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Impact of UV-B Radiation on Antioxidant and Dye Removal Capacity of *Thymus lotocephalus* green extracts

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INTRODUCTION & AIM

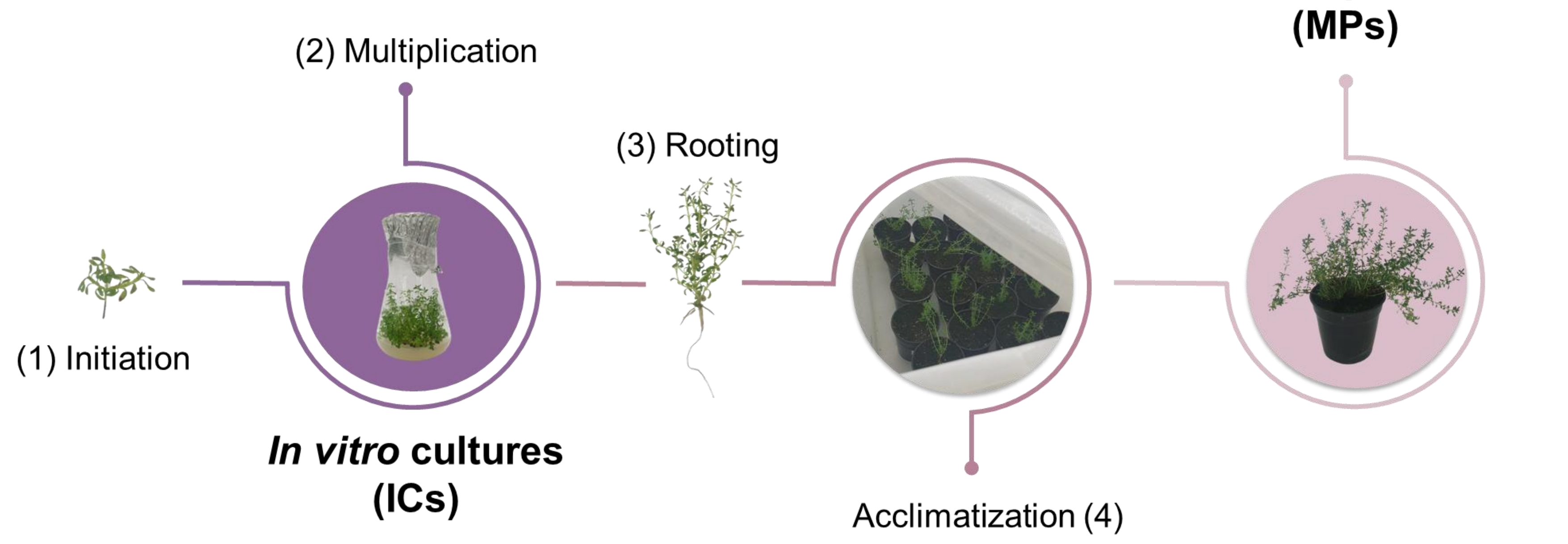
To counteract the oxidative stress induced by abiotic stress factors such as UV-B radiation, plants typically activate both enzymatic and non-enzymatic molecules (e.g., phenolic compounds) with antioxidant functions [1].

Phenolic compounds not only contribute to the plant's defense mechanisms but can also be applied in several industries due to their broad range of biological properties [1]. These compounds possess various functional groups, such as hydroxyl (–OH) and carboxyl (–COOH), which serve as active binding sites for biosorption, making them excellent candidates for the removal of contaminants [2]. This is particularly important given that a large number of synthetic dyes are produced each year. Toxic dyes like methylene blue (MB), which account for about 20% of global wastewater pollution, pose significant ecological and health risks [3].

