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Comparative Analysis of Leaves of Okra (*Abelmoschus esculentus* L. Moench) Accessions for Bioactive Compounds, Antioxidant Activities, and Antinutritional Compounds for Food Applications

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ABSTRACT

Okra (*Abelmoschus esculentus* L. Moench) is valued for its edible leaves; harvesting leaves at the optimal maturity stage is essential to maximize the accumulation of bioactive compounds. In this study, seven okra leaf accessions harvested at two maturity stages were analyzed for bioactive compounds, antioxidant activity, and antinutrients. There was a significant interaction between accession and maturity stage for most parameters ($p < 0.05$). The antioxidant capacity of mature leaves varied with assay, with young leaves often having stronger free radical scavenging activity. Mature leaves of "Clemson Spineless" recorded the highest total phenolics (633.2 mg gallic acid [GAE]/100 g), total carotenoids (100.52 mg/100 g), lutein (50.74 mg/100 g), and chlorophyll (5.87 mg/100 g). The mature leaves of "Clemson Spineless" and "33-84-113" contained the highest levels of catechins (70.99 mg/100 g) and protocatechuic acid (90.32 mg/100 g), respectively. "Clemson Spineless" had the highest FARP value (0.2 mg TEAC/100 g). The oxalate content was lowest (15.85 mg/g) in the young leaves of accession "33-84-113." This understanding is crucial for selecting the best okra accessions and determining the optimal leaf harvest maturity to enhance health benefits while minimizing undesirable antinutrients, which is essential for functional food and dietary applications.

1 | Introduction

Universal food trends are moving from food farming to well-being farming. As a result, the consumption of natural food-based products was recently concomitant with harvesting phytonutrients (primary and secondary metabolites) for improved consumer health [1]. Primary metabolites (protein, carbohydrates, fats, and vitamins) are essential for human health nourishment, while the phytochemicals, non-nutritive secondary plant metabolites such as carotenoids, and phenolic compounds, including flavonoids, are known to contribute to an improved immune system through reactive oxygen species scavenging activity, resulting in reduced incidence of diet-related chronic diseases [2].

In developing countries, indigenous food crops are increasingly consumed to promote dietary diversity and provide a rich source of phytonutrients through various vegetable varieties. Often overlooked or underutilized, these crops offer essential vitamins, minerals, and other beneficial compounds that contribute to improved nutrition and enhanced food security [3]. The response

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of vegetable varieties to climate change attributes, including drought tolerance, enhanced yield performance, and improved nutritional quality, underscores the need for ongoing development of diverse traits in vegetable genotypes [4]. The stage of leaf maturity optimum for higher phytonutrient harvest could be influenced by the surface area contributing to the biosynthesis of beneficial phytonutrients [5].

Leaves of okra (*Abelmoschus esculentus* L. Moench, Malvaceae family) are known for their phytonutrient-dense characteristics beneficial for vulnerable individuals, including pregnant women. Okra leaves are edible and widely used in cuisines across Africa and Asia, both raw and cooked. Young, tender leaves can be chopped and added raw to salads, while mature leaves are best suited to cooked applications. The leaves can be sautéed, stir-fried, or boiled and can also be boiled to make tea.

Okra leaves exhibit antidiabetic properties due to their high content of dietary fiber, minerals, and vitamins. They are rich in beneficial carotenoids, including lutein, beta-carotene, neoxanthin, and violaxanthin, along with isomers like 9-cis and 13-cis beta-carotene. These act as powerful antioxidants, supporting vision, reducing oxidative stress, and providing anti-inflammatory benefits, with their levels influenced by growing conditions such as light and water stress [6]. They also contain phytochemicals such as flavonoids, including luteoliflavan quercetin, kaempferol, and (+)-epicatechin, which contribute to antidiabetic, antioxidant [7], and anti-inflammatory effects [8]. Previous research on underpinning optimum growth and harvest time for okra leaves shows that growing conditions significantly influence the accumulation of phenolic metabolites in okra leaves and pods [9]. Mokgalabone et al. [10] showed that among the leaves grown in a microplot under open field conditions, phenolic metabolites, n-O-caffeoyl glucaric acid, feruloyl glucaric acid, feruloyl-(arabinosylxylose), feruloyl glucarate hexoside, quercetin dihexoside, and quercetin hexoside were found to be more abundant than those grown in greenhouse conditions.

Phenols are a diverse group of plant compounds with dual roles: They are beneficial bioactive compounds but can also act as antinutrients depending on the context [8]. Their health-promoting properties, such as antioxidant and anti-inflammatory effects, make them valuable in a healthy diet. However, phenols can interfere with digestion and nutrient absorption at high concentrations. Processing methods like cooking or soaking can reduce antinutrient levels [9], and in a balanced diet, the bioactive benefits of phenols typically outweigh their minor antinutrient effects. Okra leaves also contain antinutrients like phytates and oxalates. Phytates bind minerals such as iron and zinc, potentially reducing absorption, while oxalates can bind calcium. However, cooking helps mitigate these antinutrient effects, making okra leaves a nutritious dietary addition with antioxidant benefits [11].

Elshafy et al. [11] showed phytochemical constituents of newly developed okra pod accessions, leaving out the composition of the leaves. Additionally, the harvest stage of leafy vegetables has a significant impact on their phytonutrient concentration. According to some studies, young leaves accumulate more chlorophyll, while others found the opposite, and phenolic compound levels are variable, depending on the species [12]. Borokini et al. [13] reported the phytonutritional composition of okra leaf, but information on antioxidant compounds in relation to leaf age (i.e., maturity) and other common varieties remains limited.

The study aims to identify the most suitable okra accessions and leaf maturity stage for breeding programs by evaluating their phytochemical profiles, including phenols, flavonoids, carotenoids, antioxidant activity, and antinutritive compounds. This research aims to identify the genetic lines with the most desirable compounds and determine the optimal leaf maturity stage to enhance phytonutritional properties. The findings will support the development of improved okra varieties that meet market demands for use as leafy vegetables or functional ingredients in nutraceutical applications.

2 | Materials and Methods

2.1 | Plant Material

Okra accessions "Clemson Spineless," 26-71-116, 27-73-124, 28-82-118, 31-56-96, 33-74-113, and 36-74-120 from the gene bank collection were planted at the South African Agricultural Research Council-Vegetable, Industrial and Medicinal Plants (ARC-VIMP) sector in Pretoria, Gauteng province, located at 25.56° S and 28.35° E above sea level. The leaves were harvested from 90-day-old okra plants grown in an open field condition following the cultivation protocol explained by Mokgalabone et al. [10] in an open field (soil). Young leaves are tender, smaller leaves located at the apex (top) of the plant, typically harvested around 15–20 days after planting. Mature leaves are larger leaves found lower on the stem, starting from the 4th leaf from the apex down to the basal leaves, and are harvested around 80–90 days after planting.

2.1.1 | Experimental Design

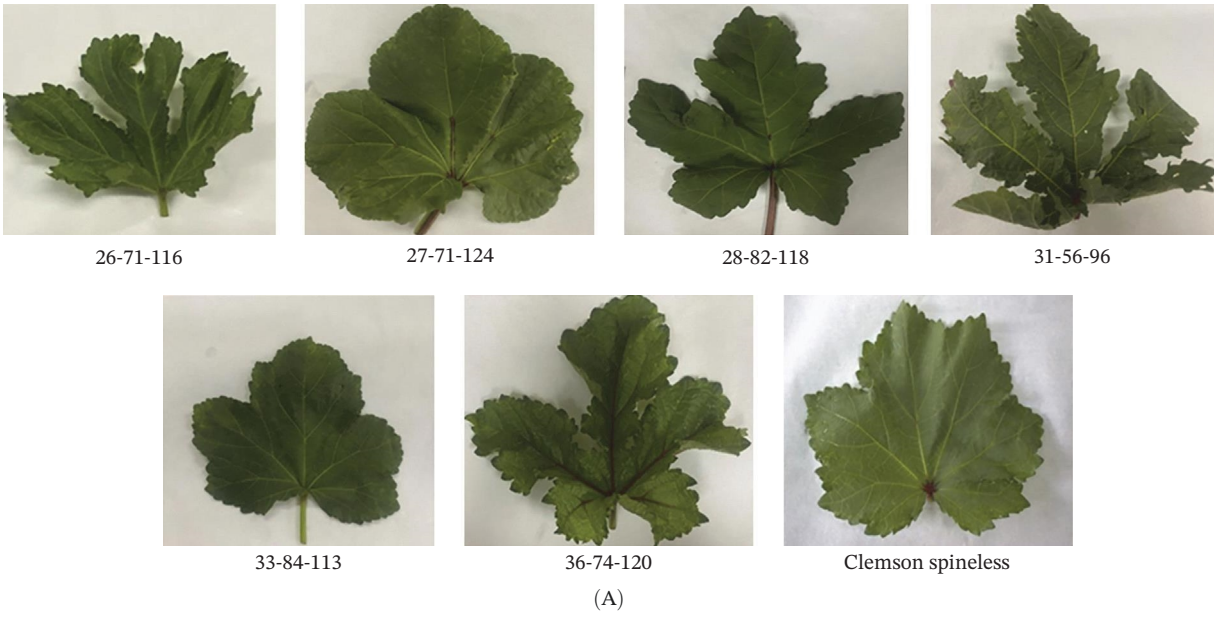
Using a completely randomized design (CRD), leaf color, bioactive compounds, and antioxidant activity were evaluated across two successive harvest cycles in summer from the same plants. A total of 20 plants per variety/accession were used. In this study, the leaf maturities were characterized by the leaf area size and color, whereby light green leaves with 150 cm^2 (15–20 days after planting) in diameter were regarded as young (Figure 1A), while those with 600 cm^2 and dark green color (80–90 days) were regarded as mature leaves, as shown in Figure 1B. For the analysis of chemical parameters, leaves from these 20 plants were pooled per accession per maturity stage. Prior to phytonutrient analysis, okra samples were freeze-dried using a benchtop freeze-drier (Benchtop Freeze Dryer, VirTis Sp Scientific, Gardiner, NY, USA, Model # 2kBTES-55) and ground into powder.

2.2 | Chemicals and Reagents

All chemicals and reagents applied in this research were obtained from Sigma–Aldrich, based in Johannesburg, South Africa.

2.3 | Chlorophyll Content

A sample of 0.2 g (fresh weight) of okra was combined with 2 mL of a 4:6 mixture of acetone and hexane for 2 h and then centrifuged (1000 rpm) for 10 min (4°C). Chlorophyll a and chlorophyll b concentrations were evaluated by measuring absorbance at 645 nm and 662 nm, respectively. The total chlorophyll content was calculated following the methods described by Mampholo et al. [14].



