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Yeast extract increases biomass, antioxidant, anti-inflammatory, and anticancer of *Cichorium intybus* L. hairy roots

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Abstract

Cichorium intybus L. hairy roots (HRs), induced from chicory plants by *Rhizobium rhizogenes*, have demonstrated potential antioxidant and antimicrobial activities. These chicory HRs exhibit bioactivities due to the presence of chemical compounds such as polyphenols and flavonoids. In the present work, yeast extract (YE) was evaluated as a biostimulant for HRs. After 25 days of culturing HRs in a medium containing YE at two different concentrations, the sample cultivated with 50 mg/L of YE showed higher biomass accumulation than the one treated with 100 mg/L. Specifically, 50 mg/L of YE increased HR biomass approximately 2.5 times compared to the control. Additionally, YE enhanced antioxidant enzyme activities (catalase and peroxidase) by increasing phenolic and flavonoid contents. The anti-inflammatory activity was assessed using the nitric oxide (NO) inhibition test, revealing that YE-elicited HRs suppressed NO synthesis at a non-toxic concentration of 5 µg/mL. Furthermore, YE-elicited HRs inhibited the proliferation of HepG2 cancer cells by approximately 20%. Overall, the present work demonstrated that YE effectively stimulated HR biomass growth while enhancing antioxidant, anti-inflammatory, and anticancer activities, providing a promising strategy for cultivating *C. intybus* L. hairy roots for therapeutic applications.

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Introduction

Cichorium intybus L., commonly known as chicory, is widely distributed across the globe, and belongs to family Asteraceae. For many years, chicory has been an important vegetable and a technologically advanced crop in temperate regions, particularly in Europe, Asia, and North America (Street *et al*., 2013). Numerous studies have reported that *C. intybus* L. exhibits a wide range of biological properties, including antibacterial, hepatoprotective, antidiabetic, gastroprotective, anti-inflammatory, antioxidant, tumour-inhibitory, and anti-allergic activities (Janda *et al*., 2021). These features are attributed to a variety of bioactive compounds synthesised by *C. intybus* L., such as sesquiterpene lactones, flavonoids, coumarins, and hydroxycinnamic acids, which are primarily concentrated in the root tissues of chicory (Imam *et al*., 2019).

Hairy roots (HRs), induced by infection with *Rhizobium rhizogenes*, serve as a valuable biotechnological tool for investigating fundamental biochemical and physiological processes in plants. HR cultures have been extensively applied in biotransformation, the production of high-value plant metabolites, and phytoremediation (Sevón and Oksman-Caldentey, 2002). The combination of *C. intybus* L. and HR culture offers an efficient system for research purposes due to HRs' rapid growth, stable genetics, and high metabolite production.

Chicory HRs are capable of synthesising polyphenol compounds such as caffeic acid and its derivatives, as well as flavonoids like quercetin and kaempferol glycosides, which have been proven to exhibit antioxidant, anti-inflammatory, and anticarcinogenic properties (Matvieieva *et al*., 2023). Moreover, studies suggest that chicory HR extracts can combat antibiotic-resistant bacteria, a major global health challenge (Häkkinen *et al.,* 2023).

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Notably, chicory HR supercritical hot water extracts have demonstrated suppression of both ampicillin-resistant *Pseudomonas aeruginosa* IBRS P001 and methicillin-resistant *Staphylococcus aureus*. Additionally, when tested against *Candida albicans*, chicory HR extracts exhibited superior antibiofilm activity compared to conventional sesquiterpene lactone compounds (Häkkinen *et al*., 2023).

Elicitors can enhance plant resilience under challenging environmental conditions by stimulating the biosynthesis of secondary metabolites (Zhao *et al*., 2005). Yeast extract (YE), a plant-derived biostimulant, is widely used to promote the accumulation of secondary metabolites in *in vitro* plant cultures due to its natural origin and cost-effectiveness (Laezza *et al*., 2024). For instance, YE has been successfully used to enhance the production of phenolic acids and tanshinones, as well as to improve the growth rate of *Salvia miltiorrhiza* HRs (Chen *et al*., 2001). Similarly, in *Orthosiphon aristatus* HRs, YE treatment led to increased root biomass, and enhanced production of secondary metabolites such as rosmarinic, caffeic, and vanillic acids (Smetanska *et al*., 2021).

