Applied Chemical Engineering (2025) Volume 8 Issue 4 ISSN: 2578-2010 (O)
doi: 10.59429/ace.v8i4.5759

ORIGINAL RESEARCH ARTICLE

Growth-stage specific metabolic adaptations in Capsicum annum: Secondary metabolite dynamics and antioxidant plasticity

Haider Abbas AbdulRedha[1*], Maallah T. AL-Husseini[2], Intisar Razzaq Sharba[3], Harith R. Al-Mousawi[4], Naser Jawad Kadhim[5]

12345 Department of Biology, Faculty of science, University of Kufa, Najaf , 540011, Iraq

***Corresponding author:** Naser Jawad Kadhim; Naseer.alzarkani@uokufa.edu.iq

ABSTRACT

This study examines growth-stage-dependent variations in secondary metabolites and antioxidant capacity in Capsicum annum. Analytical techniques including spectrophotometry were employed to quantify phenolics, tannins, flavonoids, anthocyanins, alkaloids, and DPPH radical scavenging activity. Results revealed significant metabolic shifts ($p < 0.05$) across vegetative (V) and flowering (F) stages, driven by tissue-specific demands and developmental priorities. A marked increase in phenolic content was observed in roots (1.32 to 3.24 mg/100g), stems, and leaves during flowering, correlating with oxidative stress mitigation. Fruits exhibited minimal phenolics (0.31 mg/100g), reflecting resource allocation to seed maturation. Tannins dominated vegetative roots (4354.16 mg/100g) and stems (3670.83 mg/100g), declining during flowering (roots: 2726.39 mg/100g) as defense investments shifted toward reproduction. Flavonoids increased in flowering roots (237.62 mg/100g) but remained elevated in vegetative leaves (333.16 mg/100g), supporting UV protection. Anthocyanins peaked in vegetative roots (11.40 mg/100g) and flowering flowers (11.40 mg/100g), serving dual roles in stress resistance and pollinator attraction. Alkaloids were highest in vegetative stems (842.67 mg/100g) and leaves (2373.33 mg/100g), with fruiting-stage fruits accumulating maximum levels (2536.00 mg/100g) for seed defense. Antioxidant activity (IC_{50}) varied by tissue: stems improved during flowering (IC_{50}: 2.75 to 1.95 mg), while roots declined (6.87 to 9.02 mg). Fruits displayed potent crude extract activity (IC_{50}: 0.97 mg), linked to phenolic accumulation. These findings demonstrate C. annum's adaptive metabolic strategies—vegetative tissues prioritize structural defense, while flowering stages emphasize stress tolerance and reproductive resource allocation. Tissue-specific trends (e.g., root defense hubs, fruit UV protection) underscore ecological and physiological trade-offs. Statistically validated results (LSD thresholds exceeded) inform targeted harvesting (e.g., alkaloid-rich fruits) and bioprospecting for nutraceuticals. This work enhances understanding of plant metabolic plasticity, offering strategies to optimize crop resilience and bioactive compound utilization.

Keywords: Growth-stage adaptation; Capsicum annum; metabolic plasticity; bioactive compounds; oxidative stress.

ARTICLE INFO

Received: 1 September 2025
Accepted: 26 November 2025
Available online: 05 December 2025

1. Introduction

The annual pepper plant (*Capsicum annum* L.) is a globally important economic and agricultural species with remarkable metabolic flexibility throughout its growth cycle. It possesses a key adaptation strategy to meet growth requirements and environmental challenges due to its ability to dynamically regulate the biosynthesis of secondary metabolites [1, 2]. Metabolic distribution is significantly affected between growth stages, especially during the plant's transition to the reproductive stage and fruit ripening, due to the plant's need to transfer resources between primary growth processes and the production of specialized metabolites [2,3]. This physiological reprogramming directly influences the accumulation pattern and the quantitative and qualitative presence of biologically active compounds,

such as phenolic compounds, alkaloids, and carotenoids, which collectively determine the plant's oxidative and anti-disease potential, and consequently, its therapeutic potential [3, 4]. Recent research has focused on studying growth signals and their associated specialized metabolism in the pepper plant as a complex interaction. By observing systematic changes in phenylpropanoid and alkaloid pathways during fruit progression through maturation stages [3, 5]. Metabolic adaptations are not merely passive outcomes of growth, but rather active survival strategies under changing environmental conditions [6,7]. For example, the antioxidant flexibility observed across different growth stages and the enhanced ability to scavenge free radicals during specific growth periods indicate a time-based optimization of defense mechanisms [5,8]. A dynamic characteristic of metabolism, considered an environmental adaptation of the plant, lies in its dual function of both defense and reproduction. Secondary metabolites undertake chemical defense against environmental factors such as ultraviolet radiation and opportunistic pathogens, while simultaneously contributing to fruit coloration to attract vector organisms and promote seed dispersal [6,9]. The production of biologically active compounds occurs at temporary peaks corresponding to critical developmental transitions, representing a complex balance between growth and defense [1,2]. Beyond the importance of plant metabolic transformations, understanding the specific metabolic profile of each growth stage is crucial for determining and optimizing harvest timing to achieve maximum nutritional value [4,10]. Although the secondary metabolites of *Capsicum* have been extensively studied, specialized metabolic dynamics remain poorly understood, as evidenced by the antioxidant activity of capsaicinoids and other phenolic compounds, which vary markedly across growth stages [3,8]. The lack of a well-defined regulatory mechanisms governing these fluctuations represents a significant research gap, especially given the growing pharmacological interest in plant-derived antioxidants for managing diseases associated with oxidative stress [11,12]. This study investigates the metabolic dynamics of secondary metabolites and their relationship to oxidative capacity through the quantitative characterization of the metabolic time course from vegetative growth to fruit senescence. This provides valuable insights into the plant's evolving survival strategies and promotes the use of its bioactive compounds in therapeutic applications.