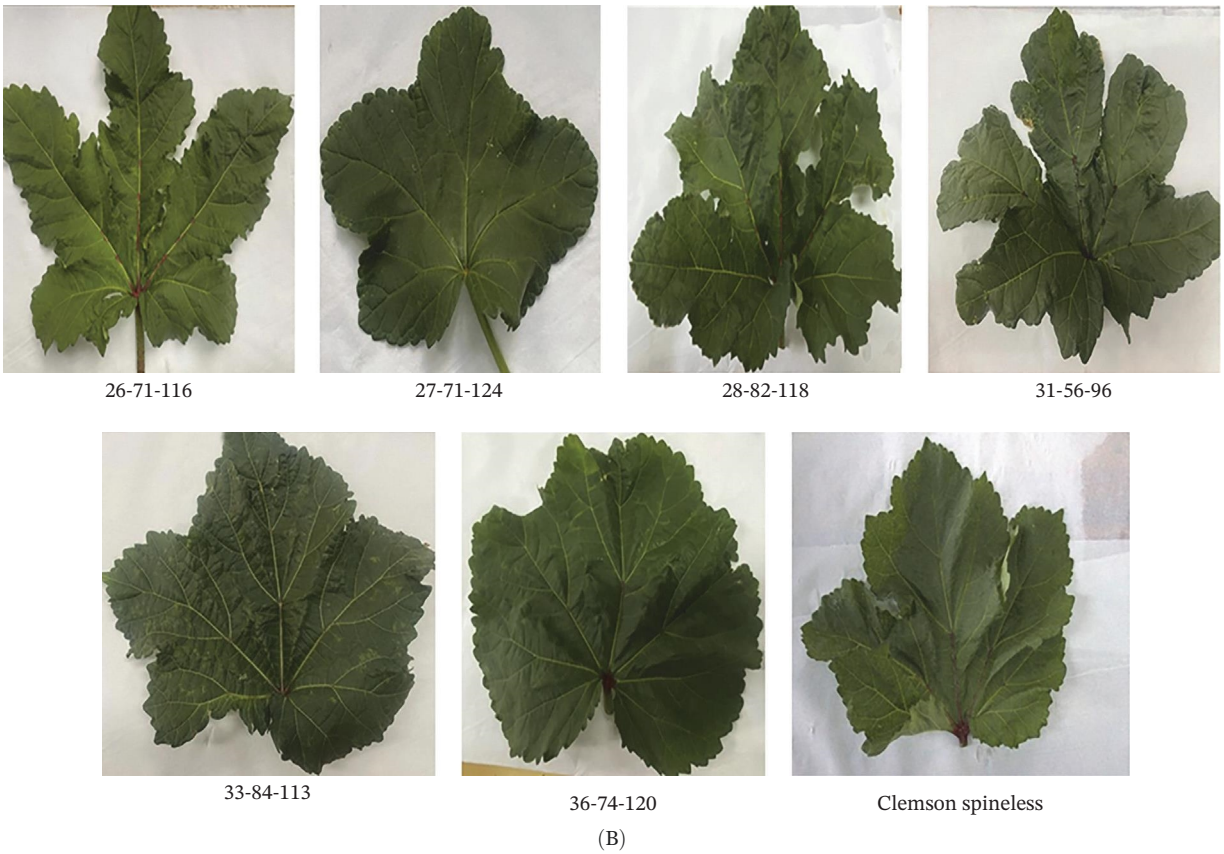


FIGURE 1 | (A) Young leaves (150 cm^2) of different okra accessions. (B) Mature leaves (600 cm^2) of different okra accessions.

2.4 | Total Phenolic Content (TPC), Phenolic Profile, and Antioxidant Activity

Extraction of biochemical compounds, including total phenols, individual phenolic acids, and antioxidant activities, was carried out following the methods described by Bhengu et al. [15]. Briefly, a total of 200 mg of the freeze-dried okra powder was homogenized with 2 mL of 80% methanol (HPLC grade), vortexed for 20 s, and sonicated for 10 min at 25°C. Afterwards, the samples were centrifuged for 20 min (10.000 rpm × g and 4°C). The supernatant was filtered through a hydrophobic PTFE syringe filter (0.22 µm pore size) for analysis of different components of phenolic compounds, TPC, and antioxidant activity (FRAP, DPPH, and ABTS).

Analyses of total phenols were determined following Folin-Ciocalteu's reagent methodology described by Singleton et al. [16] with some modifications. Briefly, 100 µL of the extract (okra leaf)

was reacted with 750 µL of 10-fold diluted Folin-Ciocalteau reagent and 750 µL of 7.5% Na_2CO_3 solution, and the absorbance of the mixture was measured at 765 nm. A calibration curve constructed with the gallic acid (GAE) standard concentrations ranging from 0 to 800 µL was generated to calculate the total phenols equivalent to GAE in mg/100 g FW.

Targeted phenolic compounds were quantified using a Shimadzu HPLC-DAD system (LC-2030C, Shimadzu Corp, Kyoto, Japan) as described by Bhengu et al. [15]. Compounds were separated on a Shimadzu C18 column (4.6 × 250 mm and 5 µm) with a gradient elution of 0.1% formic acid (solvent A) and methanol/acetonitrile (40:60, solvent B) without any modifications. A sample (10 µL) was injected three times into the HPLC. The separation was achieved on a Luna C18 column (4.6 × 250 mm, 5 µm, Phenomenex) with a gradient elution of solvent A (water +0.1% formic acid) and solvent B (methanol/acetonitrile, 40:60), starting with 0% B in A to reach 10% B at 5 min, 15% B at 10 min, 25% B at 15 min, 35% B at 25 min, 100% B at 40 min, and the flow rate of 0.6 mL/min. Targeted phenolics were quantified using the calibration curves constructed using pure external standards. The results are reported in mg/100 g FW.

The examination of DPPH free radical scavenging activity followed the methods outlined by Bhengu et al. [15]. Briefly, the solution was made by mixing 6 mg of DPPH with 25 mL of methanol-water (80:20) solvent. Then, 10 mL of this solution was mixed with a 50 mL methanol-water solvent to make the DPPH working solution. Various concentrations of phenolic extracts were prepared by serial dilution, and 100 µL of each was mixed with 200 µL of DPPH working solution in a 96-well microplate. The plate was incubated in the dark for 10 min before measuring the absorbance at 517 nm. The DPPH was expressed as IC_{50} (mg/mL).

The ABTS scavenging activity of the leaves was assessed as described by Bhengu et al. [15]. The ABTS + solution was prepared by mixing a 7 mmol/L ABTS solution with a 2.45 mmol/L potassium persulfate solution in a 1:1 ratio and keeping the mixture overnight in the dark. Afterward, in a 96-well microplate, 200 µL of this solution was added to 40 µL of the sample extract at various concentrations prepared by serial dilution of the phenolic extract. After incubation for 15 min at 37°C in the dark, the absorbance was read at 750 nm. The results are expressed as IC_{50} in mg/mL.

To calculate % inhibition from absorbance, we used the formula:

$$(\%\text{Inhibition}) = 100 \times (1 - (\text{Absorbance_Treated}/\text{Absorbance_Control})),$$

then fit this data (Concentration vs. % Inhibition, often log-transformed) to a dose–response curve (like a four-parameter logistic model) using software like GraphPad Prism, which uses nonlinear regression to find the IC_{50} value.

2.5 | Total Carotenoids and Carotenoid Profile

The total carotenoid contents (TCCs) were measured by employing the method described by Selelepoo et al. [17], with minor modifications. Okra leaves (100 mg) were mixed with 5 mL of 95% ethanol:acetone (1:1) containing 0.1% (w/v) BHT in an orbital shaker for 10 min. After that, the mixture underwent saponification for 30 min with a 20% KOH solution in methanol. Then, 3 mL of dichloromethane/hexane (3:7) containing 0.1% BHT was added to extract the carotenoids, followed by the addition of 3 mL of NaCl solution (10%, w/v) before centrifugation at 3900 × g for 5 min at 25°C. The carotenoids' extraction was repeated twice. The upper layer from the three repetitions was combined, and the solvent was evaporated using a nitrogen stream until dry. The dry extract was redissolved in 2 mL of methanol/MTBE (1:1) containing 0.1% BHT. An aliquot (200 µL) was used to read the absorbance at 453 nm. The TCC was calculated against the calibration curve of the β-carotene standard, and the results are expressed as β-carotene equivalent in mg/100 g FW.

For individual carotenoid quantification, 10 µL of filtered reconstituted extract was injected into an HPLC system, according to Bhengu et al. [15], 13 without any modification. The InertSustain C30 column (3.0 × 250 mm, 3 µm, GL Sciences, Tsukuba, Japan) was employed for chromatographic separation at 30°C. Eluent A consists of 0.1% formic acid in methanol and eluent B 0.1% formic acid in methyl tert-butyl ether. Carotenoids were identified and quantified by utilizing external standards, including β-carotene, lutein, and zeaxanthin. Chromatograms were recorded at 460 nm. The results are expressed as mg/100 g FW.

2.6 | Antinutritive Compound

Oxalate content was determined using the AOAC [18] standard procedure and Managa et al. [19]. Insoluble oxalates were extracted from 0.1 g of leaf powder with 30 mL of 2 mol/L HCl. Soluble oxalates were extracted from 0.1 g of leaf samples with distilled water. A 5% $CaCl_2$ solution was added to form a CaC_2O_4 precipitate. This precipitate was collected, washed three times with 0.35 M NH_4OH, and then dissolved in 0.5 M H_2SO_4. Subsequently, 0.1 M KMnO4 solution was used to titrate the oxalate in this solution at 60°C. Oxalate content is presented in mg/g FW.

The tannin content was measured using the method described by Managa et al. [19]. Powdered leaf (0.2 g) was mixed with 10 mL of 1% HCl. The mixture was vortexed for 20 min and then centrifuged at 2500× g for 5 min. The supernatant was collected, and 100 µL was mixed with 50 µL of vanillin-HCl in a methanol solution 8% HCl in methanol mixed with 1% vanillin in methanol in a 1:1 ratio) in a microplate. The plate was incubated for 20 min at 30°C before reading the absorbance at 500 nm. The samples turned reddish-brown. Tannic acid was used to construct a calibration curve ($Y = 0.0009x + 0.024$ and $R^2 = 0.9488$) to calculate the tannin content expressed in mg/g FW.

Phytate content was determined according to Managa et al. [19]. The sample (0.5 mg) was extracted with 100 mL of 2.4% HCl for 1 h. The extract was mixed with Wade reagent (0.03 g monohydrate ferric chloride and 0.3 g sulfosalicylic acid in 100 mL distilled water) as described by Latta and Eskin's [18] procedures. The absorbance of the pink-colored supernatant at 500 nm using a spectrophotometer against a blank solution (Wade's reagent with deionized water instead of the sample) indicates that the intensity of the pink color is inversely proportional to the amount of phytate present, as phytate binds iron and reduces the color intensity. Thereafter, a standard curve (a linear regression equation, $Y = -0.4963 + 2.8261$, and $R^2 = 0.91$) was created by

plotting absorbance values against the corresponding phytate concentrations, and a linear regression equation was used to describe the relationship. The phytate content is presented in mg/g.

2.7 | Statistical Analysis

A two-way analysis of variance was performed using Genstat 64-bit Release 24.1 (VSN International, Hemel Hempstead, UK) statistical package for Windows 10 Edition to compare the differences between two maturity stages (young and mature leaves) and genotype variation (seven different okra genotypes) over two seasons. Since a nonsignificant difference was found in 2024 and 2025 during the same season, with the main factors (accession and maturity), the data were pooled together. Mean value per accession-maturity-stage combination is $n = 14$ for the statistical test. The LSD test with $p < 0.05$ was utilized to compare the means of various biochemical components in young and mature leaves of different okra genotypes.

3 | Results and Discussion

3.1 | Chlorophyll

Figure 2 shows total chlorophyll contents in the seven studied accessions of okra leaves harvested at two maturity stages. Mature okra leaves of the "Clemson Spineless" had the highest total chlorophyll content at 5.87 mg/100 g, which was significantly higher than all other accessions ($p < 0.05$). Moreover, mature okra leaves of the "Clemson Spineless" and "28-82-118" and "31-56-96" varieties exhibited significantly higher chlorophyll levels compared to their respective young leaves. In contrast, young leaves of the "27-73-124" and "Clemson Spineless" accessions exhibited significantly ($p < 0.05$) the highest chlorophyll concentrations compared to those of "26-71-116," "28-82-118," "31-56-96," "33-84-113," and "36-74-120." Except for accessions "36-74-120," "27-73-124," and "33-84-113," where both mature and young leaves of these specific accessions exhibited similar chlorophyll content levels.

In most plant species, mature leaves typically have a higher overall chlorophyll content than young leaves because they are fully developed and specialized for maximum light absorption and photosynthesis. The cases where young leaves have higher chlorophyll content are specific and often due to species-specific adaptations or environmental factors [20]. In contrast to younger leaves, mature leaves are optimized for sustained photosynthetic performance, resulting in a complex interaction between mesophyll structure, chloroplast size, and chlorophyll content at different leaf ages and under different conditions [21]. This further suggests that mature leaves had fully grown and had an optimized green color accumulation.

