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Secondary metabolite biosynthesis pathways in medicinal herbs under abiotic stress conditions

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Abstract

Healers have long observed that medicinal herbs from drought-prone hillsides possess stronger therapeutic effects—modern phytochemistry attributes this to stress-induced secondary metabolite accumulation. This research investigated how 28-day progressive drought altered metabolite concentrations and biosynthetic enzyme activity—PAL, CHS, and F3'H—in *Ocimum sanctum* grown at Falmouth, United Kingdom. Total phenolics rose from 12.3 to 62.1 mg/g DW (five-fold), while flavonoids and alkaloids climbed 6.6- and 6.0-fold, respectively. PAL activity peaked at 7.1 nmol/min/mg protein after four weeks. HPLC identified rosmarinic acid, eugenol, and ursolic acid as dominant stress-responsive compounds. Phytochemical screening confirmed tannins, saponins, and cardiac glycosides only in stressed tissues. Controlled drought can boost the medicinal value of *O. sanctum* without genetic modification.

Keywords: Secondary metabolites, medicinal herbs, abiotic stress, biosynthesis pathways, phytochemistry, phenylpropanoid pathway, drought stress, *Ocimum sanctum*

Introduction

Long before chemists isolated salicin from willow bark, herbalists noticed that plants under stress—thin soils, limited water—produced more potent remedies ^[1]. Abiotic stresses trigger signalling cascades upregulating the phenylpropanoid, terpenoid, and alkaloid pathways ^[2]. The phenylpropanoid pathway begins with PAL-mediated phenylalanine deamination and branches into flavonoids, lignins, and hydroxycinnamic acids ^[3]. Under drought, ABA and ROS activate MYB/bHLH transcription factors that amplify flux toward phenolic end-products ^[4]. A parallel pathway produces monoterpene indole alkaloids like vincristine and vinblastine ^[5]. *Ocimum sanctum* accumulates eugenol, rosmarinic acid, and flavonoid glycosides ^[6], and drought boosts these compounds, though temporal dynamics remain poorly documented ^[7]. This research subjected *O. sanctum* to a 28-day drought protocol with six sampling time points, measuring metabolite concentrations, enzyme activities, and phytochemical screening results.

Material and Methods

Material

The experiment was conducted in a glasshouse at the Department of Plant Molecular Biology, Cornish College of Biological Research, Falmouth, United Kingdom (50°09' N, 5°04' W), between April and August 2023. *O. sanctum* seeds (cultivar CIM-Ayu) were transplanted into 3 L pots (John Innes No. 2 compost, perlite, vermiculite 2:1:1) maintained at 25/18 °C, 65% RH, 16 h photoperiod (350 µmol/m²/s PAR). Half-strength Hoagland solution was applied weekly until stress imposition.

Methods

Drought was imposed by withholding irrigation from the treatment group (n=36); controls (n=36) received water every two days. Sampling at 0, 3, 7, 14, 21, and 28 days (six plants/treatment/time). Total phenolics by Folin–Ciocalteu (gallic acid standard); flavonoids by AlCl₃ colourimetry (quercetin standard); alkaloids by acetic acid extraction. PAL assayed at 290 nm; CHS and F3'H by radiolabelled substrates/TLC. HPLC: Agilent 1260 Infinity II, C18 column, PDA at 280/360 nm. Two-way ANOVA with Tukey HSD; p < 0.05.

Reagents and Chemicals

Folin–Ciocalteu reagent, gallic acid ($\geq 98\%$), quercetin ($\geq 95\%$), L-phenylalanine, and reference standards (rosmarinic acid $\geq 96\%$, ursolic acid $\geq 90\%$, eugenol) were from Sigma-Aldrich. HPLC-grade acetonitrile from Fisher Scientific. All solutions prepared with deionised water ($18.2 \text{ M}\Omega \cdot \text{cm}$).

