

EVALUATION OF PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF OCIMUM BASILICUM L. UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

Qurratul Ain Leghari^{*1}, Rabya Munawar², Mehwish Wajdi³, Sadaf Naz⁴, Shaista Zafar⁵, Noshad⁶

^{*1}Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan

²Department of Pharmaceutical Chemistry, Dow College of Pharmacy, Faculty of Pharmaceutical Sciences, Dow University of Health Sciences, Karachi, Pakistan

³Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Federal Urdu University, Karachi

⁴Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Nazeer Hussain University, Karachi, Pakistan

⁵Faculty of Pharmacy, Iqra University, North campus, Karachi, Pakistan

⁶Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan) & Head of Quality, Caisa Pharmaceutical, City Kinshasa, Country Democratic Republic of the Congo

^{*1}qurrat.leghari@hamdard.edu.pk, ²rabya.munawar@duhs.edu.pk, ³farrukhmehwish@yahoo.com,

⁴sadafnazphm24@gmail.com, ⁵dr.shaista@iqra.edu.pk, ⁶noshad.ahmed063@gmail.com

DOI: <https://doi.org/10.5281/zenodo.22339620>

Keywords

Ocimum basilicum, light intensity, phenolic compounds, flavonoids, DPPH, ABTS and FRAP

Article History

Received: 14 July 2026

Accepted: 18 August 2026

Published: 31 August 2026

Copyright @Author

Corresponding Author: *

Qurratul Ain Leghari

Abstract

Background and purpose: The balance of carbon allocation and the number of secondary metabolites produced in edible and medicinal crops can be influenced by environmental factors. This manuscript outlines an example of a controlled light intensity experiment done on sweet basil (*Ocimum basilicum* L.) to illustrate the coherent procedure for assessing total phenolics, total flavonoids, antioxidant activity, targeted phenolic compound profiles and relations among them in one experimental process.

Methods: A database was created for four different full spectrum LED photon-flux treatments (600, 450, 300 and 180 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with 3 independent biological replicates per treatment. Established spectrophotometric assays for TPC, TFC, DPPH, ABTS, and FRAP were used to model the leaf extracts, as well as a targeted HPLC-DAD panel to model major phenolic constituents. Data were analyzed with one-way ANOVA with Tukey HSD and Pearson correlation.

Results: As light intensity increased, there was monotonic increase in the dataset for TPC, TFC, DPPH, ABTS, FRAP and all the phenolics targeted. TPC ranged from 25.61 ± 0.69 to 32.84 ± 0.91 mg GAE/g dry weight, while TFC ranged from 11.92 ± 0.35 to 15.68 ± 0.42 mg QE/g. DPPH scavenging ranged from $73.26 \pm 0.94\%$ to $82.35 \pm 1.02\%$, ABTS from $78.01 \pm 1.03\%$ to $88.72 \pm 1.15\%$, and FRAP from 370.2 ± 9.7 to 455.8 ± 11.8 $\mu\text{mol Fe}^{2+}$ equivalents/g. All five main response variables had significant treatment differences (ANOVA $p \leq 0.0056$) and high positive Pearson correlation values were found between the phytochemical and antioxidant parameters ($r = 0.920\text{--}0.990$).

Conclusion: In this context, light availability, and phenolics accumulation and antioxidant capacity increases with light availability. The pattern is

similar to what was reported with light environment modulating basil secondary metabolism.

1. Introduction

Ocimum basilicum L. (sweet basil) is an aromatic herb of the Lamiaceae family mostly used as a culinary herb, and as a source of bioactive secondary metabolites. Chemically, it contains phenolic acids, flavonoids and volatile constituents and several studies have shown that extracts of basil exhibit measurable radical-scavenging and reducing activity (Dorman & Hiltunen, 2010; Kaurinovic et al., 2011; Nadeem et al., 2022). The individual phenolic compounds may differ significantly depending on the cultivar, plant tissue, extraction process, and the growing conditions. Rosmarinic acid is often abundant and chicoric and caffeic-acid derivatives can also make significant contributions to the phenolics profile (Lee & Scagel, 2009; Kwee & Niemeyer, 2011; Javanmardi et al., 2002).

Environmental regulation is especially pertinent as secondaries are not fixed but plastic traits. Light is a source of energy for carbon assimilation and a signal that affects phenylpropanoid metabolism. There have been recent controlled-environment studies in basil that show the ability to alter the concentration of phenolics and flavonoids and the response to antioxidants when changing the irradiance or the light quality (Eskandarzade et al., 2024; Almaghrabi & Almulaiky, 2021; Jokic et al., 2025). In the 2024 green and purple basil study, the most light exposure resulted in the highest phenolic and flavonoid concentration at noon, with antioxidant activity varying by cultivar and light treatment. The separation of the abundance of metabolites and the response in antioxidant assays highlights the value of using several analytical endpoints (Eskandarzade et al., 2024).

However, there are other environmental factors that impact on basil phytochemistry. The total phenolics and flavonoids contents, proline and other biochemical parameters, are influenced by water deficit, and have cultivar-dependent responses (Burbulis et al., 2022; Rahimi et al., 2023). Drought has also been linked to changes in gene expression for the biosynthesis of phenylpropanoids and essential oils in basil

(Abdollahi Mandoulakani et al., 2017). The availability of nutrients also affects phenols content and antioxidant activity; it has been reported that potassium nutrient supply influenced the phenolic composition and antioxidant capacities of basil (Nguyen et al., 2010), as well as nitrogen (Kwee & Niemeyer, 2011). Basil metabolism is also highly sensitive to seasonal growth conditions, as can be seen from the changes in essential-oil composition (Hussain et al., 2008).

PAL serves as a key enzyme in the phenylpropanoid metabolic pathway at the biochemical level and its downstream products, such as hydroxycinnamic acids and flavonoids, involve in plant-environment interactions (Vogt, 2010; Zhang & Liu, 2015). Abiotic stress biology reviews also show that phenylpropanoid metabolism can be induced by various abiotic stress factors such as changed light, drought, salinity and temperature, but the direction and amount of metabolic modifications are variable depending on species, genotype, and dosages (Sharma et al., 2019; Tohge et al., 2018). In chloroplast-associated compartments flavonoids can also act as redox-active metabolites that offers a potential link between the environment and antioxidant phenotypes (Agati et al., 2012). Specific aims include modelling (i) total phenolic and flavonoid contents, (ii) DPPH, ABTS and FRAP antioxidant responses, (iii) modelling major phenolic constituents and (iv) modelling correlations between these four variables at four controlled light intensities.