2. Material and methods

Chili pepper plant (vegetative and flowering parts) were collected from Najaf province/Iraq, and were dried in an oven (50 °C). Chemical reagents were provided by Sigma Aldrich as Folin-Ciocalteu reagent (St. Louis, MO, USA), DPPH (2,2-Diphenylpicrylhydrazyl), gallic acid, catechin reagent and sodium nitrite (≥ 99.0% purity), sodium carbonate was obtained from Fluka Biochemika (Switzerland), absolute methanol and concentrated hydrochloric acid (37%) was purchased from Panereac Quimica S.A. (Barcelona, Spain). Absolute ethanol (≥99.8% purity) was procured from Scharlab S.L. (European Union).

2.1. Preparation of plant samples

Plant samples were collected from two distinct growth phases: the vegetative stage and the reproductive stages (flowering and fruiting). During the vegetative phase, leaves, stems, and roots were harvested. In the flowering and fruiting phase, samples from the same plant organs (leaves, stems, and roots) were collected, with the addition of floral and fruit tissues for analysis. The above parts were washed well with distilled water and then dried in an electric oven at 50°C until completely dry. Then they were ground well in a suitable grinder until a fine homogeneous powder was obtained.

2.2. Extract preparation

The ultrasonic-assisted extraction method was conducted using a Unisonics apparatus (Australia). Briefly, 0.5 g of homogenized plant material was homogenized with 50 mL ethyl acetate in a 100 mL beaker. The mixture was subjected to ultrasonic treatment in a temperature-controlled water bath (50°C) for 24 minutes. Following extraction, the samples were centrifuged at 14,000 rpm to separate phases, and the supernatant was collected. The extraction process was repeated three times, with the final volume adjusted to 50 mL. The

combined extracts were lyophilized to complete dryness, reconstituted in absolute methanol, and stored at -4°C for subsequent phytochemical analyses, including quantification of phenolics, flavonoids, tannins, anthocyanins, alkaloids, and antioxidant compounds [13].

2.3. Total phenolic content assay

The total phenolic content was quantified using the Folin-Ciocalteau method. A methanolic extract (10 mg/mL) was combined with Folin-Ciocalteau reagent, distilled water, and sodium carbonate (15%). After 2 hours of dark incubation, absorbance was measured spectrophotometrically at 765 nm. A gallic acid standard curve (100, 200, 300, 400, 500, 600, 700, and 800 mg/l) was generated to calculate phenolic levels as gallic acid equivalents (mg GAE/100 g) using triplicate measurements [14] **Figure 1**.

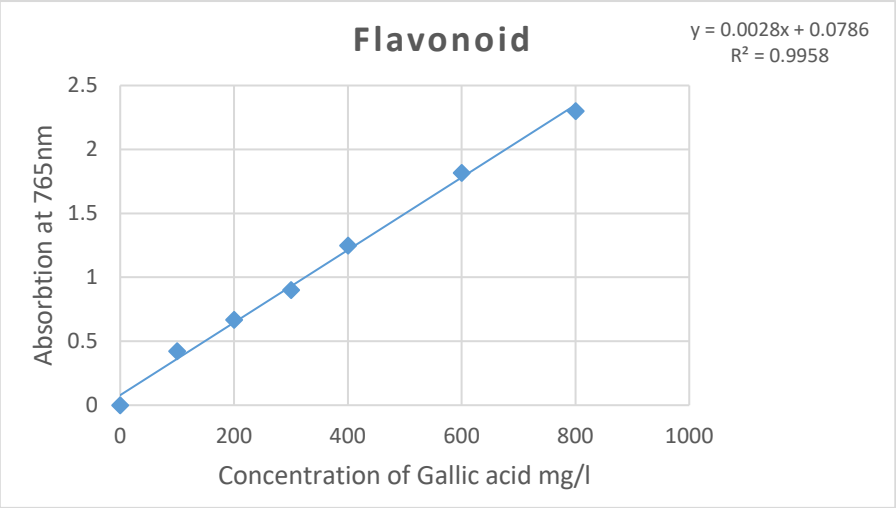

Figure 1. Calibration curve of Gallic acid (100–800 mg/l) for total phenolic quantification. Linear fit: y = 0.0028x+0.0786; R^2 = 0.9958.

2.4. Total flavonoids content assay

Total flavonoids were quantified using aluminum chloride colorimetric method. Briefly, 1 mL of extract (10 mg/mL) or quercetin standard (20–500 mg/L) was mixed with 4 mL deionized water in a 10 mL flask. Sequential additions of 0.3 mL $NaNO_2$ (5%), 0.3 mL $AlCl_3$ (10%), and 2 mL NaOH (1 M) were made at 0, 5, and 6 minutes, respectively. Adjust the final volume to 10 mL by dd H2O, and absorbance was measured via UV-Vis spectroscopy. Flavonoid content was expressed as mg quercetin equivalents (QE)/100 g dry weight, based on triplicate analyses. Figure 2. TFC (Mg Quercetin/100gram dry mass) = concentration mg/l x 10

