Effects of Drought Stress on the Chemical Composition and Bioactive Properties of *Cichorium spinosum* L.

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Abstract

Cichorium spinosum L. (spiny chicory), is one of the most well known wild edible plant (WEP) due to its valuable phytonutrient and macronutrient content. Spiny chicory leaves are harvested in nature for food and phytomedicinal purposes. In the search for sustainable agricultural systems, several studies have focused on deficit irrigation as an option for commercial cultivation of WEPs in marginal conditions. The present study aimed to chemically characterize, by chromatographic methods, the tocopherols, organic acids, and fatty acids content in the leaves of *C. spinosum* cultivated under three different irrigation levels, namely: drought stress (C1: 50% of field capacity (FC)), full irrigation (C2: 100% FC) and control (C3: rain-fed conditions). Moreover, the individual phenolic compounds profile and bioactive properties (antimicrobial, anti-inflammatory, cytotoxicity, and hepatotoxicity activities) were assessed in the hydroethanolic extracts.

The studied spiny chicory leaves presented only two tocopherol isoforms (α- and γ-), while quinic, oxalic, and succinic acids were the organic acids found in the highest concentrations. Regarding the phenolic profile, flavonoids and phenolic acids were found, mostly *O*-glycosylated derivatives of quercetin, luteolin, kaempferol, and caffeoylquinic acids, especially in C1 samples (water stress caused the accumulation of secondary metabolites). Gram-positive bacteria showed greater sensitivity to the C3 hydroethanolic extract; overall, the extracts did not show anti-inflammatory, hepatotoxic, and cytotoxic activities except for the AGS tumour cell line. These results emphasize the influence of water stress on the quality of plants, further underscoring the potential and significant added value of *C. spinosum* cultivated under marginal conditions.

Keywords: spiny chicory; wild edible plant; drought stress; chemical composition; antimicrobial.

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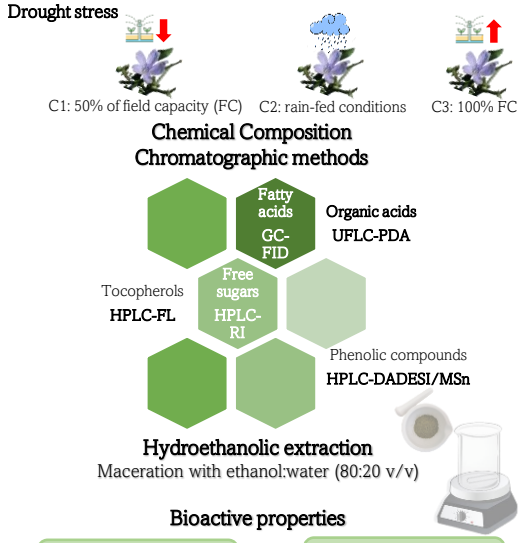
Introduction, Materials and Methods

Cichorium spinosum L. (spiny chicory), is one of the most well-known wild edible plant (WEP) due to its valuable phytonutrient and macronutrient content;

In the search for sustainable agricultural systems, several studies have focused on deficit irrigation as an option for commercial cultivation of WEPs in marginal conditions.



Antimicrobial activity

Colorimetric assay of *p*-iodonitrotetrazolium

Anti-inflammatory activity

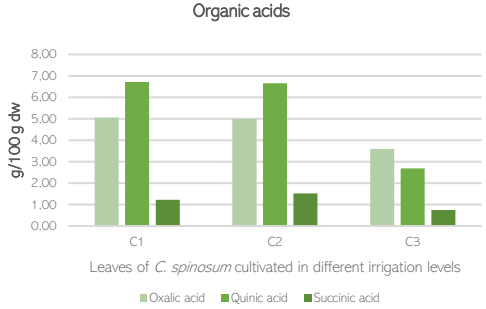
Producing nitric oxide formed in the mouse macrophage-like cell line RAW 264.7

Cytotoxicity and hepatotoxicity activities

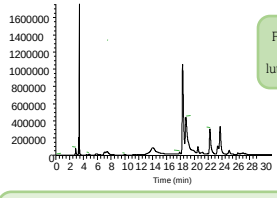
Human tumour cell lines tested: AGS, CaCo2, MCF7, NCI-H460
Non tumour cell line tested: porcine liver

Results

γ-tocopherol was detected in higher concentrations than α-tocopherol in the samples of *C. spinosum*. Therefore, the total tocopherol content was less than 0.5 g/100 g dry weight.



The sample C1 showed the highest concentration of phenolic compounds due to the accumulation of secondary metabolites



Flavonoids and phenolic acids were found: *O*-glycosylated derivatives of quercetin, luteolin, kaempferol, and caffeoylquinic acids

Gram-positive bacteria showed greater sensitivity to the C3 hydroethanolic extract. *Escherichia coli* (gram-negative bacteria) showed only sensitivity to the C1 and C2 hydroethanolic extract

No anti-inflammatory, hepatotoxicity and cytotoxicity (except for the AGS tumour cell line) effects were found.

Conclusions

These results emphasize the influence of water stress on the quality of plants, further underscoring the potential and significant added value of *C. spinosum* cultivated under marginal conditions. Additional research is required to establish the most effective cultivation methods that can enhance both yield and the quality of the plant.

Acknowledgements: The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021); to FCT for the financial support to the VALUEFARM project (PRIMA/0009/2019) - PRIMA Section 2 - Multitopic 2019; for the grant of B.H. Paschoalinotto and for the financial support within the scope of the Project PRIMA Section 2—Multi-topic 2019: VALUEFARM (PRIMA/0009/2019); L. Barros, M.I. Dias, R. Calhelha thank FCT, P.I., through institutional scientific employment program-contract for their contracts (CEEC Institutional). To MICINN for the Ramón y Cajal fellowship of M.A. Prieto (RYC-2017-22891) and for the Juan de la Cierva Formación contract for T.C.S.P.P. (FJC20120-045405-I); This work was also funded by the General Secretariat for Research and Technology of Greece (Prima 2019-11) and PRIMA foundation under the project Valuefarm (project number 1436).