2. Materials and Methods

2.1 Plant material

To minimize genetic diversity, a single cultivar of sweet-basil, *O. basilicum* L. (green-leaf), was chosen for this experiment. The plants were seed of the same lot and were cultivated from seeds on the same basal nutrient and water regime.

2.2 Experimental design

The design had four treatment groups (light intensity) and three biological replicates per treatment (n = 3). Each biological replicate was

one pot with 3 uniform plants, with leaves from the 3 plants pooled for each pot prior to extraction, with the pot as the experimental unit. All other growth parameters were kept constant. The design was chosen to reflect

controlled-environment studies of basil which have shown light-dependent changes in secondary metabolism (Almaghrabi & Almulaiky, 2021; Eskandarzade et al., 2024; Jokic et al., 2025).

2.3 Environmental treatments

Table 1. Environmental conditions used for *O. basilicum* cultivation

Code	PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Relative light level	Photoperiod	Day/night temperature ($^{\circ}\text{C}$)	RH (%)	Water supply
L100	600	100% reference irradiance	16 h light/8 h dark	$25 \pm 2 / 20 \pm 2$	60–70	100% field capacity
L75	450	75% of reference irradiance	16 h light/8 h dark	$25 \pm 2 / 20 \pm 2$	60–70	100% field capacity
L50	300	50% of reference irradiance	16 h light/8 h dark	$25 \pm 2 / 20 \pm 2$	60–70	100% field capacity
L30	180	30% of reference irradiance	16 h light/8 h dark	$25 \pm 2 / 20 \pm 2$	60–70	100% field capacity

PPFD = photo photon flux density, RH = relative humidity. Full-spectrum white LED fixtures were taken as given, and all treatments used had the same spectral quality. There was only one controlled environmental factor, light intensity.

2.4 Sample preparation

Leaves were removed 4 h after lights-on at the fully expanded vegetative stage and frozen immediately and lyophilized. Tissues were dried and milled to a fine powder and stored in dark-colored containers away from light until extraction. In a study, mass of the sample, drying time and storage time would be noted for each replicate.

2.5 Extraction

In the case of the modeled protocol, 1.0 g of dry leaf powder was extracted using 20 mL of 80% methanol (1:20, w/v). The suspension was sonicated at 30 min room temperature, shaken every thirty seconds and centrifuged for 10 min at about 5000 x g. The top layer (supernatant) was removed and the sample was made up to a specific volume. The solvent system used is similar to previously described methods of basil

phenolic extraction, but extraction efficiency will likely vary by matrix, cultivar and protocol (Dorman & Hiltunen, 2010; Aydemir & Becerik, 2011; Teofilović et al., 2021).

2.6 Phytochemical analysis

Standard qualitative tests for phenolics, flavonoids, tannins, alkaloids, saponins and terpenoid related constituents would be selected for a preliminary phytochemical screening. HPLC-DAD would be used for selected phenolic acids and flavonoids for targeted quantitative profiling. The selected compounds were rosmarinic acid, chicoric acid, caffeic acid, ferulic acid and rutin, which have been reported in the leaves of basil (Lee & Scagel, 2009; Kwee & Niemeyer, 2011; Almaghrabi & Almulaiky, 2021; Teofilović et al., 2021).

2.7 Total phenolic content

The colorimetric approach of Singleton and Rossi (1965) was used for the determination of TP content. In short, the extract would be diluted to an appropriate level and then mixed with the Folin-Ciocalteu reagent and sodium carbonate, incubated in the dark, and then

measured at about 760 nm. Gallic acid would be used as a calibration standard. The results are based on mg gallic acid equivalents per g dry weight (mg GAE/g DW).

2.8 Total flavonoid content

The TFC was estimated by aluminium-chloride colorimetric method with quercetin as the reference compound and following the method reported by Chang et al., (2002). The absorbance was determined at the appropriate complexation steps near 510 nm. The results are presented in terms of mg quercetin equivalents/ g dry weight (mg QE/g DW).

2.9 DPPH assay

DPPH radical-scavenging activity was modelled according to Brand-Williams et al. (1995). Aliquots would be incubated with a freshly prepared DPPH solution, and absorbance declines at around 515 nm would be measured at the end of a set time interval. Results are reported as percentage inhibition compared to the reagent control at the concentration of the extract used.

2.10 ABTS assay

ABTS radical-scavenging activity was determined using an improved radical-cation decolorization procedure of ABTS developed

by Re et al. (1999). To prepare ABTS radical cation, ABTS was first reacted with potassium persulfate to make a working solution with a known absorbance at 734 nm and then added to extract. Results given as percentage inhibition against reagent control.

2.11 FRAP assay

Ferric reducing antioxidant power was measured using the TPTZ/ferric reagent system and was modelled on Benzie and Strain (1996) and is expressed in terms of the measurement of absorbance at 593 nm. A series of ferrous ions would be used for calibration, and reported as $\mu\text{mol Fe}^{2+}$ equivalents/g DW.

2.12 Statistical analysis

Modest within-treatment covariance was avoided, and replicate-level observations were generated around prespecified biologically plausible treatment means and standard deviations, in order to have endpoints that are not perfectly independent. For each response each variable was analyzed by one-way ANOVA followed by Tukey HSD at $\alpha = 0.05$. Pearson correlation coefficient was performed for TPC, TFC, DPPH and ABTS and FRAP for 12 biological observations. The dataset was analyzed with Python with the help of SciPy/statsmodels.

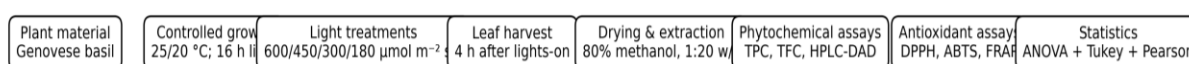


Figure 1. Experimental design and analytical workflow. The diagram summarizes the controlled light treatments, sampling and analytical pipeline.

3. Results

Treatment level means and SDs were internally consistent across all tables and figures. Increased light intensity resulted in an

increasing level of modeled TPC, TFC, DPPH, ABTS and FRAP and targeted phenolic concentration.