3.2 | TPC

The effect of okra leaves harvested at two maturity stages from different varieties or accessions on the TPC is shown in Figure 3. The "Clemson Spineless" variety exhibited the highest TPC of 633.22 mg GAE/100 g, which was significantly ($p < 0.05$) higher than all other accessions ($p < 0.05$). The TPC in mature leaves was 1.85, 1.68, 1.88, 1.37, 1.32, 1.22, and 1.69-fold higher than in young leaves for the "Clemson Spineless," "26-71-116," "28-82-118," "31-56-96," "33-84-113," and "36-74-120" accessions, respectively.

During seedling development, phenolic compounds accumulate in mature leaves at a greater rate than in young leaves, an effect attributed to UV protection and phenolic accumulation during development [22]. The biosynthesis of flavonoids, key photoprotective compounds, can be stimulated by high levels of light or

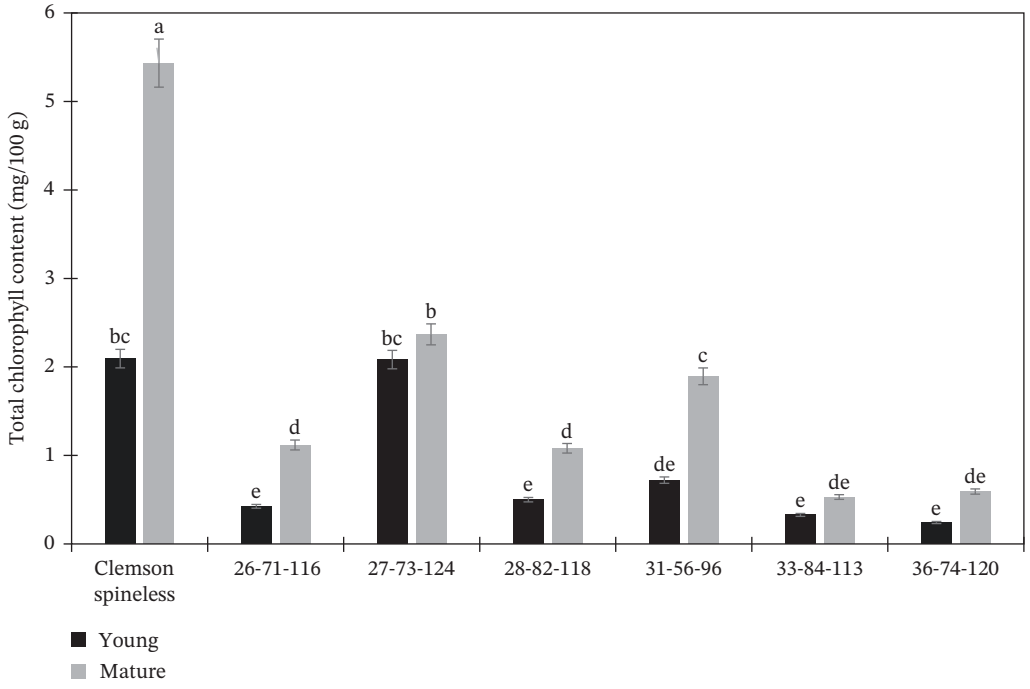


FIGURE 2 | Influence of leaf harvesting maturity on total chlorophyll content (mg/100 g FW) of seven okra accessions. Bars with different alphabets indicate significant differences at $p < 0.05$ based on Fisher's protested LSD test.

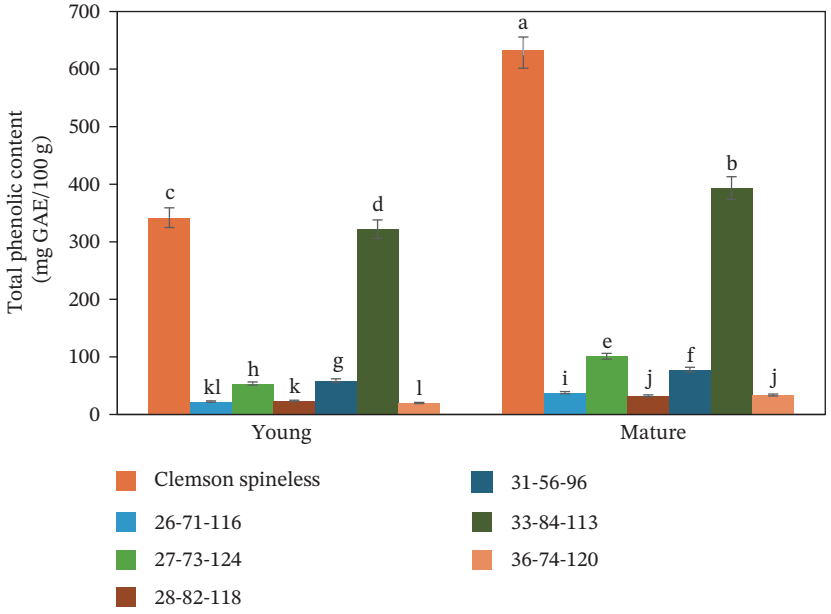


FIGURE 3 | Influence of leaf harvesting maturity on total phenolic content of seven okra accessions. Bars with different alphabetical letters are significantly different at $p < 0.05$, based on Fisher's protested LSD test.

ultraviolet radiation and imbalances in photosynthetic electron supply [22]. Further, genetic differences in the pathways for phenolic compound synthesis are likely to account for the variation in phenolic content among okra accessions [23]. However, the change in phenolic content with maturity is not a universal constant but rather a complex, species-specific physiological and biochemical response to a combination of developmental stages, genetic blueprints, and environmental cues [22].

In comparison to other indigenous vegetables, the TPC level in mature leaves of "Clemson Spineless" was higher than that of jute (313.92 mg GAE/100 g) and green *Amaranthus* (113.33 mg GAE/g), yet lower than the TPC level found in *Cleome gynandra L* (812 mg GAE/100 g) at mature leaf age [23]. Furthermore, mature leaves of accessions "27-73-124" (101 mg GAE/100 g) and "31-56-96" (78 mg GAE/100 g) showed moderate TPC levels comparable to the mature leaves of pumpkin (110 mg GAE/100 g) [24]. Okra accessions, "26-71-116," "28-82-118," and "36-74-120" exhibited the lowest TPC (37.9, 32.4, and 33.8 mg GAE/100 g), and their contents were comparable to the microgreens of cucumber vegetable (38.6 mg GAE/100 g) [23].

3.3 | Phenolic Compound Profile

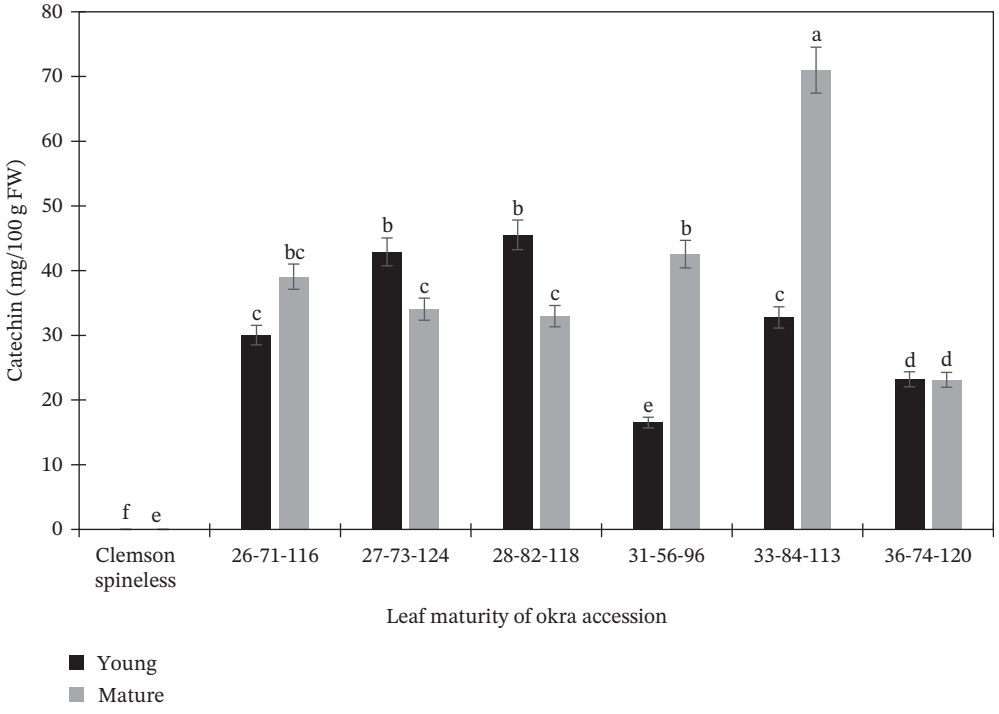
The bar graphs (Figure 4A–G) show the quantitative levels of different flavonoids and phenolic acids in young and mature leaves of okra accessions. The mature leaf of okra accession "33-84-113" contained significantly ($p < 0.05$) the highest levels of catechin (70.99 mg/100 g), compared to all other accessions (Figure 4A). The catechin content in the mature leaves of accession "33-84-113" increased by 2.17-fold as opposed to its young leaves (Figure 4A). Similarly, the catechin content in "31-56-96" increased two-fold in mature leaves compared to young leaves. In contrast, the catechin content of "27-73-124" decreased by 0.79-fold in mature leaves relative to their young leaves. Likewise, the catechin content in "28-82-118" decreased by 0.72-fold in mature leaves compared to their young leaves. However, the catechin levels in both young and mature leaves of okra accession "36-74-120" remained unchanged. Conversely, the young leaves of "27-73-124" and "28-82-118" showed statistically nonsignificant ($p > 0.05$) (similar levels) catechin content. Similarly, the mature leaves of "26-71-116" and "31-56-96" exhibited nonsignificant ($p > 0.05$) (similar levels) similar levels of catechin.

However, epicatechin was not detected in the young or mature leaves of okra accessions "28-82-118" and "36-74-120" (Figure 4B). Epicatechin content was not detected in the young leaves of accessions "27-73-124," "31-56-96," and "33-84-113," but it was present in their mature leaves. Additionally, epicatechin was also not found in the mature leaves of "Clemson Spineless," although it was detected in the young leaves at a level of 1.60 mg/100 g.

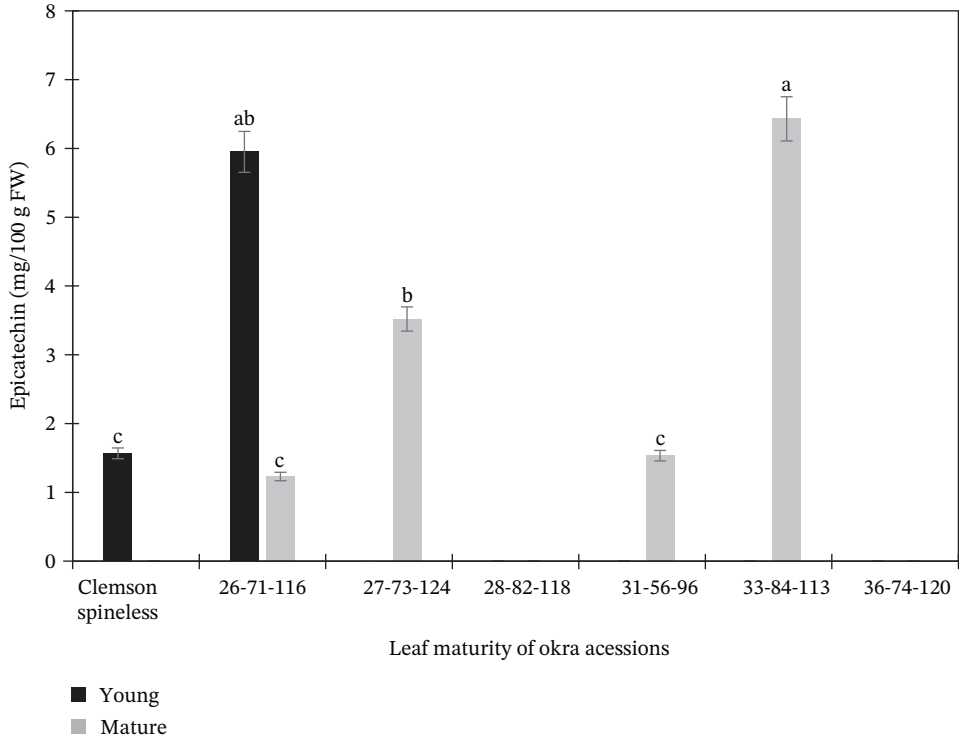
However, quercetin was not found in the young or mature leaves of "36-74-120." While quercetin was absent in the young leaves of the okra accessions "31-56-96," "33-84-113," and "Clemson Spineless," it was detected in the mature leaves of "31-56-96" (3.01 mg/100 g) and "33-84-113' (1.01 mg/100 g) (Figure 4C). Furthermore, quercetin was detected in the young leaves of okra accessions "26-71-116," "27-73-124," and "28-82-118," but it was not detected in the mature leaves.