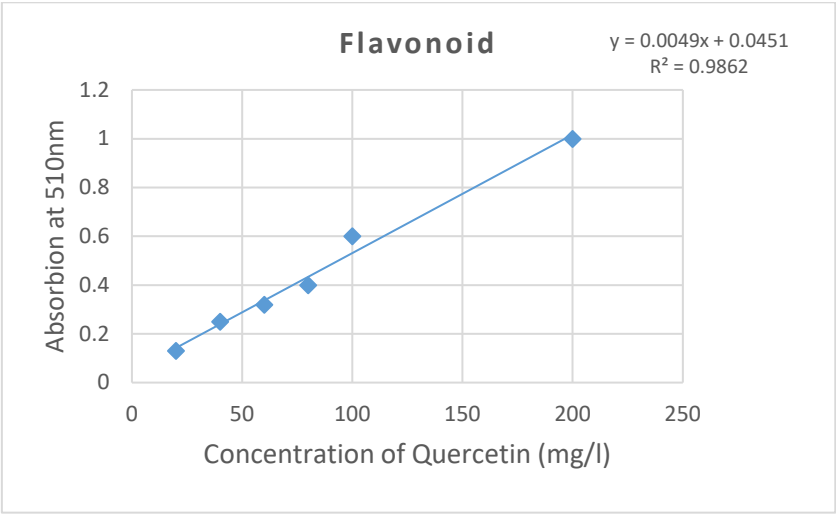

Figure 2. Calibration curve of quercetin (20–200 mg/L) for total flavonoid quantification. Linear fit: y = 0.0049x+0.0451; R^2 = 0.9862.

2.5. Total anthocyanin content assay

Total anthocyanins were quantified using a modified spectrophotometric method [15]. Absorbance was measured at 530 nm and 657 nm to correct for chlorophyll interference ($A = A_{530} - 0.25\ A_{657}$). Anthocyanin content (mg cyanidin-3-glucoside equivalents/100 g dry weight) was calculated using the formula:

$$Total\ Anthocyanins = (A \times 449.2 \times Dilution\ Factor) / (29{,}600 \times Sample\ Weight)$$

where 449.2 = molecular weight and 29,600 = molar extinction coefficient [16]. Triplicate measurements were performed.

2.6. Total tannin content assay

Tannin content was analyzed using a modified Folin-Dennis method. Extracts (10 mg/mL) were mixed with Folin-Dennis reagent and saturated Na_2CO_3, diluted to 50 mL, and incubated in darkness for 90 minutes. Absorbance was measured at 760 nm, with corrections using a reagent blank. A tannic acid standard curve (varying concentrations) enabled quantification, expressed as mg tannic acid equivalents per 100 g dry weight (triplicate measurements) **Figure 3**.

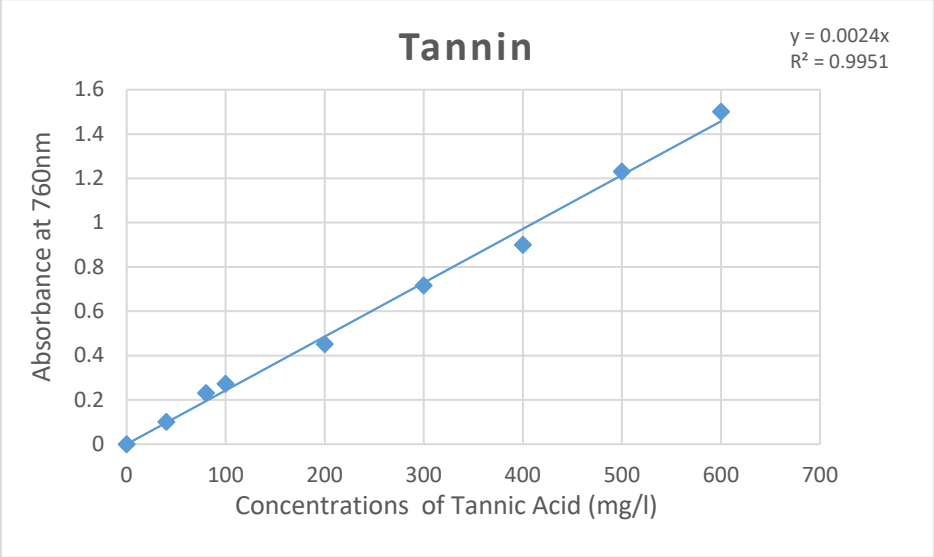

Figure 3. Calibration curve of tannic acid (0–700 mg/L) for total tannin content. Linear fit: y = 0.0024x; $R^2 = 0.9951$.

2.7. Total alkaloid content assay

The total alkaloid content was determined spectrophotometrically using the Bromocresol Green (BCG) method [17]. A standard curve was prepared using atropine (procured from Sigma Chemical Company, USA) over a concentration range of 4-13 µg/mL. The plant extracts were reacted with BCG in a phosphate buffer (pH 4.7), and the resulting yellow complex was extracted with chloroform. Its absorbance was measured at 470 nm.

2.8. Evaluation of antioxidant activity

The antioxidant activity was assessed via DPPH assay. Methanolic extract concentrations (0.01, 0.02, 0.04, 0.08, and 0.1 mg/ml), were mixed with DPPH solution (0.5 mg/mL), incubated in darkness for 30 minutes, and absorbance measured at 515 nm. Radical scavenging (%) was calculated as:

$$Inhibition\%\ (mg/ml) = (Control - Sample)/Control \times 100$$

(mg extract required to scavenge 50% of 0.1 mg DPPH) was derived from a linear standard curve (concentration vs. % inhibition).

This study relied on the antioxidant capacity was expressed as the sample mass (0.1 mg) required to scavenge 50% of the DPPH radical, calculated using the formula below, rather than utilizing concentration-based metrics.