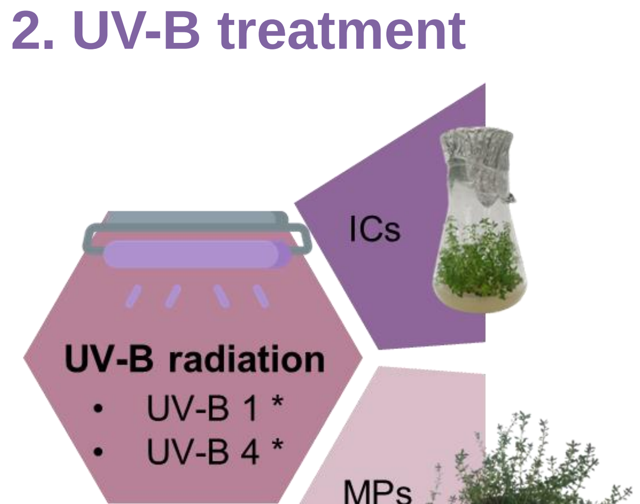
The **aim of this work** was to evaluate the impact of UV-B radiation on the phenolic production and antioxidant activity of green extracts *in vitro cultures* (ICs) and micropropagated plants (MPs) of *Thymus lotocephalus*, an endangered aromatic species endemic to the Algarve region (Portugal). Additionally, the MB removal capacity of hydrogels loaded with the produced antioxidant extracts was tested as a potential eco-friendly strategy for dye removal from water.