The levels of protocatechuic acid were significantly higher ($p < 0.05$) in the mature leaves of the okra accession "Clemson Spineless" (90.32 mg/100 g) compared to other accessions (Figure 4D). Mature leaves of "Clemson Spineless" exhibited a 5.86-fold increase compared to its young leaves.

Similarly, the mature leaves of the okra accession "31-56-96" showed a remarkable 21-fold increase in protocatechuic acid levels compared to their young leaves. In general, protocatechuic acid levels increased in mature leaves, except for the accession "28-82-118." Protocatechuic acid was not detected in the young or mature leaves of okra accession "36-74-120." Notably, "31-56-96" exhibited significantly ($p < 0.05$) the highest rutin levels in young leaves at 5.01 mg/100 g compared to all other accessions

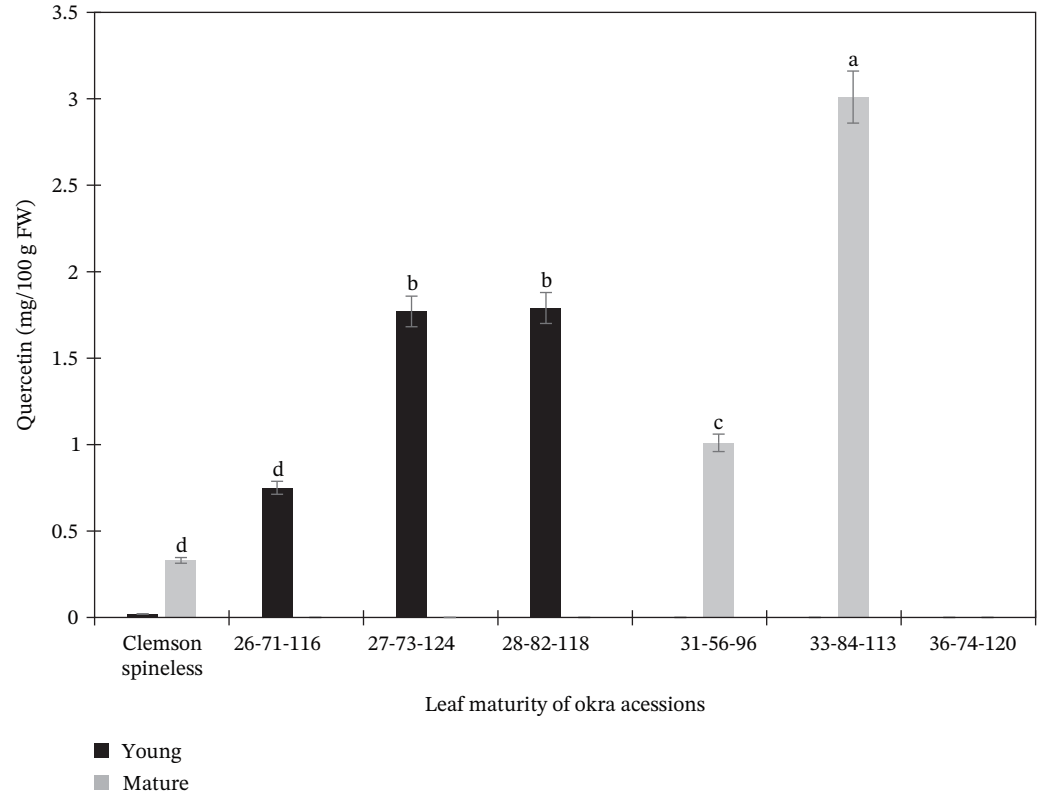


(A)

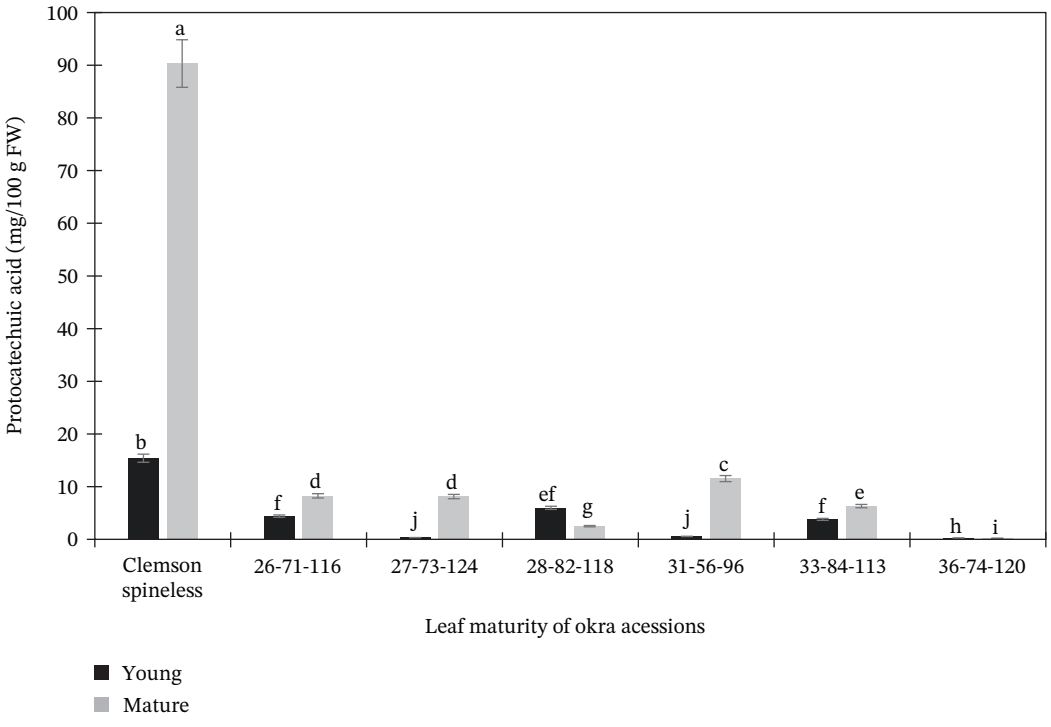


(B)

FIGURE 4 | (Continued)

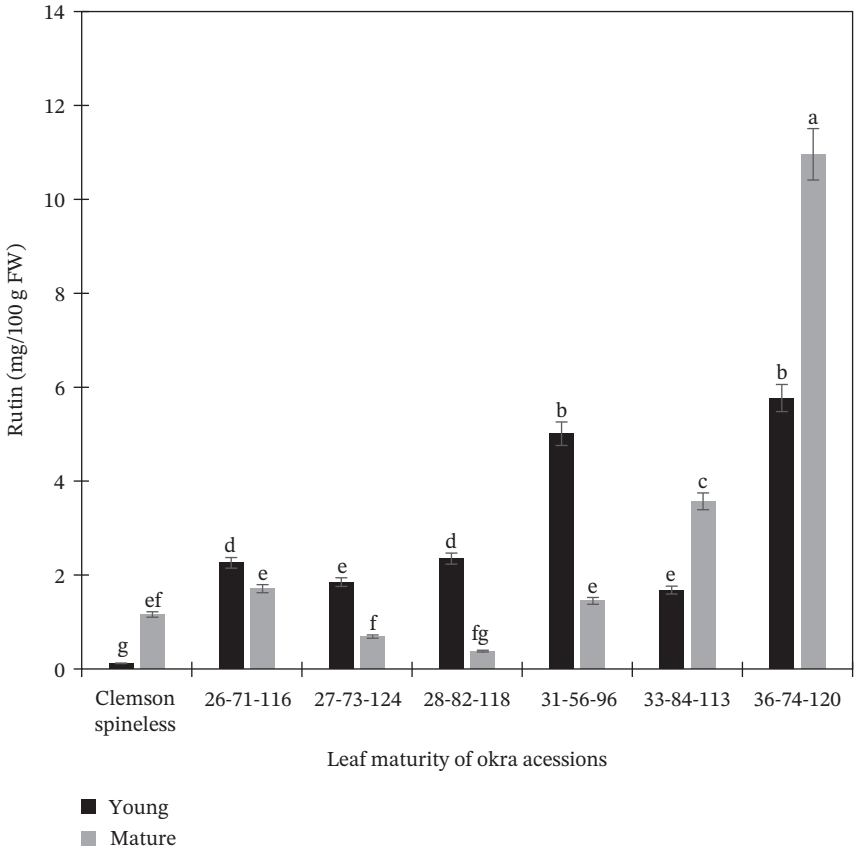


(C)

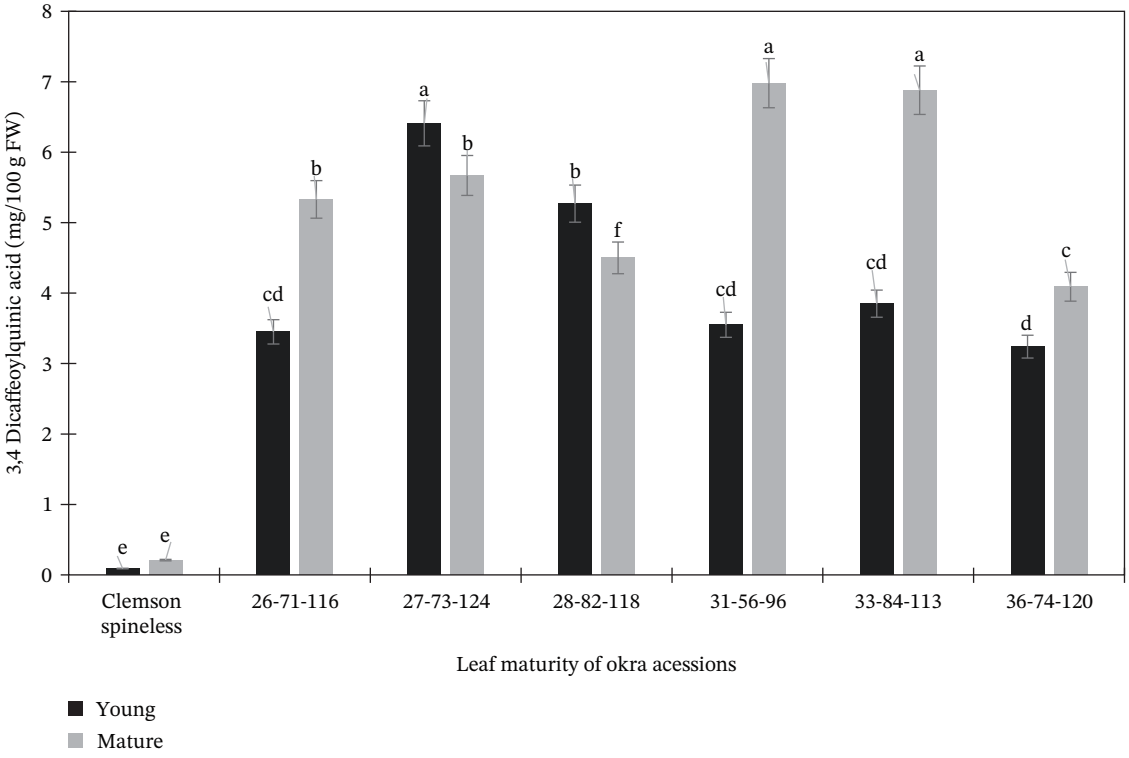


(D)