IC50% (mg) = 3.8 ml × IC50% (mg/ml)

3. The results and discussion

3.1. Secondary metabolite distribution across growth stages

The study of medicinal plants is gaining increasing importance in the field of research into bioactive compounds [18-20]. Secondary metabolite content and antioxidant activity in Chili Peppers (Capsicum annuum L.). Plants produce numerous secondary metabolites, including phenols, flavonoids, tannins, anthocyanins, and alkaloids. These metabolites are used by the plant in several crucial roles, such as defense, stress tolerance, and reproduction. These compounds are distributed and accumulated in specialized tissues with growth-related organization, reflecting the plant's adaptation and ability to balance growth, defense, and reproduction. Chili pepper species are characterized by specific secondary metabolite dynamics that vary considerably across vegetative and reproductive stages, as noted in [21, 22]. These variations directly influence antioxidant capacity. Furthermore, the genus Capsicum is distinguished by its rich phytochemical composition, including capsaicinoids, carotenoids, flavonoids, and various phenolic compounds [23, 24]. These bioactive components contribute to the well-documented antioxidant properties of pepper fruits, with free radical scavenging activity closely correlated with total phenolic and flavonoid content [25, 26].

The current study investigated the quantitative estimation of secondary metabolites in pepper tissue (C. annuum) across the vegetative and flowering stages (**Table 1**). The percentage changes in secondary metabolites (phenolics, flavonoids, tannins, and alkaloids) across plant tissues (roots, stems, and leaves) during floral transition (**Figure 1**).

3.1.1. Phenolic compound

The phenolic content increased significantly towards the flowering stage in all studied tissues (roots, stems, and leaves), with increases ranging from 145% in roots (from 1.32 ± 0.008 to 3.24 ± 0.13 mg/100g) to a higher increase of 339% in stems (from 0.92 ± 0.08 to 4.04 ± 0.07 mg/100g). The highest absolute concentration of phenolics was observed in the leaves at flowering (4.58 ± 0.03 mg/100g), representing a 203% increase. In contrast, the fruits exhibited a very low phenolic content (approximately 0.31 ± 0.001 mg/100g), **Table 1**, **Figure 1**. These results are consistent with the findings of studies [27, 28], which indicated that phenol biosynthesis increases in quantity during flowering and reproductive growth. The significant increase in concentrations during flowering is also consistent with the findings of study [29], which attributed this increase to meeting metabolic needs and the need for antioxidant protection in photosynthetic tissues.

3.1.2. Tannin compounds

In contrast to phenols, tannin concentrations decreased steadily and significantly in all tissues during the flowering stage. The leaves of the vegetative stage contained the highest tannin levels (4602.08 ± 56 mg/100 g), with a decrease of 13% during flowering. For the remaining parts, stems showed the highest percentage decrease (42%, from 3670.83 ± 37 to 2143.05 ± 17 mg/100 g) and roots (37%, from 4354.16 ± 17 to 2726.39 ± 74 mg/100 g). Flowers exhibited relatively low tannin content (2679.14 ± 35 mg/100 g), while fruits maintained moderate levels (2966.66 ± 321 mg/100 g), Table 1, Figure 1. This pattern reflects resource redistribution strategies, where defensive compound investment decreases as resources are redirected towards reproduction [30, 31]. The lower tannin levels in flowers are important for pollinator attraction and chemical defense [32].

3.1.3. Flavonoids

The current results show a 27% increase in flavonoids in the roots during the flowering period (from 182.40 ± 2.2 to 232.4 ± 2.2 mg/100 g), in contrast to the stems and leaves, where they decreased by 15% and 30%, respectively. The leaves exhibited the highest concentration during the vegetative stage (331.3 ± 3.1 mg/100 g), while the fruit had an average concentration (255.8 ± 2.2 mg/100 g), and the flowers had a flavonoid concentration of 218.5 ± 4.8 mg/100 g, Table 1, Figure 1. The high flavonoid level in the leaves is consistent with the findings of Fang et al. (2012), who demonstrated that leaves accumulate the largest quantities in the aerial parts [35]. Furthermore, Dai et al. (2023) and Li et al. have also indicated similar findings. (2024) The concentration of flavonoids varies according to the functional differentiation of tissues in different plant species [33,34].

3.1.4. Anthocyanin

Anthocyanins are regarded significant photoprotective chemicals because they protect photosynthetic tissues in leaves from high radiation damage and have antioxidant properties [36, 37]. The current study found that anthocyanin levels in leaves increased by 318% (0.91 ± 0.01 mg/100 g) compared to vegetative leaves (3.81 ± 0.30 mg/100 g). Flowers had the highest concentration and significant difference (11.40 ± 0.48 mg/100 g) compared to other parts. Unlike the leaves, the roots decreased significantly during flowering compared to the vegetative stage (from 11.40 ± 0.48 to 2.80 ± 0.43 mg/100 g), **Table 1**, **Figure 1**.

3.1.5. Alkaloids

Alkaloids are crucial substances for protecting chili pepper seeds. Concentrated in the placental tissue enclosing the seeds, capsaicin is a distinctive alkaloid of chili peppers [35, 36]. According to the current findings, the concentration of alkaloids in leaves during the vegetative stage is 2373.33 ± 19 mg/100 g, which is much greater than that of other non-reproductive portions like stems and roots. At flowering, however, their levels declined by 44% to 1317.33 ± 20 mg/100 g. In a similar vein, the roots declined by 41% (from 386.42 ± 15 to 229.33 ± 9 mg/100 g), while the stems had the greatest reduction at 53% (from 842.67 ± 17 to 394.67 ± 11 mg/100 g), Table 1, Figure 1. Research conducted by Barchenger et al. (2016) and Wahyuni et al. (2011) indicates that mature chili pepper fruits collect a greater concentration of capsaicinoids compared to other parts [40, 41].

Table 1. phenolic compounds and alkaloids content mg/100g during vegetative (V.) and flowering stage (F.)