MATERIALS & METHODS

1. Plant production – Micropropagation

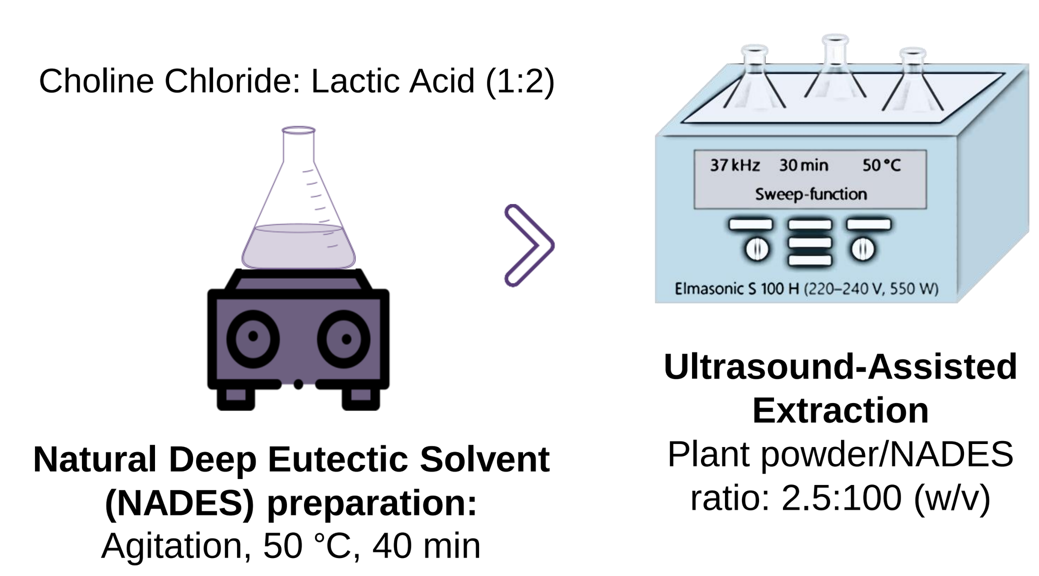


2. UV-B treatment

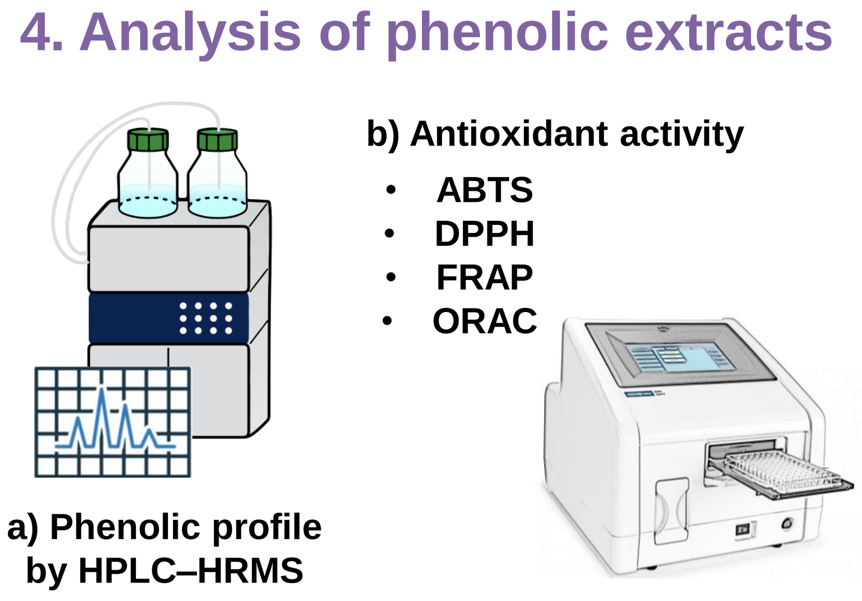


*UV-B 1: 4 h/1 day; UV-B 4: 4 h/4 days

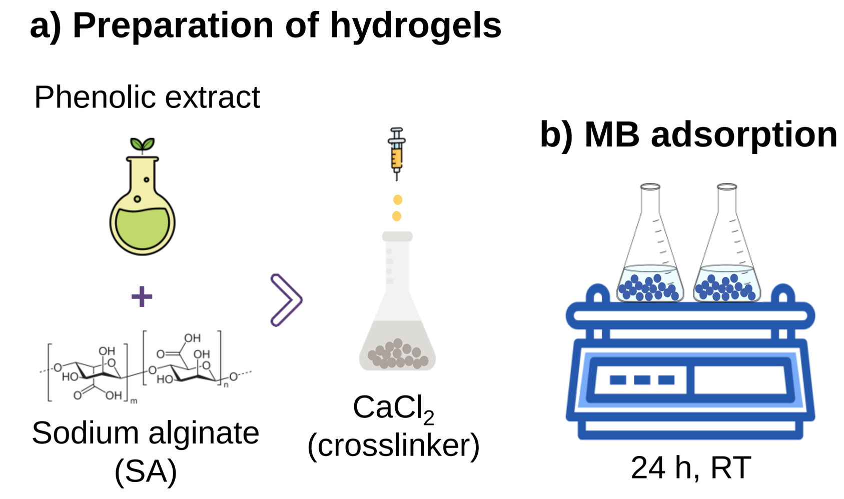
3. Phenolic extraction from ICs and MPs



4. Analysis of phenolic extracts



5. Phenolic-loaded hydrogels



RESULTS & DISCUSSION

HPLC-HRMS analysis of plant extracts revealed that rosmarinic acid (RA) is the most abundant compound in both ICs and MPs, which accounts 53% of the total phenolic compounds (Fig. 1). UV-B radiation significantly increased the production of this phenolic acid, particularly in ICs (+41% compared to the control) (Fig. 2), as well as its antioxidant activity (ABTS, DPPH, and FRAP) (Fig. 3).

Phenolic profile

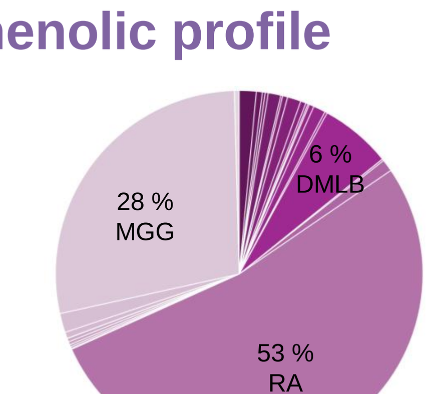


Figure 1. Phenolic profile analysis by HPLC-HRMS of green extracts from *T. lotocephalus* ICs and MPs (controls).
Notes: RA, rosmarinic acid; MGG, methyl *O*-galloyl-*D*-glucopyranoside; DMLB, dimethyl lithospermate B.

