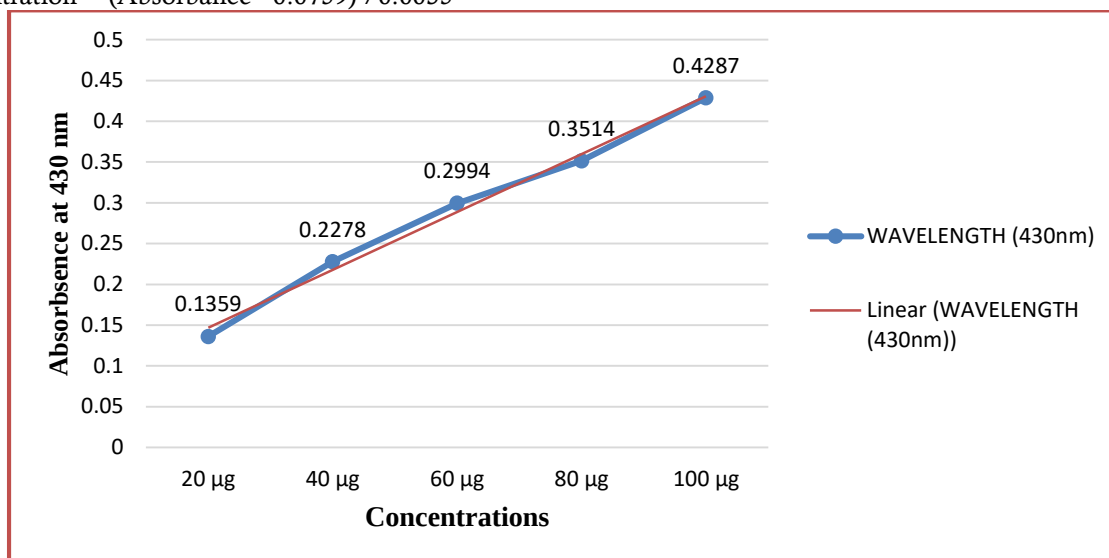


Fig 4. Content calibration graph of Saponin

Saponins have significant foaming properties and biological activities. The calibration curve for saponins, based on diosgenin standards, showed strong linearity with an R^2 of 0.9920. Sample V7 had the highest corrected saponin content at 0.1803 mg/gram. This is in line with the findings of Dikamu et al. (2025), who found that different seed extracts had variable saponin levels.

Glycoside

The calibration curve derived from standard glycoside concentrations (20–100 µg/ml) demonstrated a distinct linear relationship between absorbance and concentration (Table 10; Fig. 5), thereby confirming the assay's reliability. As shown in Table 9, the corrected glycoside content differed considerably across varieties, with values between 0.2049 and 0.3990 mg/g. The maximum value was recorded in V6 (0.3990 mg/g), with V5 coming next (0.3640 mg/g). V4 registered the lowest value at 0.2049 mg/g. V8 (0.2835 mg/g), V2 (0.2743 mg/g), V7 (0.2739 mg/g) and V3 (0.2648 mg/g) while V1 (0.2414 mg/g) also remained on the lower side. These results show that glycoside content had the most variation among the phytochemicals studied. V6 was the richest source, while V4 was the poorest.

Table 9. Glycoside Content

SAMPLE	READ-1 OD	READ-2 OD	AVG OD	GLYCOSIDE (µg/ml)	DILUTION FACTOR	CORRECTE D mg/g
V ₁	0.5776	0.6016	0.5896	60.36	4	0.2414
V ₂	0.6568	0.6840	0.6704	68.57	4	0.2743
V ₃	0.6310	0.6632	0.6471	66.21	4	0.2648
V ₄	0.4865	0.5127	0.4996	51.22	4	0.2049
V ₅	0.8732	0.9090	0.8911	91.00	4	0.3640
V ₆	0.9571	0.9975	0.9773	99.76	4	0.3990
V ₇	0.6548	0.6842	0.6695	68.48	4	0.2739
V ₈	0.6784	0.7076	0.6930	70.874	4	0.2835

Table 10: Glycoside Concentration

S.NO	CONCENTRATION (Glycoside)	WAVELENGTH (495nm)
1	20 µg	0.1914
2	40 µg	0.3865
3	60 µg	0.5736
4	80 µg	0.8198
5	100 µg	0.9588