FIGURE 4 | (Continued)

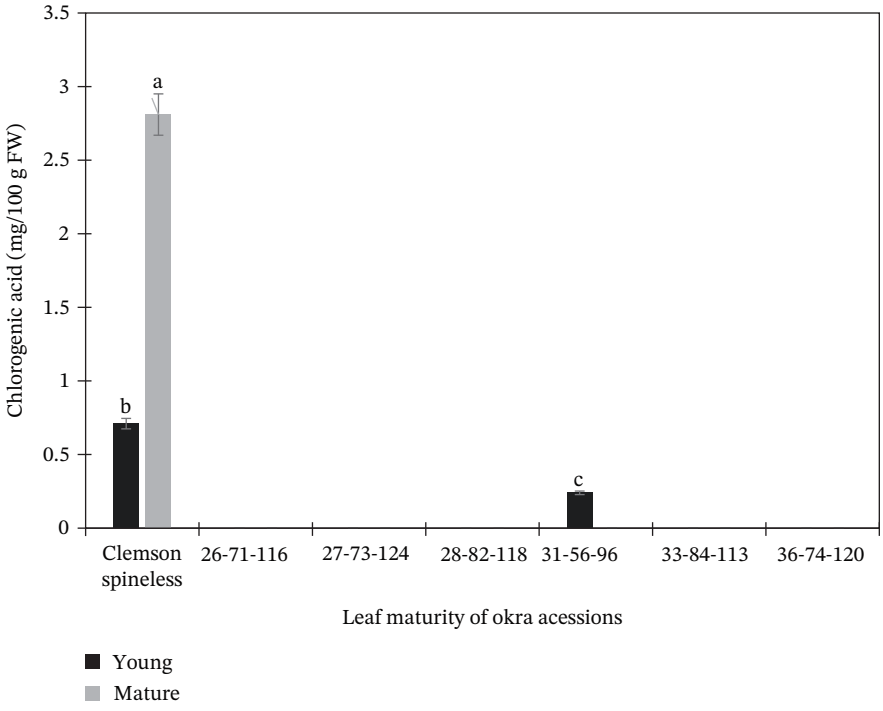


(E)



(F)

FIGURE 4 | (Continued)



(G)

FIGURE 4 | (A) Influence of leaf harvesting maturity on catechin content in the seven okra accessions. Bars with different alphabetical letters are significantly different at $p < 0.05$, based on the based on Fisher's protected LSD test. (B) Influence of leaf harvesting maturity on epicatechin in the seven okra accessions. Bars with different alphabetical letters are significantly different at $p < 0.05$, based on the based on Fisher's protected LSD test. (C) Influence of leaf harvesting maturity on quercetin content in the seven okra accessions. Bars with different alphabetical letters are significantly different at $p < 0.05$, based on the based on Fisher's protected LSD test. (D) Influence of leaf harvesting maturity on protocatechuic acid content in the seven okra accessions. Bars with different alphabetical letters are significantly different at $p < 0.05$, based on the based on Fisher's protested LSD test. (E) Influence of leaf harvesting maturity on rutin content in the seven okra accessions. Bars with different alphabetical letters are significantly different at $p < 0.05$, based on the based on Fisher's protected LSD test. (F) Influence of leaf harvesting maturity on 3,4-dicaffeoylquinic acid content in the seven okra accessions. Bars with different alphabetical letters are significantly different at $p < 0.05$, based on the based on Fisher's protected LSD test. (G) Influence of leaf harvesting maturity on chlorogenic acid content in the seven okra accessions. Bars with different alphabetical letters are significantly different at $p < 0.05$, based on the based on Fisher's protected LSD test.

(Figure 4E). Rutin content in the mature leaves of the okra accession "36-74-120" increased by 10.96-fold compared to its young leaves, while "33-84-113" showed a 2.13-fold increase. In "31-56-96," "26-71-116," "28-82-118," and "27-73-124," higher levels of rutin were observed in the young leaves compared to their respective mature leaves, with levels ranging from 5.01 to 2.26 mg/100 g. Mature leaves of "31-56-96" (6.98 mg/100 g), 33-84-113, and young leaves of "27-73-124" showed significantly ($p < 0.05$) the highest levels of 4-dicaffeoylquinic acid compared to other accessions (Figure 4F). 3,4-Dicaffeoylquinic acid (3,4-DCQAs) was significantly ($p < 0.05$) highest in the mature leaves of the okra accession "31-56-96" (6.98 mg/100 g) than all other accessions. The mature leaves of okra accessions "31-56-961.96" and "26-71-116" showed increases of 1.97-fold and 1.94-fold in DCQAs levels, respectively, compared to their young leaves. Among the young okra leaves, "27-73-124" (6.41 mg/100 g) showed the significantly ($p < 0.05$) highest concentration of 3,4-DCQAs, followed by "28-82-118" (5.27 mg/100 g). The young leaves of accessions "28-82-118" and "27-73-124" exhibited increases of 1.15-fold and 1.13-fold, respectively, compared to their mature leaves. Meanwhile, "Clemson Spineless" showed the lowest levels (0.09 mg/100 g and 0.21 mg/100 g) of DCQAs in both young and mature leaves, respectively. On the other hand, chlorogenic acid was detected only in the young leaves (0.7 mg/100 g) and mature leaves (2.81 mg/100 g) of "Clemson Spineless" and in the young leaves (0.24 mg/100 g) of "31-56-96" (Figure 4G). It is interesting to note that chlorogenic acid was detected only in the young and mature leaves of the okra variety "Clemson Spineless," as well as in the young leaves of "31-56-96" and exclusively in the mature leaves of "Clemson Spineless."

The concentration and types of phenolic acids and flavonoids in plants change significantly during maturation. Generally, TPC and the levels decrease with maturity. The phenylpropanoid and flavonoid biosynthesis pathways regulate downstream phenolic compounds. Cinnamoyl-CoA is produced by p-coumarate-CoA ligase through the action of cinnamic acid during flavonoid biosynthesis, thereby affecting the levels of catechin and epicatechin [25]. Some phenolic acids, particularly in bound or esterified forms, can increase during maturation. Certain phenolic acids, like p-hydroxybenzoic acid, show increases in esterified forms. On the other hand, some phenolic acids might show a different trend, with free fractions increasing during maturation, while bound fractions remain relatively stable or fluctuate [25]. Moreover, different accessions accumulate different types and amounts of phenolic acids, and these patterns also shift as leaves develop. These variations are influenced by genetic factors and

environmental conditions, including light exposure and other abiotic stresses [25]. Rutin, catechin, and quercetin are more abundant in mature leaves, where they accumulate in tissues exposed to stress and exhibit a stronger capacity to produce and retain protective compounds. In mature leaves, upon aging, degalloylation occurs, and galloylated catechin hydrolase activity may increase, converting galloylated phenolics (−)-epicatechin-3-gallate into (−)-epicatechin. Additionally, glycosidases (GH1, GH3, etc.) are present, releasing sugars and aglycones, contributing to the observed increase in catechin [26]. Epicatechin, a type of catechin, is particularly prevalent in mature leaves due to its role in enhancing photosynthetic capacity and defending against environmental stresses [27]. The biosynthesis of these compounds is influenced by factors such as plant cultivar, climate, and harvest time, which affect the development of photosynthetic capabilities [24]. 3,4-DCQAs is also more common in mature leaves, as it helps protect against light damage and supports lignification. Its presence in older leaf cells, especially in the sclerenchyma around the phloem, highlights its role in transporting and lignifying mature leaf tissues [28].

3.4 | TCCs

Figure 5 illustrates the TCC in the leaves of seven okra accessions harvested at two different maturity stages. The TCC increased with maturity in all okra leaf accessions, with the mature leaves of "Clemson Spineless" exhibiting significantly ($p < 0.05$) the highest levels at 100.52 mg/100 g than all other accessions and maturity stages. In contrast, the young leaves of the okra accessions "33-84-113," 36-74-120, and 28-82-118 had the significantly ($p < 0.05$) lowest TCC among all seven okra accessions. Likely, genes related to the carotenoid biosynthesis pathway are more actively expressed in mature leaves than in young leaves, and degradation of carotenoids is also lower in mature leaves. The accumulation of carotenoids serves as protection against photo-oxidative damage, and their levels can increase in mature leaves under certain conditions, such as heightened light interception or genetic predisposition for higher concentrations [29]. Overall accumulation is a balance between synthesis, degradation, and storage capacity [29]. Furthermore, the variation in carotenoid accumulation among different accessions' mature leaves may be attributed to the genetic makeup of the plants.

3.5 | Carotenoid Profiling

The distribution of carotenoid profiling in young and mature okra leaves is shown in Figure 6A–D. The results show that lutein and trans-β-carotene represent the major carotenoids in all okra accessions. The trans-β-carotene distribution varied between young and mature leaves, showing differences across accessions (Figure 6A). Mature leaves of accession "26-71-116," "28-82-118," "36-74-120," and "31-56-96" contained the significantly ($p < 0.05$) highest level of trans-β-carotene (12.86 mg/100 g) compared to all the accessions and both maturity stages. However, among young leaves, the significantly ($p < 0.05$) highest concentration was found in accession "27-73-124" (9%), compared to the other accessions. The distribution of cis-β-carotene varied among the accessions, with the mature leaves of "Clemson Spineless" containing significantly ($p < 0.05$) the highest levels of cis-β-carotene at 12.86 mg/100 g compared to all other accessions and maturity stages (Figure 6B). Overall, the mature leaves of all seven okra accessions showed significantly ($p < 0.05$) the highest levels of cis-β-carotene compared to their respective young leaves. Among the young leaves, "Clemson Spineless" showed the highest levels of cis-β-carotene (8.92 mg/100 g) significantly ($p < 0.05$), compared to the young leaves of other okra accessions.

The lutein concentrations found in okra leaves varied across different accessions and maturity stages. In the bar graphs, mature leaves of "Clemson Spineless" showed significantly ($p < 0.05$) the highest levels of lutein (50.74 mg/100 g) compared to the other accessions and both maturity stages (Figure 6C). However, young and mature leaves of accessions "26-71-116," "28-82-118," "27-73-124," "31-56-96," "33-84-113," and "36-74-120" showed lutein content lower than 10 mg/100 g.

The zeaxanthin distribution in the young leaves of 36-74-120 showed significantly ($p < 0.05$) the highest levels of 4.63 mg/100 g compared to all accessions and maturity stages (Figure 6D).

