Sustainable production of health-promoting compounds from melatonin-elicited cultures of *Echinacea purpurea*

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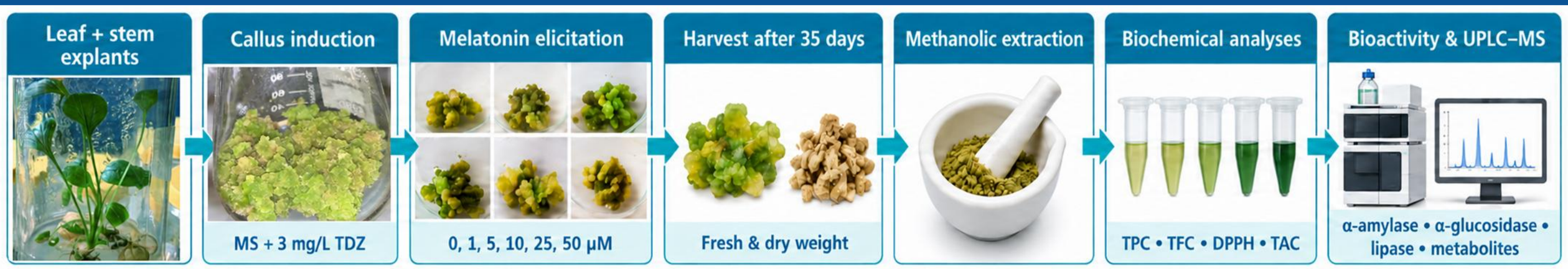
Introduction

- *Echinacea purpurea* (purple coneflower) is a valuable medicinal species rich in bioactive compounds with health-promoting properties. However, conventional cultivation depends on seasonal and environmental conditions.
- *In vitro* culture provides a controlled, year-round platform for the sustainable production of these compounds while supporting germplasm conservation and reducing dependence on field-grown plant material.
- Melatonin, a plant-signalling molecule, can regulate growth, stress responses, and secondary metabolism.

Objective: To evaluate the impact of melatonin on biomass, bioactive potential and phytochemical production in *E. purpurea* callus cultures.

Experimental Workflow



Results

- **Biomass:** Melatonin significantly enhanced callus biomass, with highest fresh weight (208.62 g/L) and dry weight (11.87 g/L) recorded at 25 µM.
- **Enzyme-inhibition activity:** Maximum α-amylase, α-glucosidase and pancreatic-lipase inhibition reached 30.09%, 22.60% and 68.33%, respectively, although their optimal melatonin doses differed.
- **Antioxidant activity:** The strongest response occurred at 25 µM, with 92.7% DPPH radical-scavenging activity and 63.22 µg AAE/mg total antioxidant capacity.

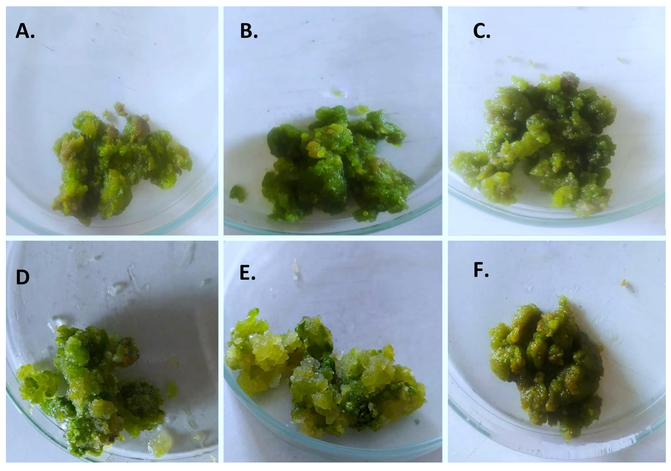


Figure 1. Biomass accumulation in *Echinacea purpurea* callus treated with 0–50 µM melatonin.

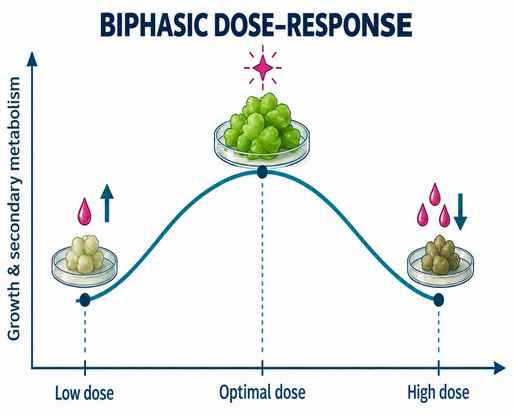


Figure 2. Schematic representation of the biphasic dose–response induced by melatonin elicitation.