The integration of YE treatment with *C. intybus* L. HR culture presents a promising approach to triggering secondary metabolite biosynthesis, which could be beneficial for medicinal applications and dietary supplements. However, this approach has not yet been investigated. The present work thus aimed to explore the effects of YE on the growth of chicory HRs. More importantly, the present work evaluated the antioxidant and anticancer activities of *C. intybus* L. HRs after 25 days of YE treatment. The successful implementation of this strategy could provide an innovative method for the large-scale production of bioactive compounds from chicory HRs for therapeutic applications.

Materials and methods

Chemical reagents

Chemicals used in the present work included Murashige and Skoog medium (MS) (Netherlands), yeast extract, Follin-Ciocalteu (Merck, Germany), guaiacol, $NaNO_2$, $AlCl_3$, Na_2CO_3 (Xilong, China), H_2O_2 (Vietnam), gallic acid (Sigma-Aldrich, USA), Dulbecco's modified Eagle's medium (DMEM), streptomycin, and penicillin (Gibco, Invitrogen, USA).

Methods

Cultivation of hairy roots

HRs of *C. intybus* L. were obtained from the BIOPI laboratory in Amiens, France. The roots were cultured in bottles containing 100 mL of half-strength Murashige and Skoog (½ MS) medium, supplemented with 30 g of sucrose (Tabatabaee *et al.,* 2021). This medium had a pH of around 5.7 - 5.8. Each bottle contained 0.33 g of roots, and was shaken continuously at 110 rpm and 26°C in a shaker machine without light. The control bottle contained root and ½ MS medium. In the treatment groups, YE was directly incorporated into the ½ MS medium before autoclaving.

Growth kinetic of hairy roots

The HRs were cultured in ½ liquid MS medium. Each bottle had 0.33 g of roots. Fresh weight measurements were conducted at 5-d intervals by carefully removing the roots from the medium. The control contained only HRs in the culture medium, while treatment groups were supplemented with different concentrations of YE. The steps were repeated over a 25-d-cultivation period to build the growth curve of *C. intybus* L. HRs. The growth rate of HRs was calculated using Eq. 1 according to Gajurel *et al*. (2022):

$$\mu = \frac{\ln\left(\frac{DWi}{DW0}\right)}{\Delta t} \quad \text{(Eq. 1)}$$

where, DWi (g) = average dry weight of the HRs at the end of the exponential growth, DW0 (g) = average dry weight of HRs at the start of the exponential growth, and t = interval of time (d) between 0 and i.

Peroxidase and catalase activities

The peroxidase (POD) and catalase (CAT) activity test was adapted from Chen and Zhang (2016) with some modifications. Their activities to detoxify H_2O_2 were performed in reaction containing 0.2% guaiacol, 100 mM PBS (pH 7.0), and 30% H_2O_2 for POD test or only 30% H_2O_2 and 100 mM PBS (pH 7.0) for the CAT test. The wavelength was set to 470 and 240 nm to measure the product produced in POD and CAT tests, respectively, using a UV-1800 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan).

Extraction of hairy roots

The harvested HRs were thoroughly washed

with distilled water to remove any residual impurities, and subsequently dried in the oven for 7 d at 60°C to reach the constant weight. Once dried, the HRs were ground into fine powder using a tissue lyser machine. The samples were extracted following Riyadi *et al.* (2023) with some modifications. Samples were immersed in absolute methanol (1:10, w/v), and sonicated at 40°C for 1 h for better extraction. Following sonication, the samples were centrifuged for 6 min to separate the solid residues from the liquid phase. The resulting supernatant, containing the crude extract, was then evaporated in an oven at 60°C to remove the methanol, and concentrate the bioactive compounds. Finally, the dried HR extracts were dissolved in methanol to achieve the final concentration of 40 mg/mL, and stored at 4°C for further analysis.