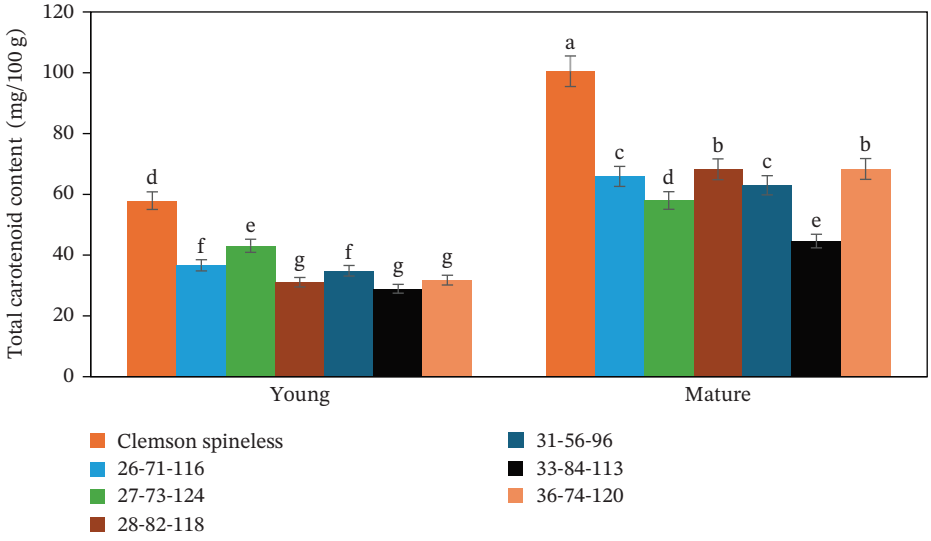
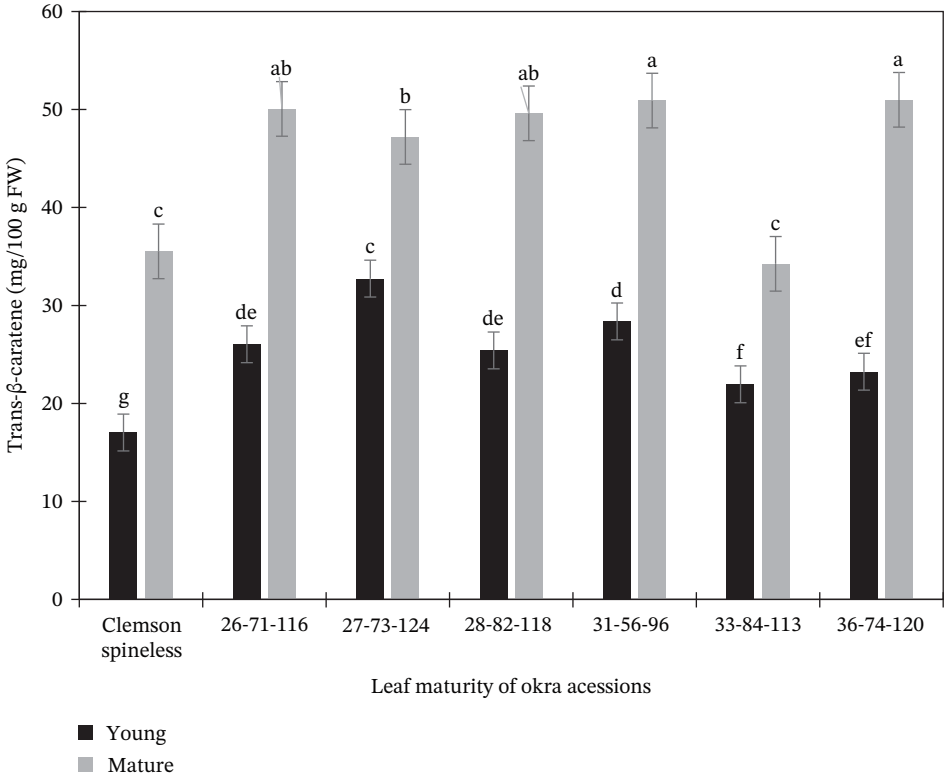
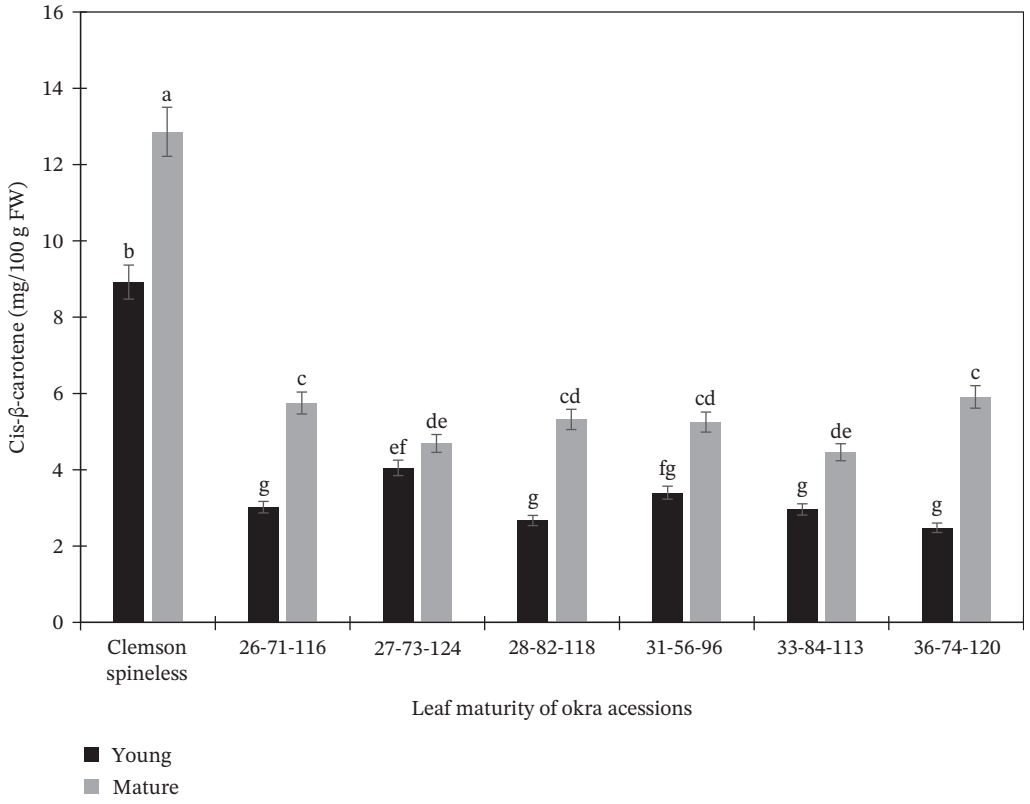


FIGURE 5 | Influence of leaf harvesting maturity on total carotenoid content of seven okra accessions. Values are presented as mean ± standard deviation ($n = 3$). Bars with different alphabetical letters are significantly different at $p < 0.05$, based on the based on Fisher's protested LSD test.

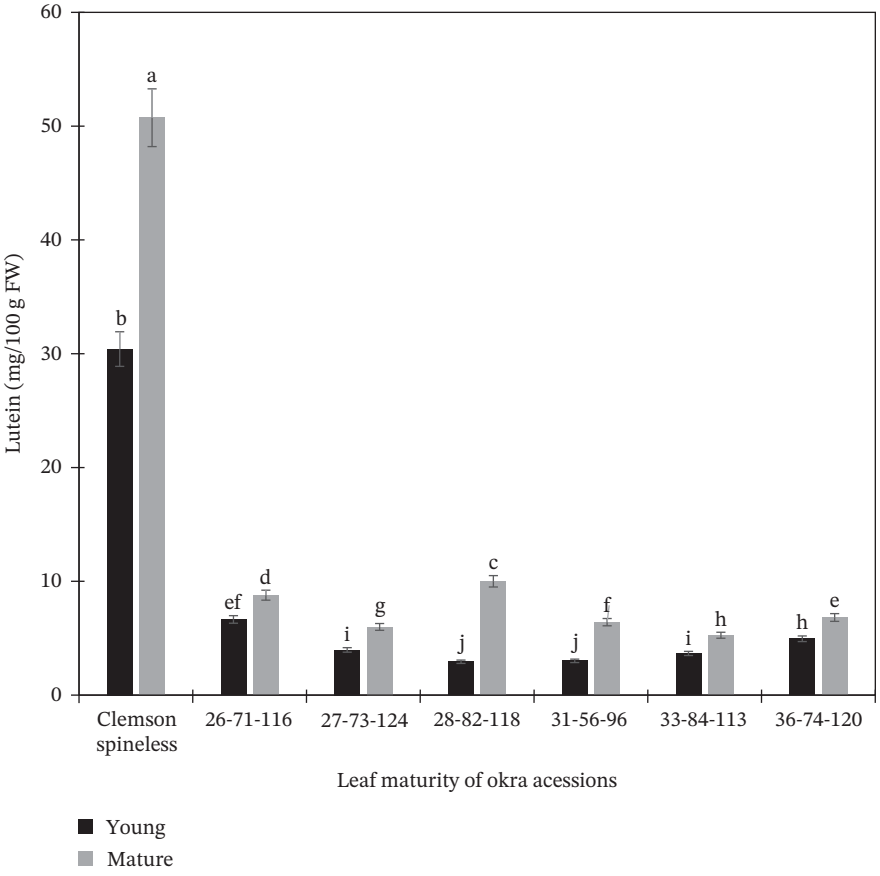


(A)

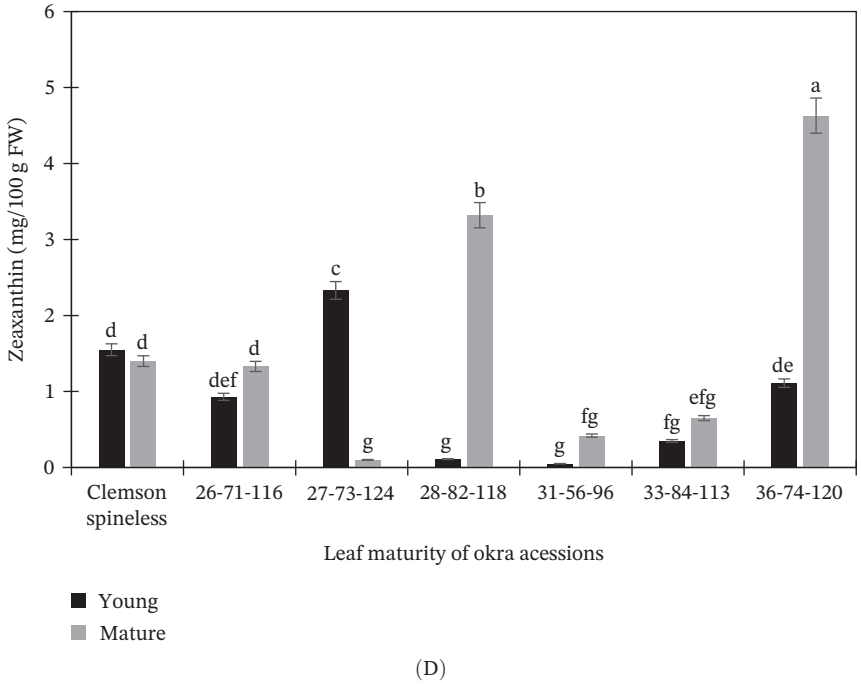


(B)

FIGURE 6 | (Continued)

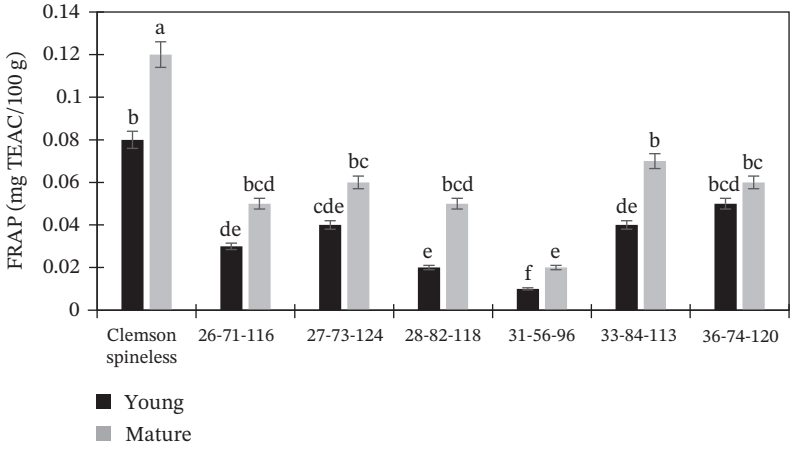


(C)

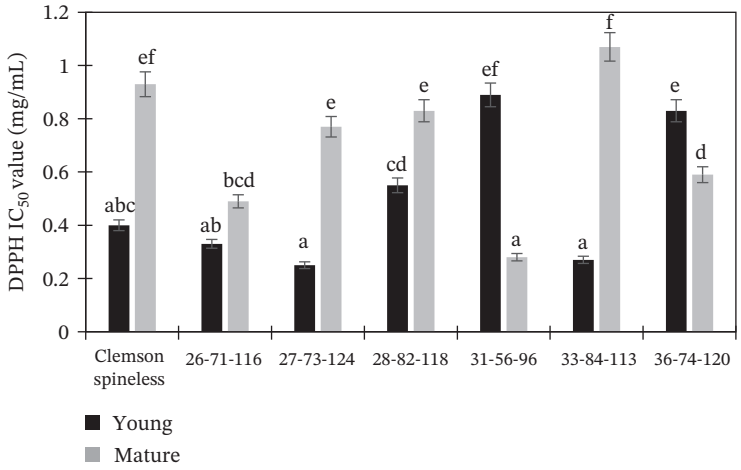


(D)