		Secondary compound assay mg/100g powder				
C. annum		Phenol	Tannin	Flavonoid	Anthocyanin	Alkaloids
Roots	V	1.32±0.008	4354.16±17	182.40±2.2	11.40±0.48	386.42±15
	F	3.24±0.13	2726.39±74	232.4±2.2	2.80±0.43	229.33±9
Stems	V	0.92±0.08	3670.83±37	202.9±2.3	0.40±0.08	842.67±17
	F	4.04±0.07	2143.05±17	173.1±3.0	0.29±0.02	394.67±11
Leaf	V	1.51±0.09	4602.08±56	331.3±3.1	0.91±0.01	2373.33±19
	F	4.58±0.03	4013.89±43	232.4±2.2	3.81±0.30	1317.33±20
Flowers		1.18±0.02	2679.14±35	218.5±4.8	3.40±0.48	1682.67±16
Fruits		0.31±0.001	2966.66±321	255.8±2.2	2.80±0.08	2536.00±13
Average		2.13	3394.52	233.43	4.22	1220.30
L.S.D. P≥0.05		1.54	62.12	8.27	1.34	21.87

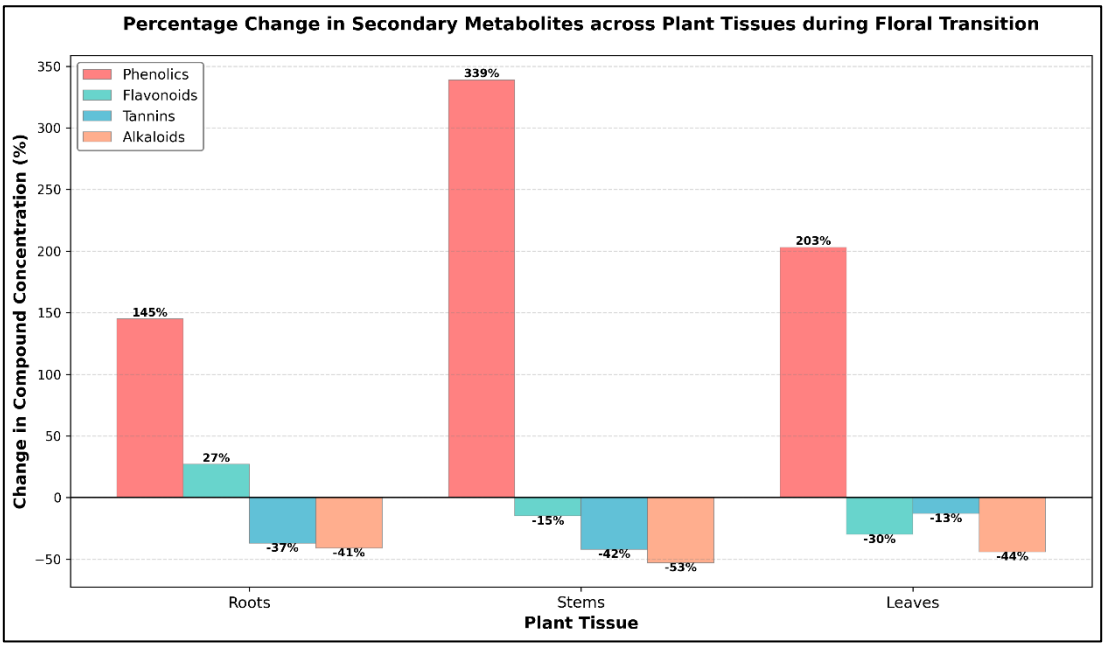

Figure 4. The percentage change in secondary metabolites (phenolics, flavonoids, tannins, and alkaloids) across plant tissues (roots, stems, and leaves) during floral transition

3.2. Antioxidant activity: tissues and growth stages

The antioxidant capacity of C. annuum tissues was assessed using the DPPH assay and expressed as IC_{50} values (lower values indicate higher activity). **Table 2** demonstrates significant differences in antioxidant capacity by tissue type and growth stage ($p < 0.05$, LSD-a = 0.449, LSD-ab = 0.635).

3.2.1. Overall antioxidant activity

The results of the current study showed that crude ethyl acetate extract (EA-Crude), containing most of the secondary metabolites, exhibited significantly stronger overall antioxidant capacity (mean IC_{50} = 0.44 mg) compared to ethyl acetate alkaloids (EA-Alk, mean IC_{50} = 3.68 mg). The superiority of EA-Crude over EA-Alk in this study may be attributed to the primary contribution of phenols and flavonoids to free radical scavenging capacity. These compounds possess structural properties that enable efficient electron or hydrogen atom donation [25, 42]. However, variations in efficacy based solely on plant parts showed that the vegetative leaves exhibited significantly higher antioxidant activity (IC_{50} = 0.46 mg/ml) compared to all other tissues and stages. This increased activity is consistent with the high concentrations of phenols (1.51 mg/100 g) and flavonoids (331.3 mg/100 g) measured in the vegetative leaves (**Table 1**).

The current results are consistent with previous studies indicating that extracts from chili pepper leaves typically exhibit strong DPPH-scavenging activity, with IC_{50} values ranging from 0.40 to 1.4 mg/ml, varying according to the cultivar and extraction solvent [25, 43]. The high antioxidant capacity of young leaves plays a role in protecting the photosynthetic machinery during rapid growth and high metabolic activity [29, 44]. The strong negative correlation between total phenolic content and DPPH IC_{50} values reported in other studies on chili peppers ($r = -0.87$) [25] is consistent with the current findings, where tissues with higher phenolic content (vegetative leaves) exhibited the strongest antioxidant activity. However, the relationship between secondary metabolite concentration and antioxidant function is complex, as structural diversity among phenolic compounds affects their free radical scavenging efficiency [45]. Flavonoids with multiple hydroxyl groups and specific conjugation patterns exhibit particularly strong antioxidant activity [46].

While EA-Crude for flowers showed moderate antioxidant activity (IC_{50} = 0.55 mg) (**Table 2**), which corresponds to average levels of phenolic and flavonoids (Table 1). It is worth noting Frey et al. (1997) stated

that biologically active compounds possess two functions in floral tissues: antioxidant protection and contributing to visual and chemical signaling to attract insects for pollination [47].