Estimation of total phenolic content

The TPC was determined using spectrophotometry through Folin-Ciocalteu's assay with gallic acid as the reference standard (Phuyal *et al.,* 2020). The HR extracts were diluted at a concentration ranging from 0.4 to 4 mg/mL. Subsequently, 40 µL of the diluted solution was mixed with 480 µL of Folin-Ciocalteu reagent (diluted with Milli Q water at a ratio of 1:10) in an Eppendorf tube, and incubated for 1 min, followed by adding 480 µL of Na_2CO_3 6%. The mixture was then thoroughly mixed and incubated at 40°C for 15 min. The absorbance was measured at 765 nm using SpectraMax iD3 Multi-Mode Microplate Readers. The TPC content was determined based on the gallic acid standard curve.

Estimation of total flavonoid content

The TFC was determined according to Le *et al.* (2022). In a reaction, 40 µL of $NaNO_2$ 10% and 40 µL of $AlCl_3$ 10% were added to 240 µL of HR extract. This mixture was thoroughly vortexed for 6 min. Subsequently, 400 µL of NaOH and 280 µL of ethanol 30% were added to the mixture, and incubated for 20 min at room temperature. The absorbance was measured at 510 nm using SpectraMax iD3 Multi-Mode Microplate Readers. The TFC content was determined based on the quercetin standard curve.

Cell culture

The RAW 264.7 murine macrophage cell line, sourced from the American Type Culture Collection (ATCC, Manassas, VA, USA), was used. Cells were cultured at 37°C in a 5% CO_2 environment in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Invitrogen, USA) supplemented with 10% FBS, 100 g/L streptomycin, and 100 IU/mL penicillin (Gibco, Invitrogen, USA) (Joo *et al.,* 2014).

Cell viability

The viability of RAW 264.7 cells was assessed based on the measurement of cell respiration by a mitochondria-dependent process of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) degradation to formazan (Wang *et al.,* 2020). Cells were seeded at a density of 5×10^4 cells per well in 96-well plates, and allowed to adhere for 24 h, or 48 h in the case of HepG2 cancer cells. Subsequently, cells were incubated with increasing concentrations of HR extracts (5, 10, and 20 µL/mg) for 24 h. Then, the culture medium was removed, and MTT solution was added to each well at a concentration of 0.5 mg/mL, and incubated further for 4 h at 5% CO_2 at 37°C. The resulting blue-violet formazan crystals were dissolved with DMSO (Laezza *et al.,* 2024). Absorbance was measured at 570 nm using SpectraMax iD5 Multi-Mode Microplate Readers (SpectraMax iD5, Molecular Devices, USA).

Estimation of nitric oxide level

To evaluate the anti-inflammation activity of the HR extract, the cells were seeded in 96-well plates at a density of 5×10^4 cells/well, and incubated in a humidified incubator at 37°C, 5% CO_2 for 24 h. After 24 h, cells were treated with different concentrations of HR extracts for 1 h, then cells were stimulated by 1 µg/mL lipopolysaccharide (LPS) (*Escherichia coli* 0111: B4; Sigma Aldrich, USA) for 24 h. Subsequently, Griess reagent was added to the 96 wells in an equal volume to 100 µL of the cell culture medium. The mixture was incubated at room temperature for 10 min. The absorbance was then read at 540 nm using a microplate reader (SpectraMax iD5, Molecular Devices, USA). Based on the standard curve of $NaNO_2$, the quantity of NO in the medium was determined (Joo *et al.,* 2014). The NO production by LPS can be considered 100%, whereas the NO inhibition by LPS was considered 0%, which served as the negative control. Cardamonin, a compound previously demonstrated to exhibit anti-inflammatory properties (Takahashi *et al.,* 2011), was used as a positive control to assess the

inhibitory effect of HR extract on LPS-induced inflammation in RAW 264.7 cells.

Statistical analysis

All the experiments were repeated three times. Data were expressed as mean ± standard deviation. The normality of the data was checked by using the Shapiro-Wilk Test. The data were statistically analysed using the One-way ANOVA followed by the Tukey's *post hoc* test ($p < 0.05$), performed using GraphPad Prism version 8.0.