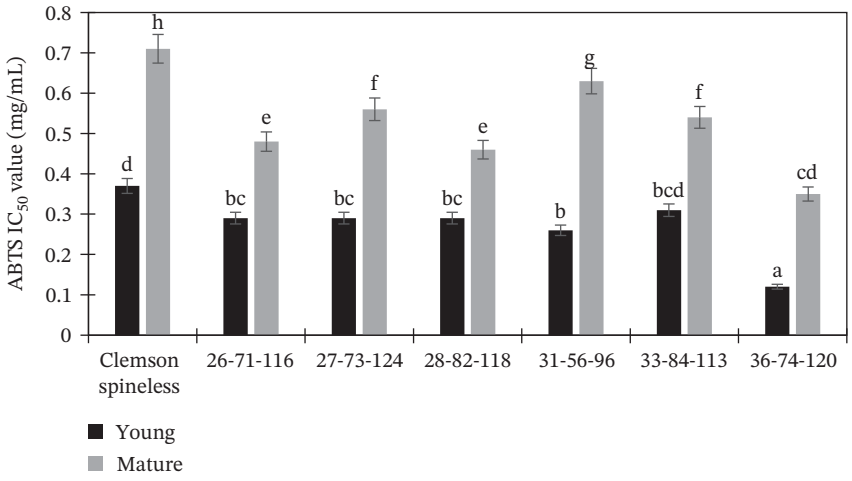
FIGURE 6 | (A) Influence of leaf harvesting maturity on trans-β-carotene of seven okra accessions. Bars with different alphabetical letters are significantly different at $p < 0.05$, based on the based on Fisher's protected LSD test. (B) Influence of leaf harvesting maturity on the different cis-β-carotene content of seven okra accessions. Bars with different alphabetical letters are significantly different at $p < 0.05$, based on the based on Fisher's protected LSD test. (C) Influence of leaf harvesting maturity on lutein content of seven okra accessions. Bars with different alphabetical letters are significantly different at $p < 0.05$, based on the based on Fisher's protected LSD test. (D) Influence of leaf harvesting maturity on zeaxanthin content of seven okra accessions in young and mature leaves. Bars with different alphabetical letters are significantly different at $p < 0.05$, based on the based on Fisher's protected LSD test.



(A)



(B)



(C)

FIGURE 7 | Influence of leaf harvesting maturity on antioxidant activities based on (A) FRAP, (B) DPPH, and (C) ABTS assays of seven okra accessions. Bars with different alphabetical letters are significantly different at $p < 0.05$, based on Fisher's protested LSD test.

In contrast, among the young leaves, "27-73-124" showed significantly ($p < 0.05$) the highest levels (2.33 mg/100 g) of zeaxanthin compared to the young leaves of other accessions.

These findings indicate that the biosynthesis of trans-β-carotene is more prominent in certain accessions during the young leaf stage, particularly in "27-73-124," showing a higher metabolic activity and the necessity for photoprotection during early development [30]. As leaves mature, carotenoid levels appear to stabilize among accessions, resulting in a more uniform distribution. The consistently lower values in "Clemson Spineless" in both stages suggest a limited genetic potential for carotenoid accumulation in this accession.

Consuming 6–10 mg of lutein daily is generally considered beneficial for eye and cognitive health, though there is no official recommended daily allowance. It has been suggested that 10 mg of lutein combined with 2 mg of zeaxanthin may help reduce the risk of macular degeneration. Therefore, consuming 20 g of mature and 33.5 g of young leaves of "Clemson Spineless" could meet the 10 mg lutein recommendation, while 40 g of mature leaves of "36-74-120" could provide 2 mg of zeaxanthin.

The results suggest a strong genotype effect, with accession "Clemson Spineless" standing out as a primary source of cis-β-carotene relative to distributed contributions from the other accessions. The high percentage in "Clemson Spineless" indicates that this accession may possess a greater enzymatic conversion rate that favors cis-isomers or may enhance the stability of cis-carotene in its leaves. Conversely, the lower levels, less than 5 mg/100 g in the young leaves of accessions "36-74-120," 33-84-113, 31-56-96, 28-82-118, and 26-71-116, suggest a limited potential for accumulation. The overall distribution of trans-β-carotene was higher in both mature and young leaves of the investigated okra accessions compared to cis-trans-β-carotene, reflecting that cis-forms are more specific to certain accessions. These results emphasize that while the accumulation of trans-β-carotene depends on both maturity stage and genotype, cis-β-carotene appears to be influenced by genetic factors, with the accession "Clemson Spineless" identified as the most contributing source. The precursor to vitamin A is β-carotene. Adults generally require 700–900 mcg retinol activity equivalents (RAE) of vitamin A daily, including provitamin A carotenoids like β-carotene [31]. The vitamin A content of 100 g of raw okra leaves could contribute to 36 mcg.

These findings suggest that leaf maturity significantly influences zeaxanthin accumulation, with accession "Clemson Spineless" and "28-82-118" exhibiting increased levels in mature leaves, while others, such as "27-73-124," show a reduced capacity to retain zeaxanthin as they mature. This physiological observation may illustrate various strategies of photoprotection and carotenoid metabolism, as higher levels of zeaxanthin in mature leaves are likely to enhance the ability to withstand prolonged exposure to light [32]. In contrast, lower concentrations in certain accessions may indicate the diversion of carotenoids to other metabolic pathways.

Research suggests that some cis-carotenoids can be more bioaccessible than all-trans-isomers, with 9-cis-β-carotene showing high bioaccessibility in some studies. Throughout photosynthesis, pigments such as carotenoids and chlorophyll play essential roles. The carotenoid biosynthesis pathway in plants involves a series of biochemical reactions, including dehydrogenation, condensation, desaturation/isomerization, hydroxylation, and epoxidation [33]. Carotenoid biosynthesis begins with geranylgeranyl diphosphate, and carotene hydroxylase converts β-carotene into lutein and zeaxanthin [34].

3.6 | Antioxidant Activity

The antioxidant activity of the leaves of young and mature seven okra accessions is given in Figure 7A–C. The antioxidant activity in the studied okra accessions and maturity stages showed a significant difference ($p < 0.05$) in both the DPPH and ABTS scavenging activities. The antioxidant power (FRAP) was similar in the young and mature leaves of the "36-74-120," "27-73-124," and 26-71-11 accessions, but it varied significantly ($p < 0.05$) between the young and mature leaves of the 28-82-118, "33-84-113," "31-56-96," and "Clemson Spineless" accessions (Figure 3A). The "Clemson Spineless" mature leaves showed a 1.13-fold increase in antioxidant power compared to the young leaves. Mature leaves often show higher antioxidant activity than younger ones due to increased accumulation of these protective phytochemicals, making them a valuable source of antioxidants [35, 36].

The DPPH assay assesses free radical scavenging activity (Figure 3B). Young leaves in accessions exhibited lower IC_{50} values compared to mature leaves. Accessions "Clemson Spineless," "26-71-116," "27-73-124," and "33-74-113" had the lowest IC_{50} in young leaves (ranging from 4 to 0.25 mg/mL), showing strong DPPH activity, which increased to the range from 1.07 to 0.77 mg/mL in mature leaves.

The ABTS assay indicates the potential for radical scavenging (Figure 3C). In all accessions, mature leaves had a lower scavenging activity compared to the young leaves. The young leaves of "36-74-120" showed the highest (0.12 mg/mL IC_{50}) ABTS scavenging activity.

Mature leaves of the accession "Clemson Spineless" (0.71 mg/mL, IC_{50}) exhibited the lowest scavenging activity, while "36-74-120" showed the highest (0.35 mg/mL, IC_{50}) activity, indicating variability in the accumulation of ABTS-reactive antioxidants.

Antioxidants play a crucial role in preventing and scavenging free radicals, thereby protecting humans from infections and degenerative diseases [36]. These variations can be attributed to the accumulation of antioxidants specific to each maturity stage. In younger tissues, a higher rate of metabolic activity and expression of defense enzymes may enhance the synthesis of antioxidants [37]. Also, several factors, such as the specific cultivar, the extraction solvent used, the plant's maturity stage, and environmental conditions, could have affected the accumulation of antioxidants during growth [38].

The ABTS assay can detect both hydrophilic and lipophilic antioxidants; thus, variations may arise from genotype-specific differences in compound profiles [39]. Plant accessions may produce higher amounts of soluble flavonoids and ascorbic acid during maturation because of genotype-specific responses to protective mechanisms against biotic and abiotic stresses [40]. In contrast to many other studies, which established a direct relationship between antioxidant capacity and TPC [41], there was no significant correlation between the antioxidant capacity of leaves and TPC.

However, the total carotenoids and total phenolics had a positive relationship with FRAP ($r = 0.72$ and 0.51, respectively) at $p < 0.05$. Phenolic compounds can readily donate electrons (or hydrogen atoms) due to the presence of their hydroxyl groups. This mechanism allows them to neutralize free radicals and specifically reduce Fe^{3+} to Fe^{2+} in the FRAP assay. The strong statistical link indicates that phenolic content is a reliable predictor of antioxidant capacity measured by the FRAP method. Additionally, the antioxidant activity of polyphenols is significantly influenced by their chemical structure, with polyphenol aglycones exhibiting higher antioxidant activity than their glycosides [42]. The correlation between total carotenoids and FRAP

activity is attributed to the chemical structure of carotenoids, particularly their long chain of conjugated double bonds. These bonds enable carotenoids to act as electron donors, reducing ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in the FRAP assay [42]. The extended system of conjugated double bonds makes carotenoids susceptible to oxidation and capable of donating electrons to the ferric ion–TPTZ complex. The reduction potential of carotenoids, influenced by the number of conjugated double bonds, determines their effectiveness in the FRAP assay [42]. For instance, lutein is particularly effective due to its specific structure and orientation in the testing medium. This structural influence leads to a positive correlation between TCC and FRAP values [42].

However, the correlation can vary depending on factors such as the specific type of carotenoid, the food matrix, and experimental conditions, including solvent choice and reaction time. These variations arise because carotenoids are lipophilic, which may result in low solubility or slower reaction kinetics under certain assay conditions [42].

3.7 | Antinutritional Compounds

Three antinutritive compounds, tannins, oxalates, and phytates, were analyzed in harvested okra accessions at two maturity stages, as presented in Table 1. Generally, the levels of these antinutritive compounds increased with maturity across most okra accessions included in this study. The mature leaves of the accession "27-73-124" showed the highest oxalate content (63.38 mg/g), followed by 26-71-11 (61.62 mg/g) and 28-82-118 (58.09 mg/g). Additionally, oxalate levels were lowest in the young leaves of "33-84-113" (15.85 mg/g) and "Clemson Spineless" (21.13 mg/g). Mature leaves of the accessions "26-71-116" and "Clemson Spineless" exhibited the highest levels of tannins (16.91 mg/g) and phytates (66.89 mg/g), respectively. The leaves of the okra accession "Clemson Spineless" had the lowest tannin content in both young (4.10 mg/g) and mature (5.77 mg/g) leaves. Similarly, young leaves of the accession "27-73-12" (3.11 mg/g) and mature leaves of "33-84-113" (5.01 mg/g) had the lowest phytate levels. Certain minerals and vitamins cannot be effectively absorbed from vegetables due to antinutritional factors [43, 44]. For example, oxalic acid impairs calcium absorption by forming insoluble calcium oxalate salts [45]. When calcium intake is insufficient, oxalate may be absorbed into the bloodstream, potentially leading to kidney stone formation [45].