Regression Line Equation

$$y = 0.0098x - 0.0044$$

- y = absorbance
- x = concentration in µg
- $R^2 = 0.9950$ (indicating excellent linearity)

$$\text{Concentration} = (\text{Absorbance} - 0.0044)/0.0098$$

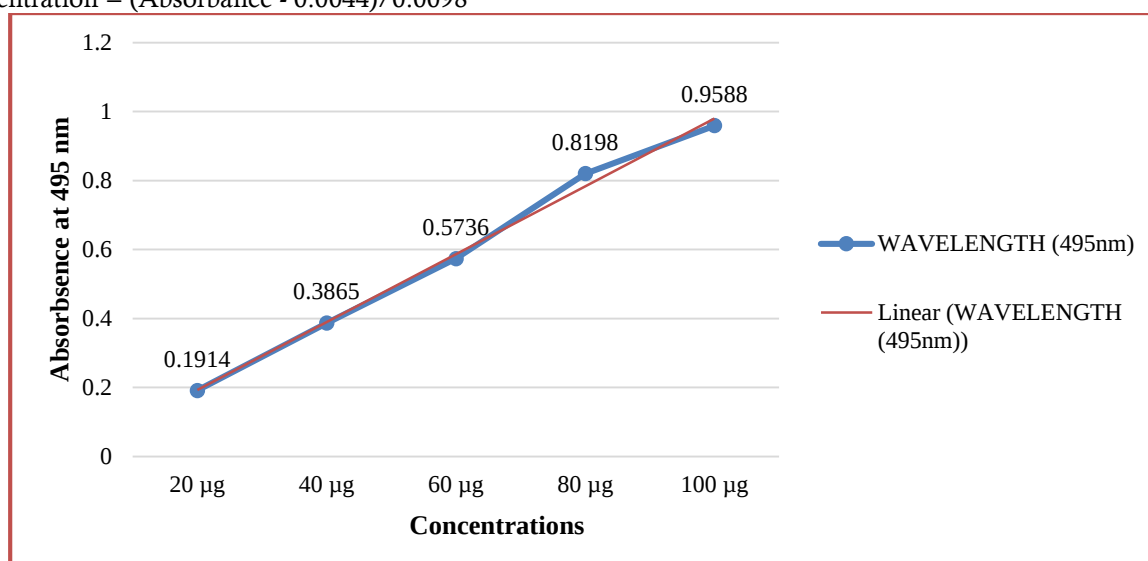


Fig 8. Content calibration graph of Glycoside

Glycosides are important due to their drug effects and medical uses. The calibration curve for glycosides, based on standard compounds, showed a strong linear relationship with an R^2 of 0.9950. Sample V6 had the highest corrected glycoside content at 0.3990 mg/gram. These results confirm previous studies that highlight the presence of glycosides in different seed species.

CONCLUSION

The current study focuses on the variety of phytochemicals found in eight finger millet (*Eleusine coracana* L.) varieties. It specifically emphasizes tannins, flavonoids, steroids, saponins, and glycosides, which were measured using spectrophotometric methods. The results showed clear differences among the varieties. V6 was notable for being the most abundant source of glycosides (0.3990 mg/g), steroids (0.2292 mg/g), and tannins (0.1829 mg/g). In contrast, V2 had the highest concentration of flavonoids (0.4411 mg/g). Saponin levels were fairly consistent across the samples, with V7 having a slight edge. These variations likely arise from genetic factors, environmental conditions, and how the plants regulate their metabolism to produce secondary metabolites. The findings highlight the nutritional and therapeutic value of finger millet, especially V2 and V6, as good options for creating functional foods, nutraceuticals, and plant-based

medicines. Their rich phytochemical profiles suggest potential uses in preventing oxidative stress, inflammation, infections, and lifestyle-related health issues. Additionally, the strong correlation of the calibration curves ($R^2 > 0.99$) confirms the reliability and consistency of the spectrophotometric methods used. Overall, this research lays the groundwork for selecting specific varieties in breeding programs and for developing value-added products, while also opening doors for future studies into the bioactive properties of finger millet.

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Conflicts of Interest

The authors state that they have no conflict of interest.

Ethical Clearance

Not applicable for this study.

Financial Grant

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Declaration

This manuscript was prepared with help from Grammarly to improve language and correct grammar only. All content has been reviewed and approved by the authors.

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