Results

Growth kinetic

HRs were cultured in ½ MS medium supplemented with YE at 50 and 100 mg/L, while control HRs were maintained in ½ MS medium without YE. After 25 d, fresh HR biomass was measured (Figure 1). YE treatment significantly enhanced HR biomass, with 50 mg/L YE yielding the highest biomass accumulation. The fresh weight of HRs increased from 0.33 g to approximately 8.32 g in this condition. In contrast, HRs cultured with 100 mg/L YE or in the control medium exhibited lower biomass accumulation. The doubling time of HRs treated with 50 mg/L YE was 3.5 d, compared to 7 d in HRs treated with 100 mg/L YE, and 9.8 d in control HRs. The corresponding growth rates were 0.197, 0.099, and 0.07, respectively.

Bioactivities of catalase and peroxidase antioxidant enzymes

YE-treated HRs exhibited significantly higher catalase and peroxidase activities compared to the control ($p < 0.05$) (Figure 2). Peroxidase activity was higher than catalase activity in all conditions. HRs treated with 50 mg/L YE exhibited the highest catalase activity (44.71 units/min/mg protein), whereas HRs treated with 100 mg/L YE exhibited the highest peroxidase activity (113.58 units/min/mg protein).

Total phenolic and flavonoid contents

The TPC was quantified to determine the concentration of gallic acid equivalents produced by HRs grown in different culture media using the Folin-Ciocalteu reagent, while the TFC was assessed to determine the concentration of quercetin equivalents. The highest TPC (46.08 ± 2.19 mg gallic acid equivalent/g) and TFC (22.62 ± 3.4 mg quercetin equivalent/g) were observed in HRs treated with 100 mg/L YE, whereas control HRs and HRs treated with 50 mg/L YE exhibited lower phenolic and flavonoid contents (Figure 3).

Cytotoxicity of HR extracts on RAW 264.7 cells

HRs treated with 100 mg/L YE were selected for further evaluation due to their superior antioxidant enzyme activity, TPC, and TFC accumulation. RAW 264.7 cells were treated with HR extracts at 5, 10, and 20 µg/mL for 24 h, and cell viability was assessed *via* MTT assay (Figure 4). The highest viability (85.5 ± 0.89%) was observed in cells treated with 5 µg/mL HR extract, while viability decreased to 66.84 ± 4.9% and 61.12 ± 1.11% at 10 and 20 µg/mL, respectively. Control HR extracts yielded higher viability than YE-treated HR extracts. All treatment conditions showed significantly lower cell viability than the untreated negative control ($p < 0.05$).

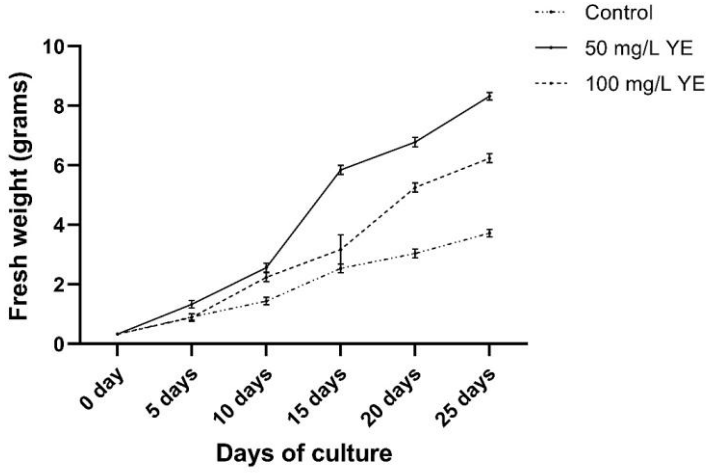

Figure 1. Growth curve of *Cichorium intybus* L. hairy roots grown in treatment conditions (½ MS supplemented with yeast extract at 50 and 100 mg/L) and in control condition (with only ½ MS) for 25 days. Data are mean of three replicates with error bars showing standard deviation.