Rosmarinic acid (RA) content

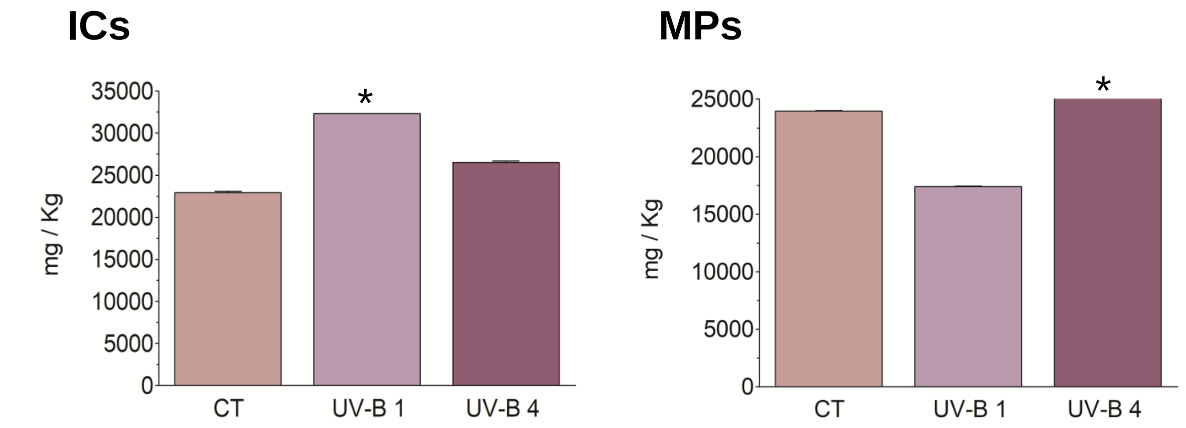


Figure 2. Effect of UV-B radiation on rosmarinic acid production in green extracts of *T. lotocephalus.*
Values are expressed as mean ± SE. For each micropropagated stage (ICs or MPs), the values followed by asterisk (*) are significantly higher than control ($p < 0.05$, Dunnett t-test).

Antioxidant activity

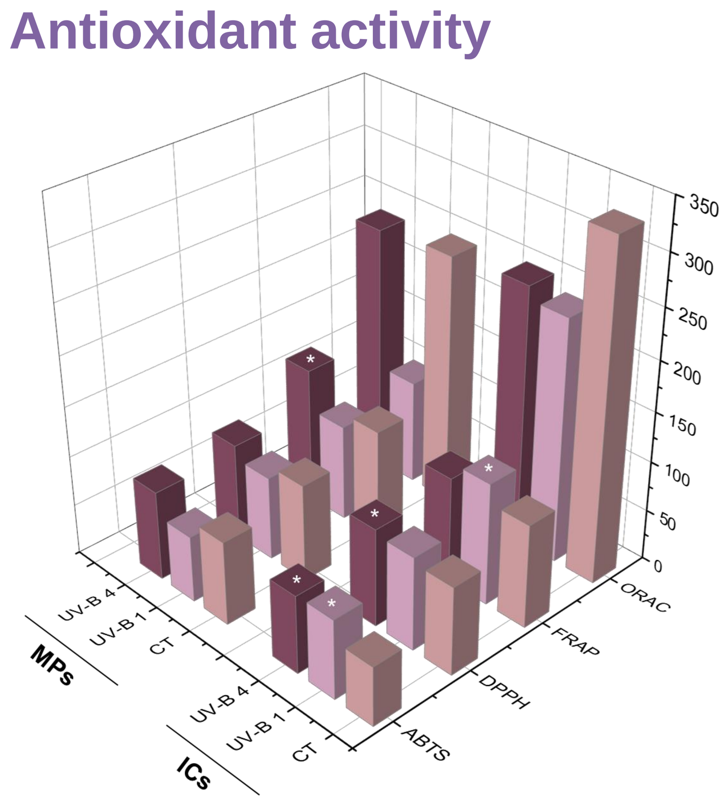


Figure 3. Effect of UV-B radiation on antioxidant activity evaluated by 2,2-azino-bis(3- ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1- picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) methods of green extracts of *T. lotocephalus*.
ABTS, DPPH and ORAC are expressed as milligrams of Trolox equivalents per gram of dry weight and FRAP as milligrams of ascorbic acid equivalents per gram of dry weight. Values are expressed as mean ± SE. For each micropropagated stage (ICs or MPs), the values followed by asterisk (*) are significantly higher than control ($p < 0.05$, Dunnett t-test).