Higher tannin levels in mature leaves are often linked to increased activity of enzymes involved in tannin biosynthesis. These enzymes, such as phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS), play a crucial role in synthesizing tannin precursors. As leaves mature, the activity of these enzymes can increase, resulting in higher tannin concentrations [46]. The differences in tannin content between young and mature leaves across various okra accessions may primarily stem from genetic variations in their biosynthesis pathways and the regulation of these pathways. Environmental factors, such as nutrient availability, can trigger differing responses in tannin production among genotypes [47, 48]. Antioxidative properties of tannins may make them anticarcinogenic and antimutagenic. The effectiveness of tannins in these roles depends on the type and dosage consumed [48, 49].

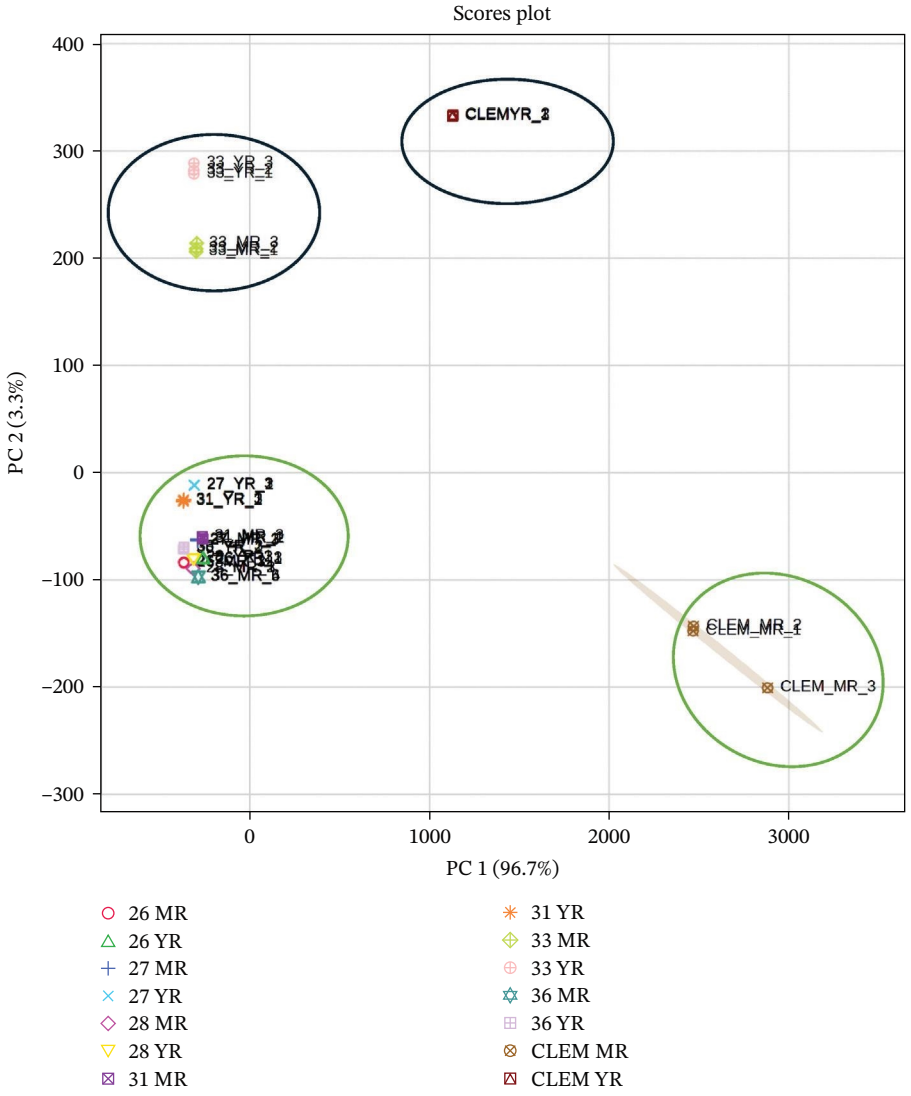
3.8 | Multivariate Analysis

Figure 8A shows an exploratory PCA of the seven okra accessions/varieties harvested at two different growth maturity stages (young and mature). The PCA revealed four major clusters based

TABLE 1 | Impact of leaf harvesting maturity on antinutritive compounds of seven okra accessions.

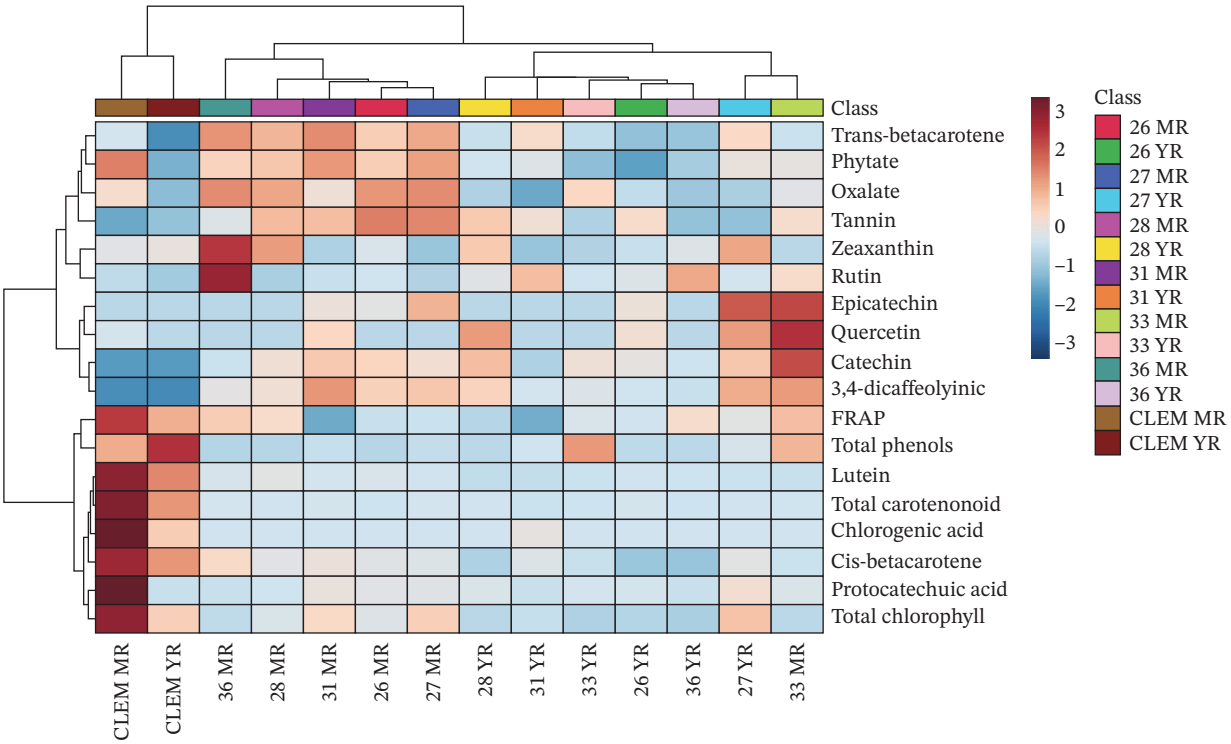
Accessions	Tannins (mg/g)	Phytate (mg/g)	Oxalate (mg/g)
		Young leaves	
Clemson spineless	4.10 ± 0.12^{m}	3.39 ± 0.02^{l}	21.13 ± 0.10^{l}
26-71-116	11.60 ± 0.07^{f}	5.05 ± 0.01^{g}	31.69 ± 0.50^{h}
27-73-124	5.65 ± 0.01^{l}	3.11 ± 0.03^{m}	26.76 ± 1.00^{j}
28-82-118	12.88 ± 0.09^{e}	4.58 ± 0.01^{i}	28.17 ± 1.10^{i}
31-56-96	10.86 ± 0.01^{h}	4.85 ± 0.02^{h}	38.73 ± 1.00^{g}
33-84-113	5.77 ± 0.01^{k}	3.64 ± 0.03^{k}	15.85 ± 2.00^{m}
36-74-120	7.24 ± 0.80^{j}	3.98 ± 0.02^{j}	24.65 ± 1.20^{k}
		Mature leaves	
Clemson spineless	5.76 ± 0.04^{k}	6.89 ± 0.01^{a}	42.25 ± 0.01^{f}
26-71-116	16.91 ± 0.06^{a}	5.71 ± 0.05^{e}	61.62 ± 1.01^{b}
27-73-124	16.49 ± 0.13^{b}	6.42 ± 0.02^{c}	63.38 ± 2.01^{a}
28-82-118	13.75 ± 0.12^{c}	5.82 ± 0.03^{d}	58.09 ± 1.00^{c}
31-56-96	13.72 ± 0.01^{d}	6.55 ± 0.02^{b}	44.54 ± 0.01^{e}
33-84-113	11.43 ± 0.01^{g}	5.01 ± 0.02^{g}	47.53 ± 1.11^{d}
36-74-120	9.57 ± 0.60^{i}	5.61 ± 0.02^{f}	63.37 ± 0.01^{a}

Note: The data show the means and standard deviations. Different letters in the same column for okra leaves represent significant differences at $p < 0.05$.



(A)

FIGURE 8 | (Continued)



(B)

FIGURE 8 | (A) The principal component analysis showed the clustering effect of the leaves of different okra accessions based on the distribution of bioactive compounds, antioxidant power, and antinutritive compounds. (B) The heatmap presents major phytonutrients contributing to okra accession variations at two maturity stages. The phenolic compounds, carotenoids, antioxidant activity, and antinutritional factors found in young (YR) and mature (MR) leaves of various okra accessions. The color gradient indicates relative abundance (red = higher intensity and blue = lower intensity), while clustering illustrates the similarities among the compounds and samples.

on their accession and maturity stage differences, with both PC1 and PC2 contributing 100% predictive model statistics. The first and second clusters grouped samples of the five okra accessions, including 26–71-116, 27-73-124, 28-82-118, 31-56-96, and 36-74-120, along the PC1, together with accession "33-74-113" young and mature samples, which were parallel to the third and fourth clusters showing "Clemson Spineless" (matured and young) samples. Total phenols segregate the young leaves of "Clemson Spineless" from the mature leaves. Contrary, PC2 separated the samples of accession "Clemson Spineless" based on maturity stages, and the samples of accession "33-74-113" showed similar heterogeneity differences against the five accession clusters, yielding a minor distinction contribution to an overall 3.3% predictive model statistics.

To observe the phytonutrients' contribution to an overall variation of the accessions, a heatmap model was generated as shown in Figure 8B. Accession "Clemson Spineless" harvested at the mature stage exhibited the strongest positive abundance of phytonutrients, including total carotenoids, which could have been attributed to the presence of individual phenolic and carotenoid compounds, chlorogenic acid, protocatechuic acid, lutein, cis-β-carotene, total chlorophyll, and FRAP value. Samples of the "Clemson Spineless" at the young maturity stage showed an abundance of TPC compared to their mature leaves. Mature leaves of "33-74-113" were predominated by the epicatechin, quercetin, catechin, 3,4-, and rutin compounds on the abundance stage. The mature leaves of 36-74-120 are abundant in zeaxanthin and rutin.

4 | Conclusion

This study presents the effects of harvesting maturity and genetic variability on the phytochemicals and antinutritional compounds of okra leaves. The results demonstrate that antioxidant activity due to maturity was complex and assay-dependent. While FRAP activity tended to be higher in mature leaves of some accessions, often, radical scavenging capacities were stronger in young leaves (DPPH and ABTS). Mature leaves generally exhibited higher TPC, although the accumulation of specific individual phenolic compounds was highly accession dependent. Moreover, mature accession "Clemson Spineless" showed significantly better traits than the other accessions, demonstrating high levels of particularly lutein and cis-β-carotene. In summary, our study supports the use of particular okra accessions and leaf maturation phases for functional foods, for nutrition-conscious farming methods, and for food policies that prioritize health. The mature leaves of "Clemson Spineless" and "33-84-113" are recommended for nutritional inclusion and food applications because of their ideal ratio of high bioactive content to low antinutritional elements.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Research data are not shared.

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