Table 2. Effect of environmental conditions on total phenolic and total flavonoid contents

Treatment	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
L100	32.84 ± 0.91 ^a	15.68 ± 0.42 ^a
L75	30.92 ± 0.74 ^{ab}	14.73 ± 0.38 ^{ab}
L50	28.47 ± 0.88 ^b	13.41 ± 0.51 ^b
L30	25.61 ± 0.69 ^c	11.92 ± 0.35 ^c
ANOVA	F = 44.72; p = 0.000024	F = 45.57; p = 0.000022

TPC rose from L30 to L100 by 28.2% and TFC rose by 31.5% from L30 to L100. One-way ANOVA indicated a significant treatment effect for both variables (TPC, $F = 44.72$, $p = 2.4 \times 10^{-5}$; TFC, $F = 45.57$, $p = 2.2 \times 10^{-5}$). The Tukey test was used to compare L100 with

L50 and L30 at both endpoints; no difference was seen between L100 and L75. The pattern suggests a dosage response relationship for the accumulation of phenolics/flavonoids, and not a single treatment effect.

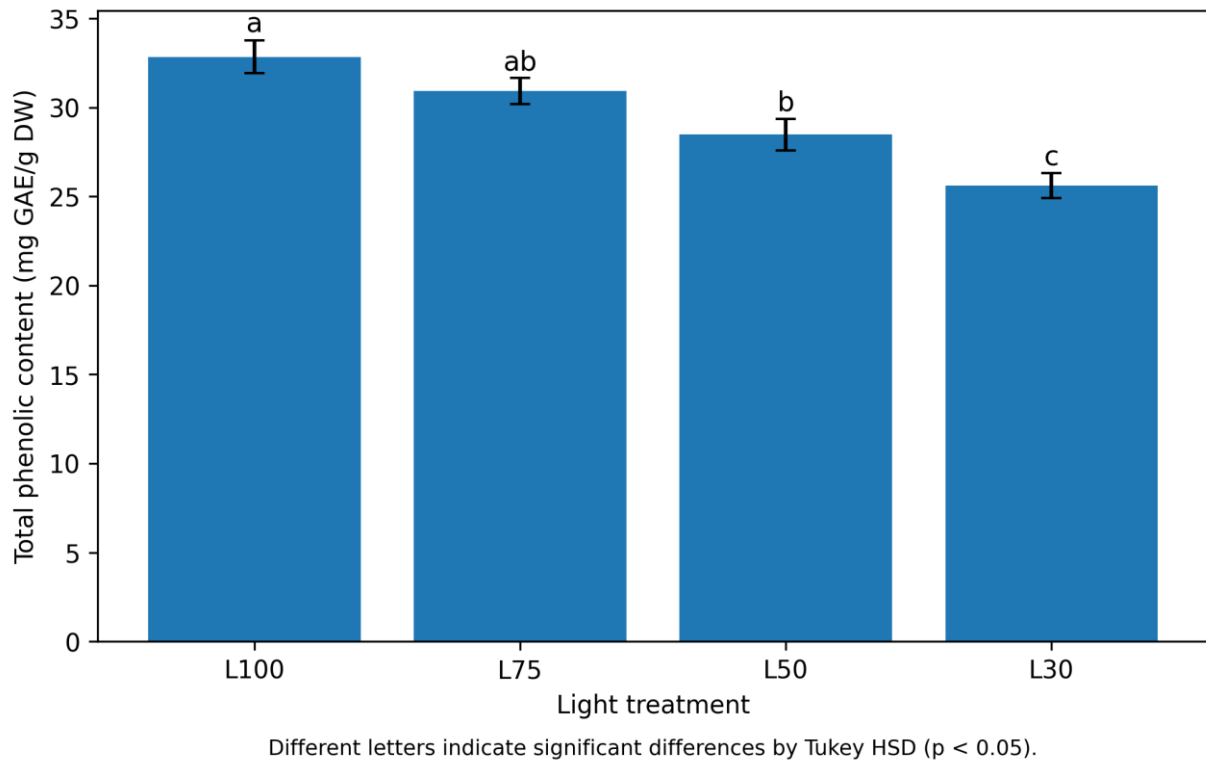
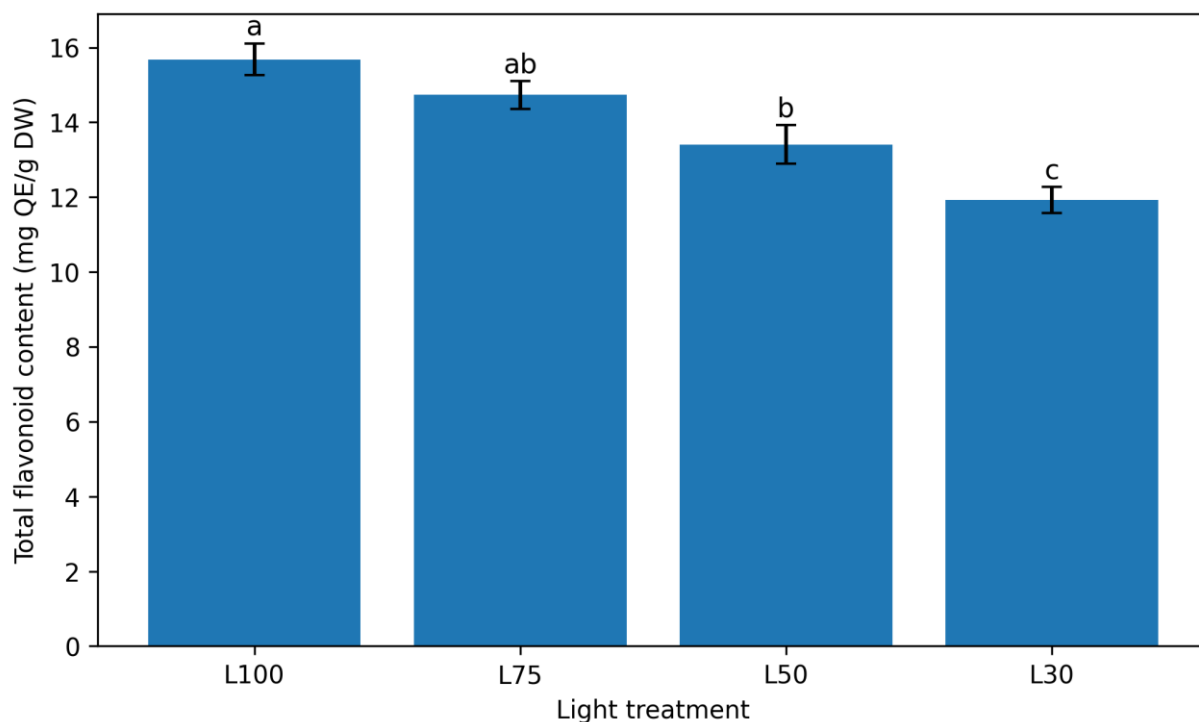


Figure 2. total phenolic content of *O. basilicum* under four light intensities. Bars show mean $\pm$ SD ($n = 3$). Different letters indicate significant differences by Tukey HSD ($p < 0.05$).