3.2.2. Developmental changes of Antioxidant Activity

It was observed that EA-Alk activity in leaves and roots decreased during the transition to flowering, with IC_{50} values increasing in roots from 6.87 to 9.02 mg and in leaves from 0.80 to 1.50 mg (**Table 2**) with decline percentage of efficacy (-31, -88%) respectively, **Figure 2**. This parallels the decrease in alkaloids during the flowering stage, indicating a degree of dependence of these tissues on alkaloids. This confirms what Wahyuni et al. (2011) suggested regarding a metabolic redistribution of alkaloids towards fruit development, most likely [41]. This redistribution of alkaloids from vegetative tissues to fruits represents a significant shift in resource allocation strategies, prioritizing seed protection over vegetative defense during the reproductive stages [38, 48]. Conversely, when the concentration of alkaloids in the stems increased during the flowering stage, the antioxidant capacity of its extract (EA-Alk) increased significantly (IC_{50} concentration decreased from 2.75 to 1.95 mg). This aligns with Tang et al. (2021) finding that the enhanced stress-induced alkaloid synthesis in stems is essential to support the increased transport requirements during fruit development and maintain its structural integrity [49].

Table 2. antioxidant capacity (mg) to scavenge 50% of 0.1mg DPPH during vegetative (*V.*) and flowering stage (*F.*)

Parts	G stage	Antioxidant assay (DppH)		Average b
		EA-Alk	EA-Crude	
Roots	V.	6.87	0.24	3.55
	F.	9.02	0.23	4.62
Stems	V.	2.75	0.58	1.66
	F.	1.95	0.56	1.25
Leaf	V.	0.80	0.13	0.46
	F.	1.50	0.31	0.90
Flowers		2.55	0.55	1.55
Fruits		3.97	0.97	2.47
Average a		3.68	0.44	
L.S.D. 0.05			a=0.449 b=0.224 ab=0.635	

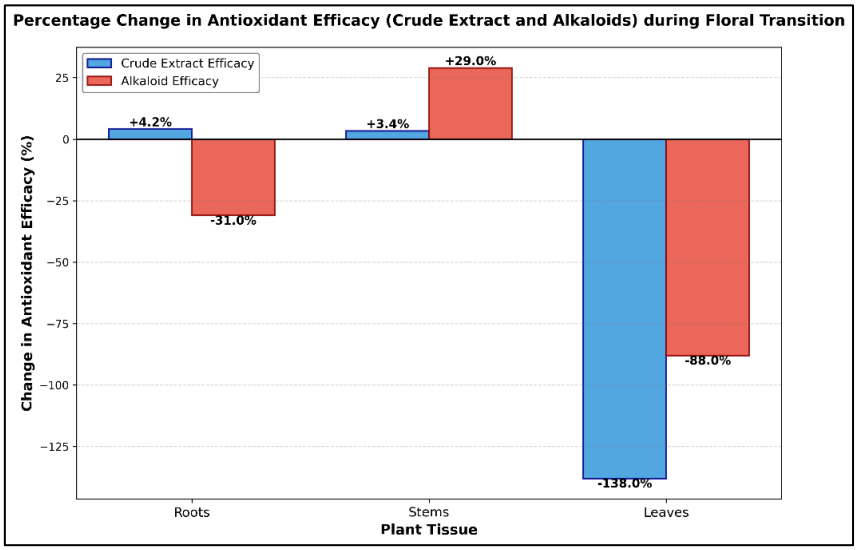

Figure 5. The percentage change in antioxidant efficacy (EA-Crude and EA-Alk) across plant tissues (roots, stems, and leaves) during floral transition

4. Conclusion

The study reveals clear changes in the secondary metabolite content of chili peppers during their various growth stages. Phenolic compounds and anthocyanins increased significantly in the roots, stems, and leaves during the flowering stage, while conversely, tannin and alkaloid levels decreased in the vegetative tissues and increased in the fruit. The leaves exhibited the highest flavonoid levels, confirming their primary role as a reservoir of antioxidant activity. These findings underscore the functional dynamics of secondary metabolism in chili peppers and reinforce their value as a source of natural antioxidants with potential nutritional and therapeutic applications.