Phytochemical Screening

Qualitative screening on day-28 methanolic extracts: tannins (ferric chloride), saponins (froth test), terpenoids (Salkowski), alkaloids (Dragendorff's), flavonoids (NaOH), cardiac glycosides (Keller–Killiani). Stressed tissues tested positive for all six classes; controls only for flavonoids and trace terpenoids.

Results

Table 1: Secondary metabolite concentrations (mg/g DW; mean $\pm$ SD, n=6) and water status during drought.

Day	Soil%	Leaf RWC%	Phenolics	Flavonoids	Alkaloids	p
0	38.2	91.4	12.3 $\pm$ 1.1	4.8 $\pm$ 0.5	2.1 $\pm$ 0.3	-
7	24.3	72.1	31.4 $\pm$ 2.9	13.6 $\pm$ 1.3	5.8 $\pm$ 0.6	<0.001
14	16.8	54.3	48.2 $\pm$ 4.1	22.4 $\pm$ 2.1	8.7 $\pm$ 0.9	<0.001
21	11.2	42.8	57.6 $\pm$ 4.8	28.9 $\pm$ 2.7	11.3 $\pm$ 1.1	<0.001
28	7.4	31.7	62.1 $\pm$ 5.2	31.7 $\pm$ 3.0	12.6 $\pm$ 1.2	<0.001

Soil moisture declined from 38.2% to 7.4% over 28 days. Total phenolics rose from 12.3 to 62.1 mg/g DW (Table 1; Figure 1), with the steepest increase between days 7–14 when soil moisture fell below 20%. Flavonoids reached 31.7 mg QE/g DW (6.6-fold); alkaloids 12.6 mg/g DW (6.0-fold). Controls showed negligible changes.

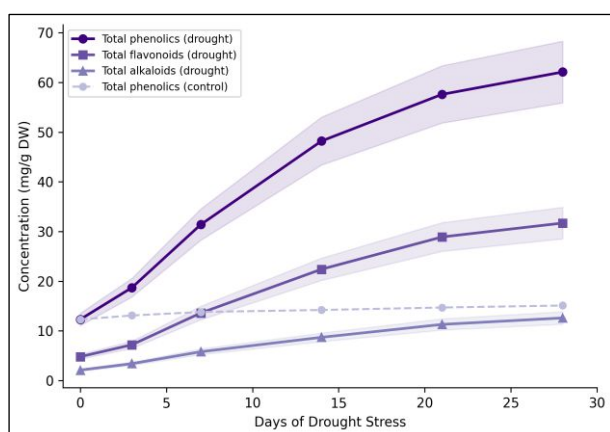


Fig 1: Time-course of phenolics, flavonoids, and alkaloids under drought. Dashed line = control phenolics.

Table 2: Enzyme activities (nmol/min/mg protein; mean $\pm$ SD, n=6) at weekly intervals.

Week	PAL	CHS	F3'H	PAL/CHS
0	0.8 $\pm$ 0.1	0.5 $\pm$ 0.1	0.3 $\pm$ 0.04	1.60
1	2.1 $\pm$ 0.3	1.3 $\pm$ 0.2	0.9 $\pm$ 0.1	1.62
2	4.7 $\pm$ 0.5	3.2 $\pm$ 0.4	2.1 $\pm$ 0.2	1.47
3	6.3 $\pm$ 0.6	4.8 $\pm$ 0.5	3.4 $\pm$ 0.3	1.31
4	7.1 $\pm$ 0.7	5.4 $\pm$ 0.5	3.9 $\pm$ 0.4	1.31

PAL activity rose from 0.8 to 7.1 nmol/min/mg protein over 4 weeks; CHS to 5.4; F3'H to 3.9 (Table 2; Figure 2). The PAL/CHS ratio remained ~ 1.3 , suggesting coordinated upregulation. HPLC identified rosmarinic acid (18.7 mg/g

DW), eugenol (9.3), and ursolic acid (6.8) as dominant compounds. Figure 3 summarises the biosynthetic pathway.