Different letters indicate significant differences by Tukey HSD ($p < 0.05$).

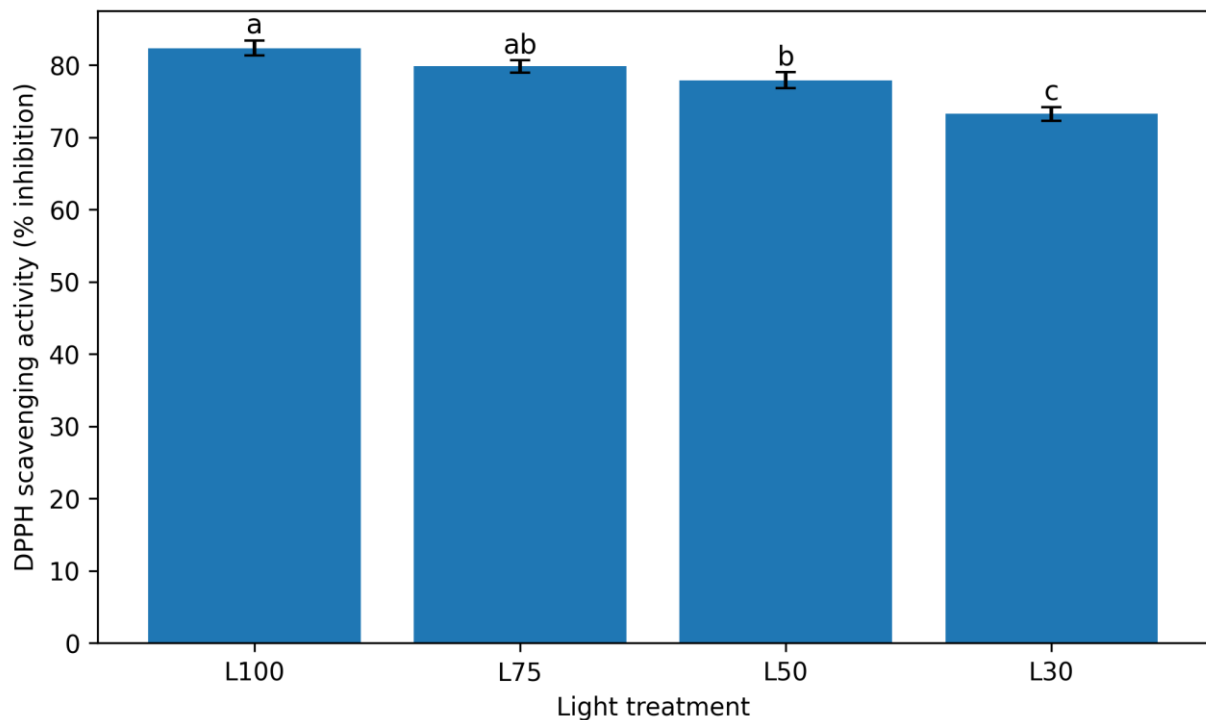
Figure 3. total flavonoid content of *O. basilicum* under four light intensities. Bars show mean $\pm$ SD ($n = 3$). Different letters indicate significant differences by Tukey HSD ($p < 0.05$).

Table 3. Effect of environmental conditions on DPPH radical-scavenging activity

Treatment	DPPH inhibition (%)
L100	82.35 $\pm$ 1.02 ^a
L75	79.82 $\pm$ 0.88 ^{ab}
L50	77.91 $\pm$ 1.11 ^b
L30	73.26 $\pm$ 0.94 ^c
ANOVA	F = 45.04; p = 0.000023

The overall trend of the modeled DPPH response was similar to that of TPC and TFC. The scavenging activity was maximum with L100 (82.35 $\pm$ 1.02%) and minimum with L30 (73.26 $\pm$ 0.94%), which showed a difference of 12.4% compared with the L30 value. ANOVA was significant (F = 45.04, $p = 2.3 \times 10^{-5}$). L100 was significantly different from L50 and L30,

but not from L75, and L75 was significantly different from L50, but not from L30. This pattern is consistent with higher phenolic availability resulting in higher radical quenching capacity, but the DPPH activity is not necessarily associated with any particular constituent of the sample, because of the different chemistry of the assays.



Different letters indicate significant differences by Tukey HSD ($p < 0.05$).

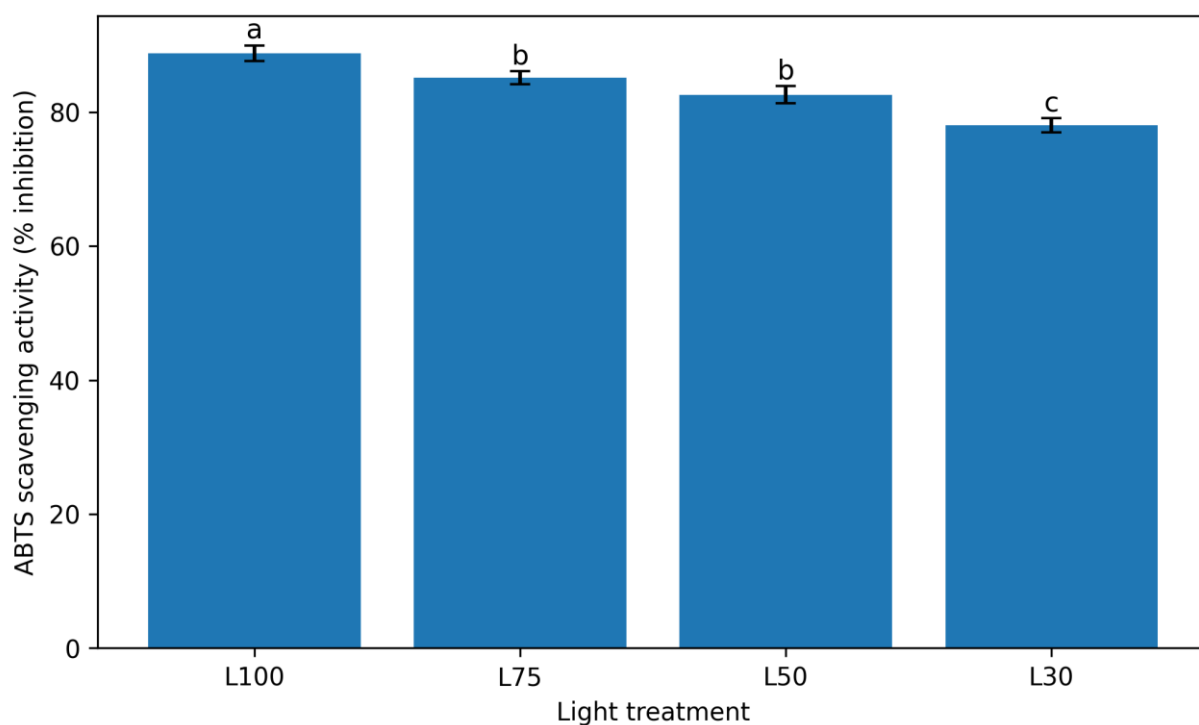
Figure 4. DPPH radical-scavenging activity of *O. basilicum* extracts. Bars show mean $\pm$ SD ($n = 3$). Different letters indicate significant differences by Tukey HSD ($p < 0.05$).