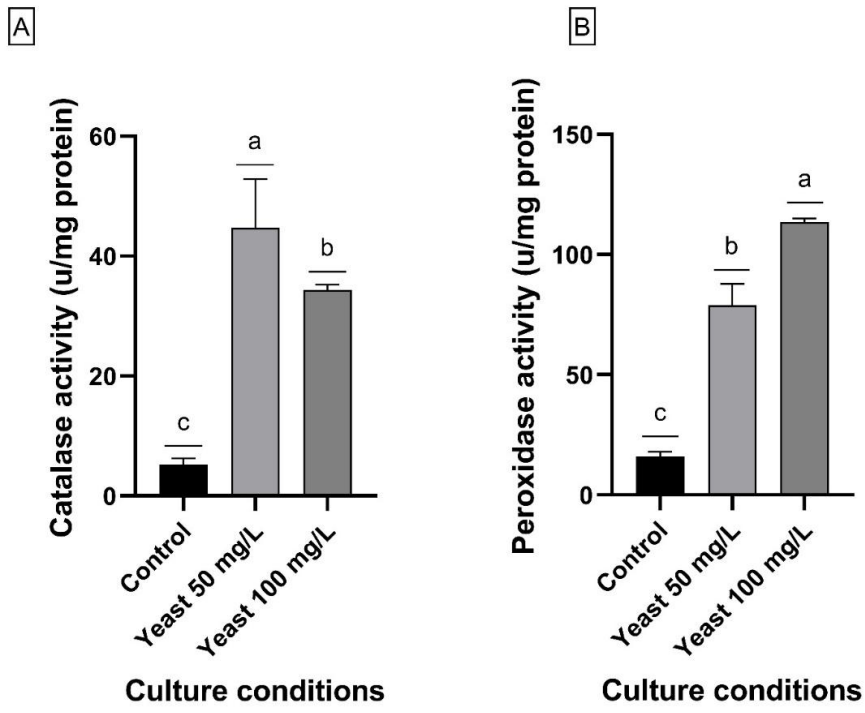

Figure 2. Catalase **(A)** and peroxidase **(B)** activity of *Cichorium intybus* L. hairy roots grown in treatment conditions (½ MS supplemented with yeast extract at 50 and 100 mg/L) and in control condition (with only ½ MS). Data are mean of three replicates with error bars showing standard deviation. Different lowercase letters indicate significant differences ($p < 0.05$).

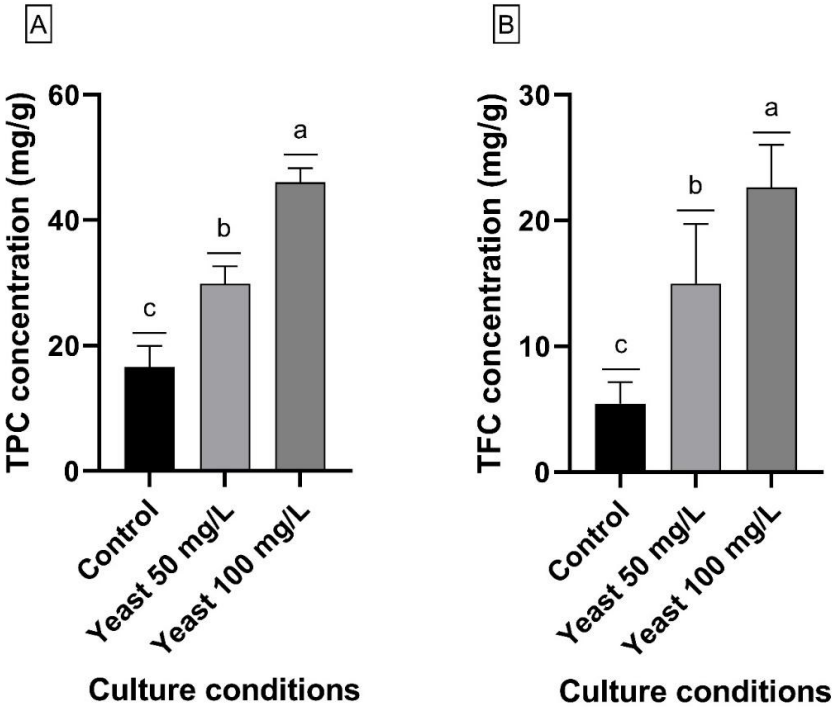

Figure 3. Total phenolic **(A)** and total flavonoid **(B)** contents of *Cichorium intybus* L. hairy roots grown in treatment conditions (½ MS supplemented with yeast extract at 50 and 100 mg/L) and in control condition (with only ½ MS). Data are mean of three replicates with error bars showing standard deviation. Different lowercase letters indicate significant differences ($p < 0.05$).