All hydrogels demonstrated a significantly higher MB adsorption capacity (+52–60%) than SA, a natural copolymer known to be a highly efficient adsorbent for MB (Fig. 4).

The highest MB adsorption was observed in the hydrogels loaded with ICs extracts. Furthermore, the hydrogels made with the higher UV-B-exposed MP extracts demonstrated superior MB adsorption compared to the control.

Methylene blue adsorption

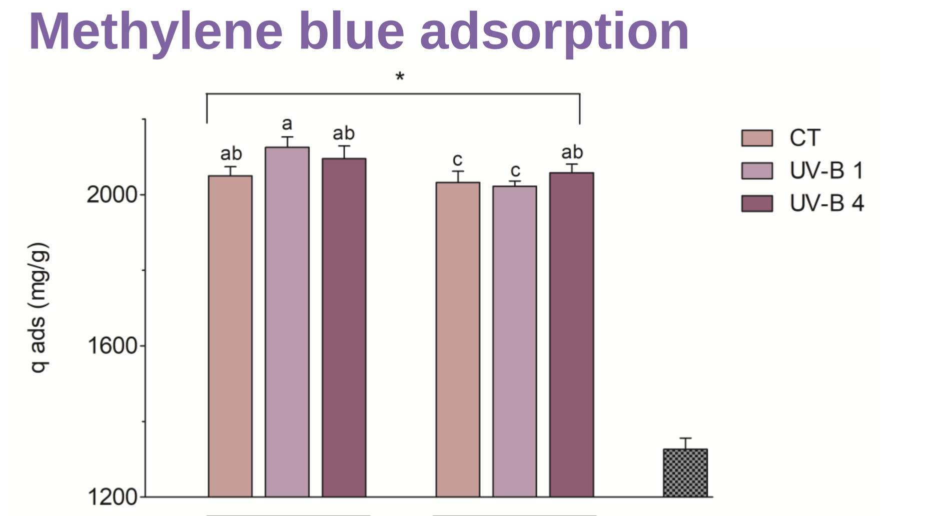


Figure 4. Quantity of methylene blue adsorbed (mg/g) by phenolic-loaded hydrogels from *T. lotocephalus* subjected to two distinct UV-B treatments (UV-B 1 and UV-B 4).
Values are expressed as mean ± SE. For each micropropagated stage (ICs or MPs), the results were analyzed using one-way analysis of variance (ANOVA) and the graph bars followed by different letters are significantly different ($p < 0.05$) (Duncan's New Multiple Range Test). (*) To compare isolated SA with each phenolic-loaded hydrogel, a Dunnett t-test was performed ($p < 0.05$).

In general, antioxidant activity, RA content and MB adsorption were positively correlated (Fig. 5). This suggests that the antioxidant properties of phenolics enhance the stability of the adsorption complex, maintaining the integrity of the adsorption sites and ensuring the hydrogel's long-term stability.

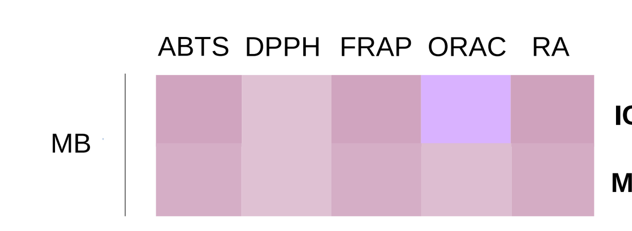


Figure 5. Pearson's correlation between antioxidant capacity assays/ RA and MB adsorption.

CONCLUSIONS

The results highlight the potential of a novel eco-friendly hydrogel rich in antioxidants as an effective adsorbent for the removal of MB, providing a sustainable approach to addressing wastewater pollution caused by synthetic dyes, thereby contributing to environmental remediation efforts.

References: [1] Mansinhos, Gonçalves & Romano, 2024, Front. Plant Sci. 15:1370810; [2] Mishra et al., 2021, J. Environ. Chem. Eng. 9, 104901; [3] Hassani et al., 2024, Polymers 16, 3055.

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