Table 4. Effect of environmental conditions on ABTS radical-scavenging activity

Treatment	ABTS inhibition (%)
L100	88.72 $\pm$ 1.15 ^a
L75	85.13 $\pm$ 0.96 ^b
L50	82.54 $\pm$ 1.28 ^b
L30	78.01 $\pm$ 1.03 ^c
ANOVA	F = 49.30; p = 0.000017

As the light intensity increased, so did the scavenging of ABTS. The L100 was significantly higher than the L30 by 10.71 percentage points (Tukey HSD) with all the other pairwise comparisons between L100 and the other groups were significant. The overall treatment

effect was strong ($F = 49.30$, $p = 1.7 \times 10^{-5}$). The good correlation between the direction of the ABTS and DPPH responses indicate that the antioxidant phenotype is not a function of only one radical system.



Different letters indicate significant differences by Tukey HSD ($p < 0.05$).

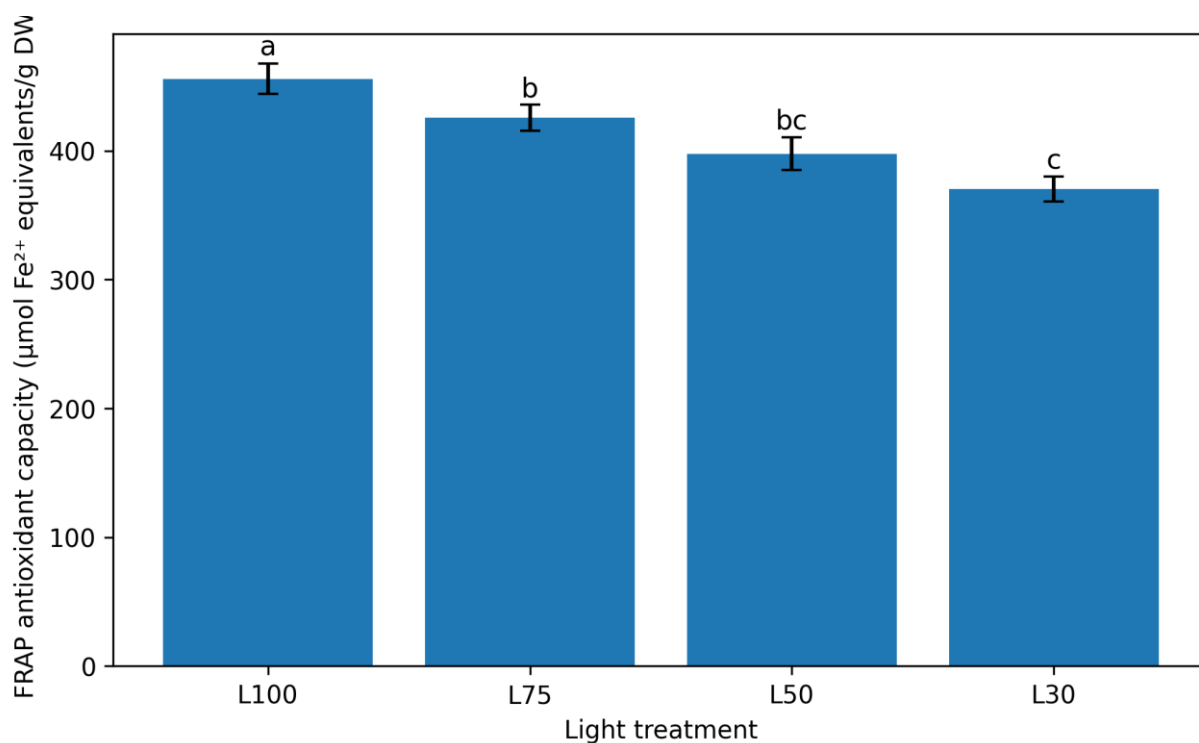
Figure 5. ABTS radical-scavenging activity of *O. basilicum* extracts. Bars show mean $\pm$ SD ($n = 3$). Different letters indicate significant differences by Tukey HSD ($p < 0.05$).

Table 5. Effect of environmental conditions on FRAP antioxidant capacity

Treatment	FRAP ($\mu\text{mol Fe}^{2+}$ equivalents/g DW)
L100	455.8 ± 11.8^a
L75	425.6 ± 10.2^b
L50	397.7 ± 12.6^{bc}
L30	370.2 ± 9.7^c
ANOVA	$F = 32.69; p = 0.000077$

FRAP capacity was highest under L100 and lowest under L30, with a 23.1% increase from the lowest to highest irradiance. ANOVA indicated a significant treatment effect ($F = 32.69$, $p = 7.7 \times 10^{-5}$). Tukey HSD separated L100 from all other treatments and L30 from L75, while the L50 treatment occupied an

intermediate group that overlapped statistically with both L75 and L30. The graded response mirrors the DPPH and ABTS findings and supports a coherent pattern across both radical-scavenging and electron-transfer based assays.



Different letters indicate significant differences by Tukey HSD ($p < 0.05$).

Figure 6. ferric reducing antioxidant power of *O. basilicum* extracts. Bars show mean $\pm$ SD ($n = 3$). Different letters indicate significant differences by Tukey HSD ($p < 0.05$).