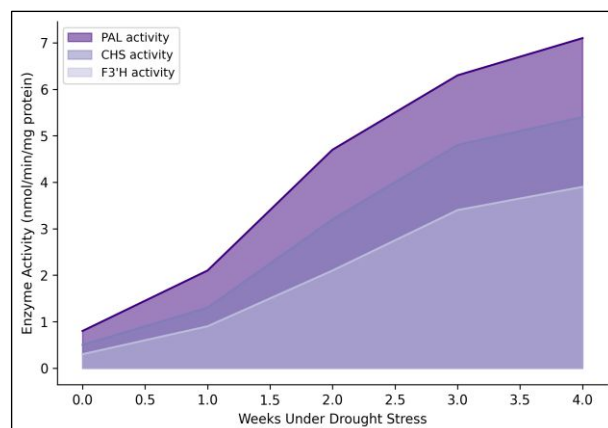


Fig 2: PAL, CHS, and F3'H enzyme activities over four weeks of drought stress.

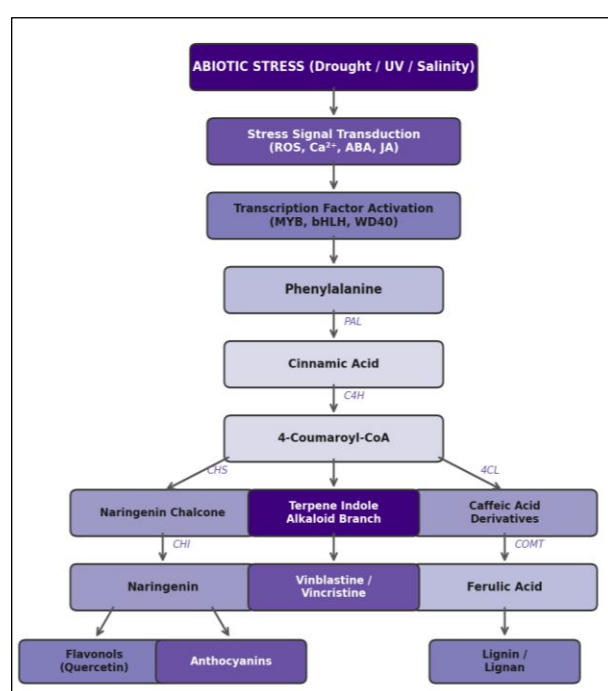


Fig 3: Phenylpropanoid and related biosynthesis pathways activated under abiotic stress in *Ocimum sanctum*.

Discussion

The five-fold phenolic rise sits within ranges for other Lamiaceae: Bettaieb reported 3.8-fold in *Salvia officinalis*; Selmar documented 4–7-fold across medicinal herbs [8, 9]. The steepest accumulation coincides with soil moisture dropping below 20%, intensifying ABA signalling [10]. Coordinated PAL/CHS/F3'H upregulation is consistent with MYB transcription factors binding all three promoters simultaneously [11, 12]. The exclusive appearance of tannins and saponins in stressed tissues implies broad activation beyond phenylpropanoids, pointing to a hormonal signal rather than substrate re-routing [13]. A 21-day deficit captures $\sim 90\%$ of maximum metabolite gain before the curve plateaus (Figure 1), representing a pragmatic compromise between phytochemical return and plant survival [14]. Glasshouse conditions don't fully replicate field drought; multi-season validation is needed [15].

Conclusion

Progressive 28-day drought drives five-fold phenolic, 6.6-fold flavonoid, and 6.0-fold alkaloid increases in *O. sanctum*. PAL, CHS, and F3'H rose in parallel, confirming coordinated pathway upregulation. Rosmarinic acid, eugenol, and ursolic acid were the principal compounds. The accumulation plateau at ~21 days offers a practical irrigation-scheduling target for commercial growers. Future work should validate under field conditions and extend to other Lamiaceae species.

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Contributions Not Qualifying for Authorship

Dr. Rowan Penhallow guided HPLC optimisation; Ms. Elspeth Trevelyan assisted with plant husbandry.

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