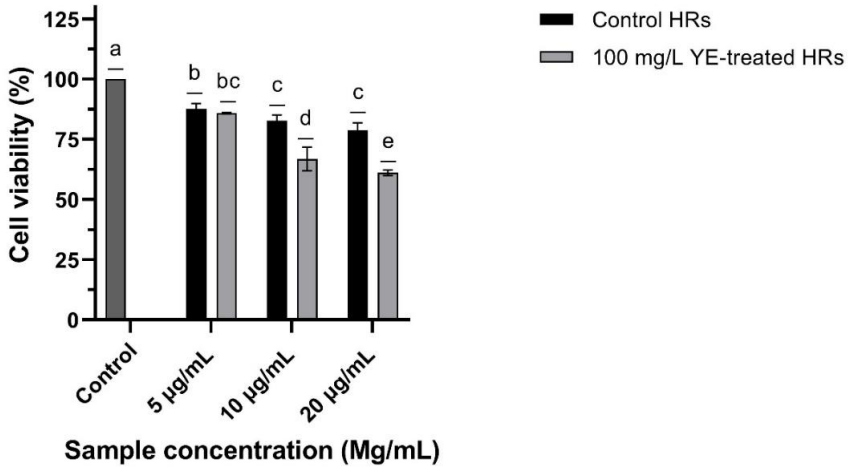

Figure 4. Cell viability percentage of RAW 264.7 cells treated with *Cichorium intybus* L. extracts. Data are mean of three replicates with error bars showing standard deviation. Different lowercase letters indicate significant differences ($p < 0.05$).

Nitric oxide inhibition in LPS-stimulated RAW 264.7 cells

Since 5 µg/mL HR extract exhibited minimal cytotoxicity, this concentration was used to evaluate NO inhibition in LPS-stimulated RAW 264.7 cells. The highest inhibitory activity on NO synthesis was demonstrated by 100 mg/L YE-elicited HR extract, with an inhibition percentage of approximately 15.73 ± 0.5%. In contrast, the control HRs exhibited an NO inhibition of 5.5 ± 0.35%. However, its NO inhibition effect was lower than the positive control, cardamonin (50% inhibition).

Inhibition of HepG2 cancer cell viability

The anticancer potential of YE-treated HR extracts was assessed in HepG2 cells (Figure 5). At 5 µg/mL, HR extracts from control and 100 mg/L YE-treated HRs reduced cell viability to 85.76 ± 0.61% and 81.17 ± 0.98%, respectively, which was significantly lower than the negative control ($p < 0.05$). YE-treated HR extracts exhibited stronger cancer cell inhibition than control HRs, indicating their potential for cancer therapy.

Discussion

The present work aimed to investigate the efficacy of YE in enhancing the growth rate as well as the antioxidant and anticancer activities of chicory HRs.

In the growth curve of chicory HRs, the lag phase was observed from day 0 to day 10. Following this period, biomass accumulation increased significantly, with a steeper curve from day 10 to day 25, representing the log phase of HR proliferation. The growth of HRs was predicted to enter the

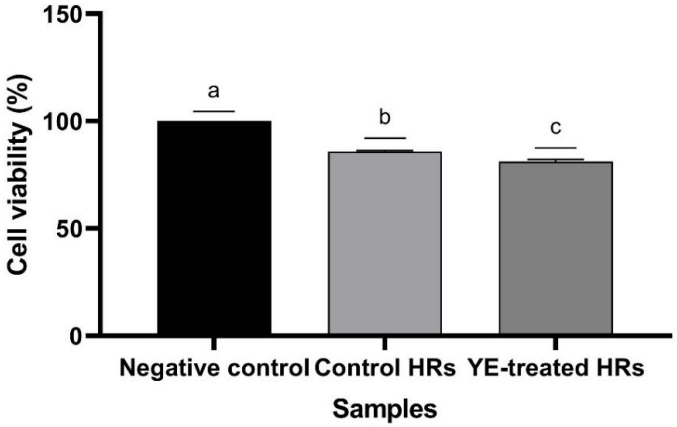

Figure 5. Viability percentage of HepG2 cells treated with *Cichorium intybus* L. extracts. Data are mean of three replicates with error bars showing standard deviation. Different lowercase letters indicate significant differences ($p < 0.05$).