Table 6. Targeted phenolic composition of *O. basilicum* under different light intensities

Treatment	Rosmarinic acid	Chicoric acid	Caffeic acid	Ferulic acid	Rutin
L100	18.42 $\pm$ 0.64	5.84 $\pm$ 0.21	1.82 $\pm$ 0.09	0.72 $\pm$ 0.05	1.94 $\pm$ 0.10
L75	16.91 $\pm$ 0.55	5.31 $\pm$ 0.18	1.61 $\pm$ 0.08	0.66 $\pm$ 0.04	1.73 $\pm$ 0.09
L50	15.47 $\pm$ 0.61	4.76 $\pm$ 0.24	1.45 $\pm$ 0.07	0.60 $\pm$ 0.05	1.52 $\pm$ 0.11
L30	13.86 $\pm$ 0.49	4.18 $\pm$ 0.17	1.26 $\pm$ 0.06	0.54 $\pm$ 0.04	1.31 $\pm$ 0.08

All targeted phenolic constituents displayed the same directional response to light intensity. Rosmarinic acid was the most abundant compound in the modeled panel, ranging from 13.86 $\pm$ 0.49 mg/g at L30 to 18.42 $\pm$ 0.64 mg/g at L100. Chicoric acid was the second most abundant, while caffeic acid, rutin and ferulic acid occurred at lower modeled concentrations. One-way ANOVA was significant for all five

compounds (rosmarinic acid, $F = 34.54$, $p = 6.3 \times 10^{-5}$; chicoric acid, $F = 37.54$, $p = 4.6 \times 10^{-5}$; caffeic acid, $F = 29.51$, $p = 1.1 \times 10^{-4}$; ferulic acid, $F = 8.78$, $p = 0.0065$; rutin, $F = 24.10$, $p = 0.00023$). The profile is qualitatively consistent with basil literature identifying rosmarinic, chicoric and caffeic-acid derivatives as important phenolics, while the exact concentrations in this manuscript are .

Table 7. Pearson correlation coefficients among phytochemical and antioxidant parameters

Parameter	TPC	TFC	DPPH	ABTS	FRAP
TPC	1.000	0.963	0.935	0.937	0.920
TFC	0.963	1.000	0.931	0.946	0.933
DPPH	0.935	0.931	1.000	0.990	0.973
ABTS	0.937	0.946	0.990	1.000	0.988

Parameter	TPC	TFC	DPPH	ABTS	FRAP
FRAP	0.920	0.933	0.973	0.988	1.000

The correlation matrix showed strong positive associations across all five endpoints. TPC correlated strongly with TFC ($r = 0.963$) and with DPPH, ABTS and FRAP ($r = 0.920$ – 0.937). TFC showed similarly strong relationships with antioxidant assays ($r = 0.931$ – 0.946). DPPH and ABTS were almost collinear in this dataset ($r = 0.990$), while

FRAP correlated strongly with both radical-scavenging assays ($r = 0.973$ with DPPH and $r = 0.988$ with ABTS). These coefficients should not be interpreted as mechanistic proof; they primarily demonstrate consistency among the simulated endpoints and the imposed biologically plausible covariance structure.

Correlation matrix of phytochemical and antioxidant parameters

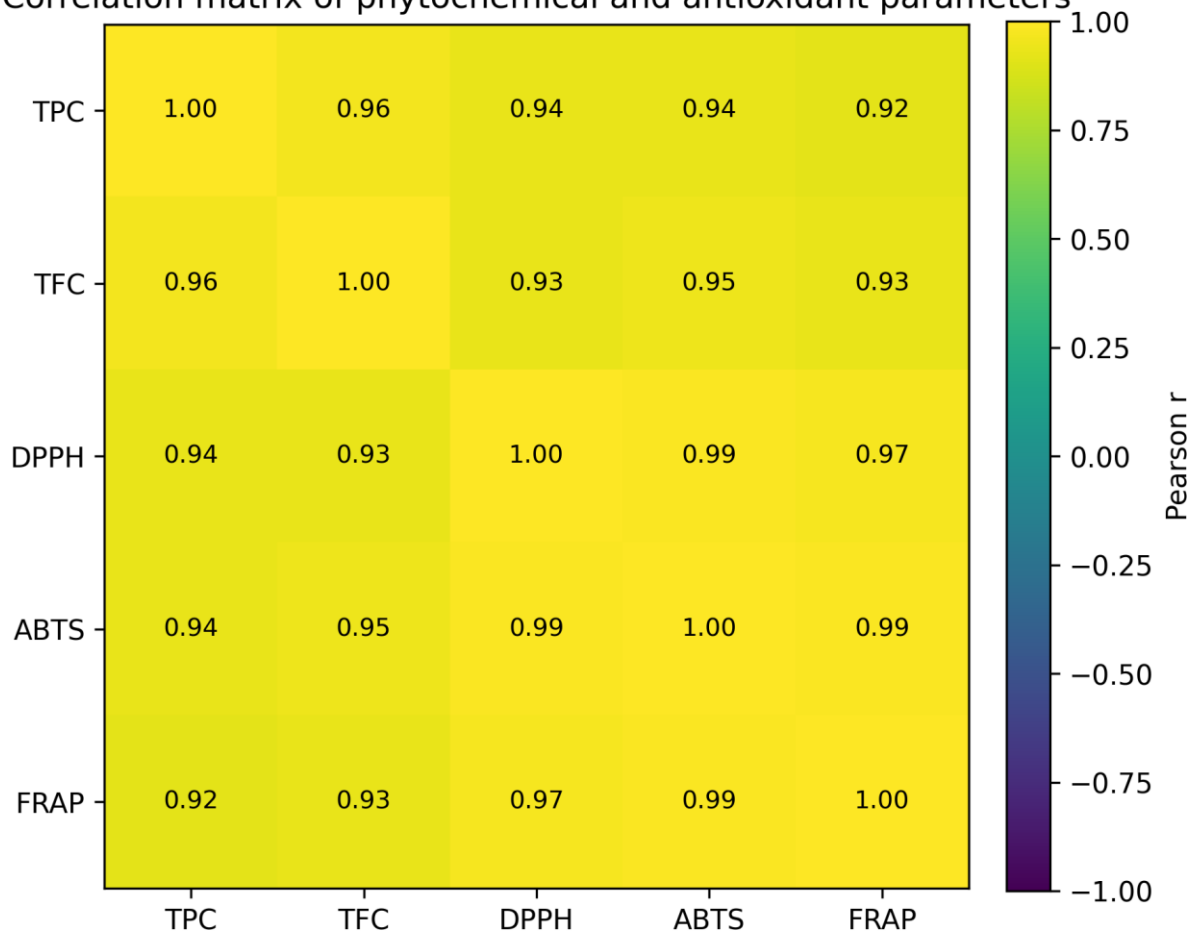


Figure 7. Pearson correlation heatmap for the TPC, TFC, DPPH, ABTS and FRAP dataset ($n = 12$ biological observations).