Conflict of interest

The authors declare no conflict of interest

References

1. Chen, Y., Wang, L., & Zhang, Q. (2021). Leaf senescence and photosynthetic efficiency in Capsicum annum: Trade-offs between growth and defense. Journal of Agricultural and Food Chemistry, 69(12), 3456–3464. https://doi.org/10.1021/acs.jafc.1c00877
2. Mansouri, H., Ghorbani, A., & Karimi, M. (2021). Metabolic reallocation in plant roots during reproductive growth: Implications for defense and nutrient transport. Plant Physiology and Biochemistry, 165, 123–130. https://doi.org/10.1016/j.plaphy.2021.05.001
3. García-Castro, E., Pérez-Ramírez, I. F., & Mora-Escobedo, R. (2023). Phenolic and alkaloid dynamics in Capsicum annum fruits during maturation: Implications for UV and pathogen protection. Food Chemistry, 405(Part A), 134825. https://doi.org/10.1016/j.foodchem.2022.134825
4. Batiha, G. E. S., Alqahtani, A., Ojo, O. A., Shaheen, H. M., Wasef, L., Elzeiny, M., & Hetta, H. F. (2020). Biological properties, bioactive constituents, and pharmacokinetics of some Capsicum spp. and capsaicinoids. International journal of molecular sciences, 21(15), 5179.
5. Materska, M. (2024). "Flavone C-glycosides from Capsicum annuum L. – Influence on antioxidant capacity and lipoxygenase activity." Food Chemistry, 2024, 196, 680-687.
6. Lattanzio, V., Lattanzio, V. M. T., & Cardinali, A. (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Natural Product Communications, 1(11), 993–996.
7. Mithöfer, A., & Boland, W. (2012). Plant defense against herbivores: Chemical aspects. Annual Review of Plant Biology, 63, 431–450.
8. Gülçin, İ. (2020). Antioxidant activity of plant alkaloids: Mechanisms and applications in food systems. Critical Reviews in Food Science and Nutrition, 60(15),2500–2515. https://doi.org/10.1080/10408398.2020.1853034
9. Gould, K. S., Dudle, D. A., & Neufeld, H. S. (2009). Why some stems are red: Cauline anthocyanins shield photosystem II against high light stress. Trends in Plant Science, 14(6), 334–342.
10. Saleh B., Omer A., Teweldemedhin B. Medicinal uses and health benefits of chili pepper (Capsicum spp.): A review. MOJ Food Process Technol. 2018;6:325–328. doi: 10.15406/mojfpt.2018.06.00183.
11. Al-nuani, R. M. A., & Kadhim, N. J. (2020, November). The effect of Capparis Spinosa L. Plant on the cytochrome and glutathione to reduce the hepatotoxicity induced by paracetamol in mice. In Journal of Physics: Conference Series (Vol. 1664, No. 1, p. 012121). IOP Publishing.
12. Aziz, D. Z., Kadhim, N. J., Majeed, A. A., & Abood, A. H. (2020, November). Protective Effects of Ginger extract against Methotrexate induced cytotoxicity in mice. In Journal of Physics: Conference Series (Vol. 1664, No. 1, p. 012103). IOP Publishing.
13. Kadhim, N. J., Al-Rekaby, L. S., Redha, A. A., & Chappell, J. (2019, September). Chemical composition and antioxidant capacity of eggplant parts during vegetative and flowering stage. In Journal of Physics: Conference Series (Vol. 1294, No. 9, p. 092013). IOP Publishing.
14. Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. (1999) cited in Milan S. Stanković, 2010, Total Phenolic Content, Flavonoid Concentration and Antioxidant Activity of Marrubium peregrinum L. Extracts
15. Taha M., Banat F., Rababah A., Ereifej K. and Wade Y. (2010). Optimization of Extraction Conditionsof Total Phenolics, Antioxidant Activities,and Anthocyanin of Oregano, Thyme,Terebinth, and Pomegranate, Journal of Food Science, Institute of Food Technologists. 75(7):1750-3841.
16. Rabino, I. and Mancinelli, A. (1986) Light, Temperature and Anthocyanin Production. Journal of Plant Physiology, 81, 922-924.
17. Shamsa, F., Monsef, H. R., Ghamooshi, R., Verdian, R. M., & Mohammad, R. (2007). Spectrophotometric determination of total alkaloids in Peganum harmala L. using bromocresol green. Res J Phytochem, 1(2), 79-82.
18. Al-Husseini, M. T., Al-Mousawi, H. R., Kadhim, N. J., Madhloom, A. A. A. R., Aziz, D. Z., & Muha, A. J. K. (2020, November). Biological activity of Pomegranate peels Punica granatum Silver nanoparticles AgNPs extract