stationary phase after 25 days (Häkkinen *et al*., 2023). This 25-day milestone is critical because it coincides with the peak synthesis of bioactive compounds during the log growth phase. Therefore, 25 days was considered the optimal harvesting time for biomass. The supplementation of YE in the HR culture medium significantly increased biomass production. Notably, HR biomass elicited by 50 mg/L YE was significantly higher than that of 100 mg/L YE ($p < 0.05$). A similar trend was observed in a previous study where YE enhanced the root biomass of *Orthosiphon aristatus* (Smetanska *et al*., 2021). Furthermore, the doubling time results indicated that HRs grown in a medium containing 50 mg/L YE exhibited the fastest growth, followed by those in 100 mg/L YE and the control medium. The doubling time of HRs varies by plant species and culture conditions. For instance, carrot HRs were reported to have a doubling time of 6.5 days in MS medium (Mai *et al*., 2022).

POD and CAT are key enzymes in plant antioxidant defence mechanisms (Dvořák *et al*., 2021). The POD activity in YE-elicited HRs exhibited significant increases compared to the control. Specifically, HRs treated with 100 mg/L YE showed an eight-fold increase in POD activity relative to the control, while those treated with 50 mg/L YE exhibited a five-fold increase. Similarly, CAT activity in YE-elicited HRs was significantly higher than in the control. The observed increase in CAT and POD activities may be attributed to the stress-inducing effect of YE, which triggers the production of reactive oxygen species (ROS). Excessive ROS accumulation can lead to oxidative damage in lipids, nucleic acids, and proteins, ultimately resulting in cell death (Lehmann *et al*., 2015). The upregulation of CAT and POD activities suggests an adaptive response to scavenge ROS by catalysing the decomposition of H_2O_2 to H_2O and O_2, thereby enhancing detoxification and maintaining redox homeostasis in plants (Dvořák *et al*., 2021).

YE treatment also stimulated TPC and TFC production in a concentration-dependent manner, with higher YE concentrations leading to increased accumulation of these compounds in chicory HRs. A similar trend was reported in *Aster scaber* HRs, where optimal YE concentration enhanced TPC and TFC production (Ghimire *et al*., 2019). Our findings agreed with this observation, as 100 mg/L YE-elicited HRs exhibited higher TPC and TFC production compared to 50 mg/L YE-elicited HRs. Furthermore, when YE was combined with another biotic elicitor, jasmonic acid, *Orthosiphon aristatus* HRs exhibited increased synthesis of specific phenolic compounds such as rosmarinic, vanillic, and chlorogenic acids (Smetanska *et al*., 2021). The induction of TPC and TFC in YE-elicited HRs may be attributed to the plant's defence response to YE, a known biostimulant.

To evaluate the anti-inflammatory and anticancer potential of chicory HRs, a cell viability assay was conducted. Since 5 µg/mL of extract exhibited minimal cytotoxicity, this concentration was chosen for further studies. NO is a signalling molecule generated during immune and inflammatory responses (Coleman, 2001). In the present work, all tested HR extracts exhibited anti-inflammatory activity, with the highest NO inhibition (approximately 15%) observed in YE-elicited HRs. Higher extract concentrations (> 5 µg/mL) demonstrated greater NO inhibition; however, they also caused cytotoxic effects, making them unsuitable for further investigation. The enhancement of secondary metabolite production by elicitors can contribute to anti-inflammatory activity (Lee *et al*., 2013; Shah *et al*., 2021). The obtained results suggested that the observed NO inhibition was not due to cytotoxic effects but rather a direct NO-inhibitory property of the tested extracts (Rao *et al*., 2007). The increased TPC and TFC levels were likely responsible for the NO inhibition, as demonstrated in previous studies on *Scutellaria radix* (Chi *et al*., 2001), citrus (Lee *et al*., 2020), and *Malva sylvestris* (Irfan *et al*., 2021). The anti-inflammatory properties of chicory roots have been documented in conditions such as pyorrhoea and gingival inflammation (Papetti *et al*., 2013), TNF-α-mediated cyclooxygenase induction (Cavin *et al.,* 2005), and cytokine inhibition (Rizvi *et al*., 2014). While various elicitors, including iron and zinc oxide nanoparticles (Mohebodini *et al*., 2017) and methyl jasmonate (Bernard *et al*., 2020), have been used to enhance chicory HR biomass and secondary metabolite production, the application of YE as an elicitor for these purposes has not been previously explored. The obtained results would thus provide novel insights, and contribute significantly to this field.