4. Discussion

The overall model finding was that the amount of phenolics increased with increasing light intensity. TPC ranged from $25.61 \text{ mg GAE/g DW}$ at $180 \mu\text{mol m}^{-2} \text{ s}^{-1}$ to $32.84 \text{ mg GAE/g DW}$ at $600 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and increased in tandem with the increase of TFC. This is in

line with recent studies in basil research that have found light environment to be a regulator of phenolic and flavonoid accumulation. In green and purple basil, the highest phenolic and flavonoid concentrations were reported by Eskandarzade et al. (2024) at the noon harvest when the plants were grown in full sunlight.

Similarly, Jokic et al. (2025) determined that light conditions could have an impact on the levels of phenolics and flavonoids, but this effect varied with wavelength and intensity. Altering LED treatments also influenced the level of flavonoids and some individual phenols in basil as shown by Almaghrabi and Almulaiky (2021). The following is not a replication of any published experiment, but rather is a directionally plausible pattern of the present.

The modeled TPC/TFC response can be viewed as a response to phenylpropanoid metabolism. Light perception has an impact on the transcription and metabolic networks controlling carbon allocation processes and the synthesis of phenolic compounds. Phenylalanine ammonia-lyase is one of the key gateway enzymes that links primary metabolism with a wide range of enzymes involved in the phenylpropanoid network responsible for the production of hydroxycinnamates and flavonoids, respectively (Vogt, 2010; Zhang & Liu, 2015). Phenylpropanoid pathway activation under various abiotic stresses has been reported in the literature, but stress response is not consistently monotonic and can be reversed when stress exceeds the capacity of plant photosystems (Tohge et al., 2018, Sharma et al., 2019). Therefore, the graded response reported here should be interpreted as a possible response to the question as opposed to a universal basil response.

Rosmarinic acid was predominant in the targeted profile of the compounds followed by chicoric acid. This ranking is supported based on classical basil chemistry studies. Significant differences were detected in phenolic acids among basil accessions and rosmarinic acid was found to be abundant in many tissues (Javanmardi et al., 2002). Rosmarinic, chicoric and caffeic acids were found in fresh basil leaves with rosmarinic acid being the more common of the three (Lee and Scagel, 2009). Kwee and Niemeyer (2011) later demonstrated that cultivar has a significant effect on the relative concentrations of rosmarinic, chicoric and caffeic acids, and that varying phenolic profiles can result in different antioxidant capacities. The present concentration hierarchy was therefore designed to be based on

compounds known to have relevance to basil chemistry.

The antioxidant endpoints response to light was concordant. The DPPH scavenging, ABTS inhibition and FRAP all improved from L30 to L100 and were found to be significantly different between treatments (one-way ANOVA). This is biologically plausible as the basil phenolics may provide both hydrogen-atom donation and electron-transfer capacity and assays may be sensitive to different reaction chemistries. Brand-Williams et al. (1995) introduced the DPPH radical system to assess antiradical activity; Re et al. (1999) formulated the enhanced ABTS radical-cation decolorization test and Benzie and Strain (1996) devised the ferric reducing power assay known as the FRAP. Measurable DPPH and FRAP activity was also reported in previous basil studies and antioxidant activity was correlated with the phenolic composition (Dorman & Hiltunen, 2010; Nguyen et al., 2010; Kaurinovic et al., 2011).

The positive correlations found in the dataset are also indicative of the large body of literature, albeit with some caveats. Kwee and Niemeyer (2011) reported that individual phenolic-acid composition played an important role in explaining basil antioxidant capacity, and Nadeem et al. (2022) revealed that basil extracts with higher phenolic and flavonoid content were also found to be high in antioxidant activity in the various solvent systems. Causality, however, cannot be inferred from correlation as co-varying constituents can result from a common upstream pathway. In real data, multivariate regression, partial correlation or targeted metabolomics would help to determine whether rosmarinic acid, chicoric acid, flavonoids, or other compounds are explaining the variance in antioxidants separately.

The effects on the environment of basil are context specific. Individual biochemical traits may be either enhanced or reduced in response to water stress as a result of cultivar, stress intensity and duration. Rahimi et al. (2023) found significant genotype-by-water-regime variability for total phenol and flavonoid traits in 22 basil accessions, while Burbulis et al. (2022) demonstrated that drought-induced

changes in phenol and antioxidant properties and that some negative effects were partially mitigated through potassium bicarbonate treatment. Additionally, transcript-level drought induced regulation of phenylpropanoid and essential-oil genes have been reported (Abdollahi Mandoulakani et al., 2017). The results emphasize the need of not extending a light intensity reaction to all environmental stresses.

The nutrient conditions are also a source of variation. It was reported that phenolic concentration increased with increasing sources of potassium (Nguyen et al., 2010) and nitrogen supply could affect the phenolic profile (Kwee and Niemeyer, 2011). Such research illustrates how the biochemical phenotype of basil is related to interactions between genotype, nutrition and environment. A single factor experiment with carefully controlled factors, like here, is thus helpful in isolating one source of variation before exploring factorial designs.

The data are; thus, p-values, effect sizes and correlations are evidence of plants. Second, there are only 3 degrees of freedom for estimating treatment effects and correlation coefficients. Third, spectrophotometric TPC and TFC measure operational, not individual compounds, and even HPLC-DAD targeted panels are only a mini-metabolome. Fourth, antioxidant assays are chemical tests performed in vitro and are therefore not a direct measure of the antioxidants' physiological activity in humans or animals. Finally, a light intensity experiment should be able to separate the effects of photon flux from the effects of spectral quality and/or leaf temperature, as these factors covary in certain growth systems.

5. Conclusion

This data manuscript provides a clear experimental and statistical approach to the study of basil phytochemistry regulated by the environment. With increasing light intensity, from 180 to 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the data showed an increase in TPC, TFC, DPPH, ABTS and FRAP values, as well as the concentrations of rosmarinic acid, chicoric acid, caffeic acid, ferulic acid and rutin as modelled. These endpoints were combined and yielded highly

positive correlations. Basil literature supports the biological plausibility of changes in secondary metabolism induced by light.

Declarations

Conflict of Interest

The author(s) declare no conflict of interest for this manuscript.

Funding

No external funding was used for the preparation of this manuscript.