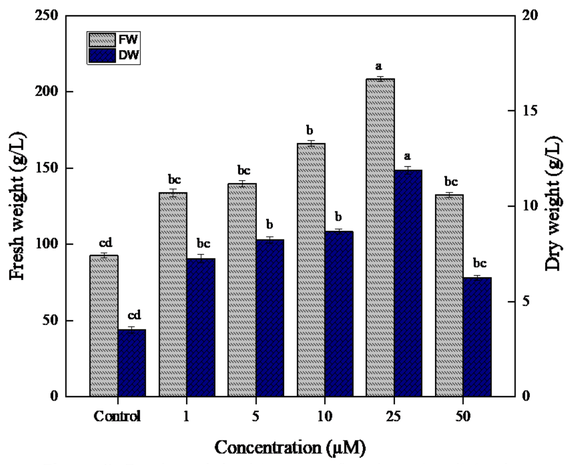


Figure 3. Fresh and dry biomass of melatonin-elicited callus cultures. Values represent mean ± SD (*n* = 3).

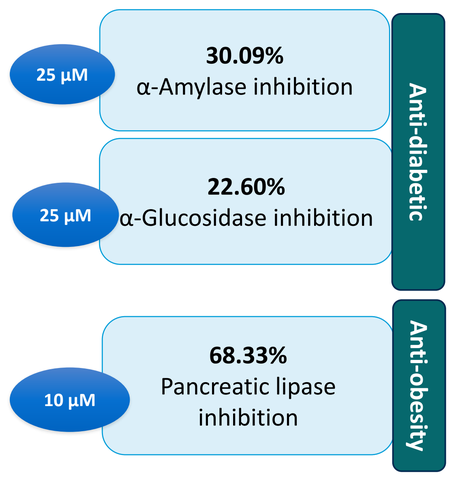
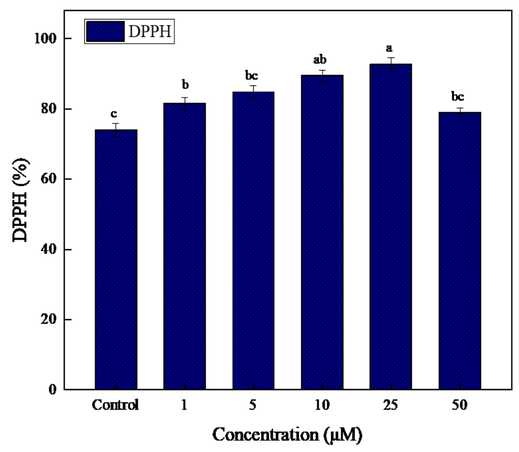


Figure 4. Maximum *in vitro* enzyme-inhibitory activities of melatonin-elicited callus extracts



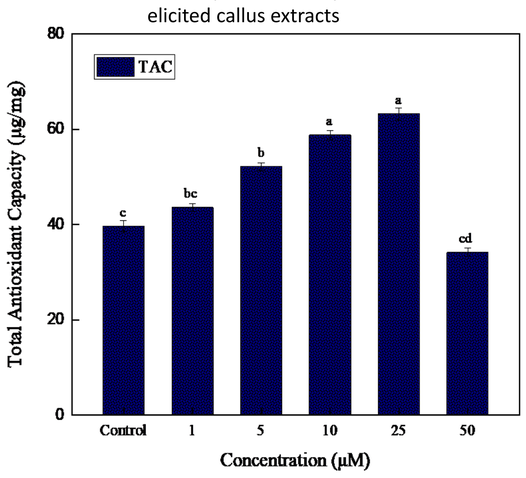


Figure 5. DPPH free-radical-scavenging activity and Total antioxidant capacity of melatonin-elicited *E. purpurea* callus cultures. Values represent mean ± SE (*n* = 3).

- **Secondary metabolites production:** The 25 µM treatment produced the highest total phenolic content and production, reaching 132.8 mg GAE/L. Total flavonoid production increased markedly at 25 µM melatonin, reaching a maximum of 585.9 mg QE/L.
- **UPLC–MS metabolite profile:** Melatonin altered metabolite accumulation in a dose-dependent manner: 25 µM enhanced several caffeoylquinic acids, whereas 50 µM maximized several other medicinally relevant phenolic compounds.

Figure 6. a) Total phenolic content (TPC) and production (TPP), b) Total flavonoid content (TFC) and production (TFP), under different melatonin concentrations. Values represent mean ± SD (*n* = 3).

UPLC–MS Metabolite Analysis

Compound	Control (AU × 10³)	Best dose (µM)	Maximum (AU × 10³)	Change vs control
Caffeic acid	27.51	50	49.62	+80.4%
Caftaric acid	124.16	50	156.18	+25.8%
Chicoric acid	945.22	50	1034.79	+9.5%
Neochlorogenic acid	9.70	25	12.37	+27.5%
3,4-Dicaffeoylquinic acid	113.25	25	150.50	+32.9%
1,4-Dicaffeoylquinic acid	27.21	25	44.214	+62.5%
Quercetin 3-O-rutinoside	1.04	50	8.03	+672.1%

Table 1. UPLC–MS results show high accumulation of valuable metabolites in *E. purpurea* callus cultures under three different melatonin treatments

Conclusions

- Melatonin elicitation offers a sustainable strategy for enhancing health-promoting compounds in *Echinacea purpurea* callus cultures, while supporting germplasm conservation and reducing reliance on field-grown plant material.
- **25 µM melatonin was identified as the optimum dose** for enhancing biomass, phytochemical production, and antioxidant activity.

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