against Fourth larvae of Culex quinquefasciatus mosquito (Diptera: Culicidae). In Journal of Physics: Conference Series (Vol. 1660, No. 1, p. 012013). IOP Publishing.
19. Hadi, M. A., Hameedi, E. H., Kadhum, N. J., Aziz, D. Z., Al-Saddi, A. H., & Zaidan, H. K. (2016). Ameliorative Effect of Curcuma longa L. Rhizomes against Biochemical Toxicity Induced by Dichlorvos in Female Albino Rats. J. Chem. Pharm. Sci, 9, 1098-1106.
20. Khshan, K. T., & Alkafaje, H. A. (2021, April). Biosynthesis of silver nanoparticles using Calendula officinalis (L.) extract and evaluating their antioxidant activity. In IOP Conference Series: Earth and Environmental Science (Vol. 735, No. 1, p. 012073). IOP Publishing.
21. Osorio, S., Alba, R., Damasceno, C. M. B., Lopez-Casado, G., Lohse, M., Zanor, M. I., Tohge, T., Usadel, B., Rose, J. K. C., Fei, Z., Giovannoni, J. J., & Fernie, A. R. (2012). Integrative comparative analyses of transcript and metabolite profiles from pepper and tomato ripening and development stages uncovers species-specific patterns of network regulatory behavior. Plant Physiology, 159(4), 1713-1729.
22. Tang, Y., Liu, X., Ke, S., & Hang, J. (2022). Metabolomic analysis identifies differences between wild and cultivated pepper species during fruit development. Frontiers in Plant Science, 13, 893055.
23. Materska, M., & Perucka, I. (2005). Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (Capsicum annuum L.). Journal of Agricultural and Food Chemistry, 53(5), 1750-1756.
24. Barchenger, D. W., & Bosland, P. W. (2016). Review of the species and infraspecific taxonomy of Chile peppers (Capsicum spp.). HortScience, 51(3), 292-299.
25. Biratu, K. S., Selvaraj, S., Hailemichael, H. D., Chaithanya, K. K., Hanson, P., & Eyasu, A. (2024). Total phenolic and flavonoid content, lipophilic components, and antioxidant activity of Capsicum annuum L. cultivars. Natural Product Communications, 19(12), 1-10.
26. Ghasemnezhad, M., Sherafati, M., & Payvast, G. A. (2011). Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (Capsicum annum) fruits at two different harvest times. Journal of Functional Foods, 3(1), 44-49.
27. Döll, S., Kuhlmann, M., Rutten, T., Mette, M. F., Scharfenberg, S., Petridis, A., Bekel, T., Diekmann, H., & Krähmer, A. (2021). Tissue-specific signatures of metabolites and proteins in asparagus roots and exudates. Horticulture Research, 8(1), 87.
28. Palma, J. M., Sevilla, F., & Corpas, F. J. (2020). Antioxidant profile of pepper (Capsicum annuum L.) fruits at different ripening stages. Antioxidants, 9(9), 898.
29. Han, N., Joo, H. J., Kim, M. Y., Lee, J., Lee, Y., Moon, Y., Chung, D., Lee, Y., & Sung, J. (2021). Tissue-specific distribution of primary and secondary metabolites of Baemoochae (×Brassicoraphanus) and its changes as a function of developmental stages. Food Chemistry, 363, 130311.
30. Tong, Z., He, W., Fan, X., & Guo, A. (2022). Biological function of plant tannin and its application in animal health. Frontiers in Veterinary Science, 8, 803657.
31. Mora, J., Pott, D. M., Osorio, S., & Vallarino, J. G. (2022). Regulation of plant tannin synthesis in crop species. Frontiers in Genetics, 13, 870976.
32. Molino, S., Fernández-Miyakawa, M. E., Anzulovich, A. C., & Rufino-Palomares, E. (2023). Why is it important to understand the nature and chemistry of tannins? A perspective for the food and nutraceutical industries. Food Chemistry, 403, 134394.
33. Dai, Y., Wang, Y., Zhang, Y., Wu, Y., Xu, M., Li, X., Peng, X., Zang, Y., & Jiang, Y. (2023). The chemical profiling of Salvia plebeia during different growth periods and the strong correlation of phenolic acids and flavonoids with its antioxidant activity. Foods, 12(16), 3021.
34. Li, Y., Ma, J., Chen, Q., Wang, X., Zhou, W., Zhang, L., Yang, Y., & Wang, H. (2024). Accumulation patterns of flavonoids during multiple developmental stages in two tea cultivars. Beverage Plant Research, 4(1), 1-13.
35. Fang, J., Reichelt, M., Hidalgo, W., Agnolet, S., & Schneider, B. (2012). Tissue-specific distribution of secondary metabolites in rapeseed (Brassica napus L.). PLoS ONE, 7(10), e48006.
36. Zhu, H., Zhang, T. J., Zhang, P., & Peng, C. L. (2016). Pigment patterns and photoprotection of anthocyanins in the young leaves of four dominant subtropical forest tree species in two successional stages under contrasting light conditions. Tree Physiology, 36(9), 1092-1104.
37. Steyn, W. J., Wand, S. J. E., Holcroft, D. M., & Jacobs, G. (2002). Anthocyanins in vegetative tissues: A proposed unified function in photoprotection. New Phytologist, 155(3), 349-361.
38. Maharjan, A., Kim, Y. J., Shim, J. S., Yang, E. Y., Kang, Y. P., & Paek, N. C. (2024). A comprehensive review of capsaicin: Biosynthesis, industrial applications, and therapeutic potentials. Phytochemistry Reviews, 23, 1-28.
39. Supalkova, V., Huska, D., Diopan, V., Hanustiak, P., Zitka, O., Stejskal, K., Baloun, J., Pikula, J., Havel, L., Zehnalek, J., Adam, V., Trnkova, L., Beklova, M., & Kizek, R. (2007). Study of capsaicin content in various parts of pepper fruit by liquid chromatography with electrochemical detection. Acta Chimica Slovenica, 54(1), 55-59.
40. Barchenger, D. W., Naresh, P., & Kumar, S. (2016). Exogenous applications of capsaicin inhibits seed germination in Capsicum species. Scientia Horticulturae, 203, 29-31.
41. Wahyuni, Y., Ballester, A. R., Sudarmonowati, E., Bino, R. J., & Bovy, A. G. (2011). Metabolite biodiversity in pepper (Capsicum) fruits of thirty-two diverse accessions: Variation in health-related compounds and implications for breeding. Phytochemistry, 72(11-12), 1358-1370.

42. Agati, G., Brunetti, C., Fini, A., Gori, A., Guidi, L., Landi, M., Sebastiani, F., & Tattini, M. (2021). Are flavonoids effective antioxidants in plants? Twenty years of our investigation. Antioxidants, 9(11), 1098.
43. Howard, L. R., Talcott, S. T., Brenes, C. H., & Villalon, B. (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivars (Capsicum species) as influenced by maturity. Journal of Agricultural and Food Chemistry, 48(5), 1713-1720.
44. Zhang, Q., Zhai, J., Shao, L., Lin, W., & Peng, C. (2019). Accumulation of anthocyanins: An adaptation strategy of Mikania micrantha to low temperature in winter. Frontiers in Plant Science, 10, 1049.
45. Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. Journal of Nutritional Biochemistry, 13(10), 572-584.
46. Adler, L. S. (2000). The ecological significance of toxic nectar. Oikos, 91(3), 409-420.
47. Frey, M., Chomet, P., Glawischnig, E., Stettner, C., Grün, S., Winklmair, A., Eisenreich, W., Bacher, A., Meeley, R. B., Briggs, S. P., Simcox, K., & Gierl, A. (1997). Analysis of a chemical plant defense mechanism in grasses. Science, 277(5326), 696-699.
48. Edreva, A. M., Velikova, V. B., & Tsonev, T. D. (2007). Phenylamides in plants. Russian Journal of Plant Physiology, 54(3), 287-301.
49. Tang, M., Sun, J., Shimizu, H., & Ping, Y. (2021). Resource allocation strategies among vegetative growth, sexual reproduction and defense in response to nitrogen input in Phragmites australis. The Plant Journal, 106(4), 1035-1048.

w