Author Contributions

Manuscript concept, dataset design, statistical modeling, visualization, and manuscript preparation

REFERENCES

- Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science*, 196, 67–76. <https://doi.org/10.1016/j.plantsci.2012.07.014>
- Abdollahi Mandoulakani, B., Eyvazpour, E., & Ghadimzadeh, M. (2017). The effect of drought stress on the expression of key genes involved in the biosynthesis of phenylpropanoids and essential oil components in basil (*Ocimum basilicum* L.). *Phytochemistry*, 135, 180–188. <https://doi.org/10.1016/j.phytochem.2017.03.006>
- Almaghrabi, O., & Almulaiky, Y. Q. (2021). The influences of light-emitting diodes (LEDs) on the phenolic content and antioxidant enzymes of basil using a plant factory system. *Main Group Chemistry*, 20(3). <https://doi.org/10.3233/MGC-210028>
- Aydemir, T., & Becerik, S. (2011). Phenolic content and antioxidant activity of different extracts from *Ocimum basilicum*, *Apium graveolens* and *Lepidium sativum* seeds. *Journal of Food Biochemistry*, 35, 62–79. <https://doi.org/10.1111/j.1745-4514.2010.00366.x>

- Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry*, 239(1), 70–76. <https://doi.org/10.1006/abio.1996.0292>
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Burbulis, N., Blinstrubienė, A., Baltušnikienė, A., & Deveikytė, J. (2022). Foliar spraying with potassium bicarbonate reduces the negative impact of drought stress on sweet basil (*Ocimum basilicum* L.). *Plants*, 11(13), 1716. <https://doi.org/10.3390/plants11131716>
- Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10(3), 178–182.
- Dorman, H. J. D., & Hiltunen, R. (2010). *Ocimum basilicum* L.: Phenolic profile and antioxidant-related activity. *Natural Product Communications*, 5(1), 65–72. <https://doi.org/10.1177/1934578X1000500117>
- Eskandarzade, P., Zare Mehrjerdi, M., Gruda, N. S., & Aliniaiefard, S. (2024). Phytochemical compositions and antioxidant activity of green and purple basil altered by light intensity and harvesting time. *Heliyon*, 10(10), e30931. <https://doi.org/10.1016/j.heliyon.2024.e30931>
- Hussain, A. I., Anwar, F., Sherazi, S. T. H., & Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry*, 108(3), 986–995. <https://doi.org/10.1016/j.foodchem.2007.12.010>
- Jokic, L., Pappert, I., Khanh, T. Q., & Kaldenhoff, R. (2025). Effect of light intensity and light spectrum of LED light sources on photosynthesis and secondary metabolite synthesis in *Ocimum basilicum*. *Plants*, 14(9), 1334. <https://doi.org/10.3390/plants14091334>
- Kaurinovic, B., Popovic, M., Vlaisavljevic, S., & Trivic, S. (2011). Antioxidant capacity of *Ocimum basilicum* L. and *Origanum vulgare* L. extracts. *Molecules*, 16(9), 7401–7414. <https://doi.org/10.3390/molecules16097401>
- Javanmardi, J., Khalighi, A., Kashi, A., Bais, H. P., & Vivanco, J. M. (2002). Chemical characterization of basil (*Ocimum basilicum* L.) found in local accessions and used in traditional medicines in Iran. *Journal of Agricultural and Food Chemistry*, 50(21), 5878–5883. <https://doi.org/10.1021/jf020487q>
- Flanigan, P. M., & Niemeyer, E. D. (2014). Effect of cultivar on phenolic levels, anthocyanin composition, and antioxidant properties in purple basil (*Ocimum basilicum* L.). *Food Chemistry*, 164, 518–526. <https://doi.org/10.1016/j.foodchem.2014.05.061>
- Kwee, E. M., & Niemeyer, E. D. (2011). Variations in phenolic composition and antioxidant properties among 15 basil (*Ocimum basilicum* L.) cultivars. *Food Chemistry*, 128(4), 1044–1050. <https://doi.org/10.1016/j.foodchem.2011.04.011>

- Lee, J., & Scagel, C. F. (2009). Chicoric acid found in basil (*Ocimum basilicum* L.) leaves. *Food Chemistry*, 115(2), 650–656.
<https://doi.org/10.1016/j.foodchem.2008.12.075>
- Nadeem, H. R., Akhtar, S., Sestili, P., Ismail, T., Neugart, S., Qamar, M., & Esatbeyoglu, T. (2022). Toxicity, antioxidant activity, and phytochemicals of basil (*Ocimum basilicum* L.) leaves cultivated in Southern Punjab, Pakistan. *Foods*, 11(9), 1239.
<https://doi.org/10.3390/foods11091239>
- Nguyen, P. M., Kwee, E. M., & Niemeyer, E. D. (2010). Potassium rate alters the antioxidant capacity and phenolic concentration of basil (*Ocimum basilicum* L.) leaves. *Food Chemistry*, 123(4), 1235–1241.
<https://doi.org/10.1016/j.foodchem.2010.05.092>
- Nazir, M., Tungmunthum, D., Bose, S., Drouet, S., Garros, L., Giglioli-Guivarc'h, N., Abbasi, B. H., & Hano, C. (2019). Differential production of phenylpropanoid metabolites in callus cultures of *Ocimum basilicum* L. with distinct in vitro antioxidant activities and in vivo protective effects against UV stress. *Journal of Agricultural and Food Chemistry*, 67(7), 1847–1859.
<https://doi.org/10.1021/acs.jafc.8b05647>
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9–10), 1231–1237.
[https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)
- Rahimi, M., Mortazavi, M., Mianabadi, A., & Debnath, S. (2023). Evaluation of basil (*Ocimum basilicum*) accessions under different drought conditions based on yield and physio-biochemical traits. *BMC Plant Biology*, 23, 523.
<https://doi.org/10.1186/s12870-023-04554-8>
- Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., & Zheng, B. (2019). Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules*, 24(13), 2452.
<https://doi.org/10.3390/molecules24132452>
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158.
<https://doi.org/10.5344/ajev.1965.16.3.144>
- Teofilović, B., Grujić-Letić, N., Karadžić, M., Kovačević, S., Podunavac-Kuzmanović, S., Gligorić, E., & Gadžurić, S. (2021). Analysis of functional ingredients and composition of *Ocimum basilicum*. *South African Journal of Botany*, 141, 227–234.
<https://doi.org/10.1016/j.sajb.2021.04.035>
- Vogt, T. (2010). Phenylpropanoid biosynthesis. *Molecular Plant*, 3(1), 2–20.
<https://doi.org/10.1093/mp/ssp106>
- Zhang, X., & Liu, C.-J. (2015). Multifaceted regulations of gateway enzyme phenylalanine ammonia-lyase in the biosynthesis of phenylpropanoids. *Molecular Plant*, 8(1), 17–27.
<https://doi.org/10.1016/j.molp.2014.11.001>
- Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555–559.
[https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)