Additionally, the anticancer potential of HR extracts was assessed using HepG2 cell viability assays. At 5 µg/mL, both control and YE-elicited HR

extracts exhibited cytotoxic effects, reducing cell viability by 15 - 20%. Notably, the YE-elicited extract demonstrated a greater inhibitory effect on HepG2 cell viability, reducing it by 18.74 ± 1.33% compared to the control extract. The anticancer properties of different chicory plant parts have been linked to their bioactive metabolite content (Imam *et al*., 2019). The present work, for the first time, employed YE to enhance the anticancer activity of chicory HRs, further highlighting the potential of YE as an effective elicitor for improving bioactive compound production in plant cell cultures.

Conclusion

The present work evaluated the potential of YE as a biostimulant to enhance the antioxidant, anti-inflammatory, and anticancer properties of *Cichorium intybus* L. HRs. The findings demonstrated that YE significantly promoted HR biomass accumulation. Enzymatic activity assays revealed that YE elicitation increased catalase and peroxidase activities, strengthening the antioxidant defence system. Furthermore, YE-elicited HR extracts exhibited notable anti-inflammatory and anticancer effects by suppressing NO production, and inhibiting HepG2 cancer cell growth. These results highlighted the potential of YE-elicited *Cichorium intybus* L. HRs as a promising source of bioactive compounds for therapeutic applications.

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References

Bernard, G., Dos, H. A. S., Etienne, A., Samaillie J., Neut, C., Sahpaz, S., … and Rambaud, C. 2020. MeJA elicitation of chicory hairy roots promotes efficient increase of 3,5-diCQA accumulation, a potent antioxidant and antibacterial molecule. Antibiotics 9(10): 659.

Cavin, C., Delannoy, M., Malnoe, A., Debefve, E., Touché, A., Courtois, D. and Schilter, B. 2005. Inhibition of the expression and activity of cyclooxygenase-2 by chicory extract. Biochemical and biophysical research communications 327: 742-749.

Chen, H., Chena, F., Chiu, F. and Lo, C. 2001. The effect of yeast elicitor on the growth and secondary metabolism of hairy root cultures of *Salvia miltiorrhiza*. Enzyme and Microbial Technology 28: 100-105.

Chen, T. and Zhang, B. 2016. Measurements of proline and malondialdehyde contents and antioxidant enzyme activities in leaves of drought stressed cotton. Bio-Protocol 6: e1913.

Chi, Y. S., Cheon, B. S. and Kim, H. P. 2001. Effect of wogonin, a plant flavone from *Scutellaria radix*, on the suppression of cyclooxygenase-2 and the induction of inducible nitric oxide synthase in lipopolysaccharide-treated RAW 264.7 cells. Biochemical Pharmacology 61: 1195-1203.

Coleman, J. W. 2001. Nitric oxide in immunity and inflammation. International Immunopharmacology 1: 1397-1406.

Dvořák, P., Krasylenko, Y., Zeiner, A., Šamaj, J. and Takáč, T. 2021. Signaling toward reactive oxygen species-scavenging enzymes in plants. Frontiers in Plant Science 11: 618835.

Gajurel, G., Nopo-Olazabal, L., Hendrix, E. and Medina-Bolivar, F. 2022. Production and secretion of isowighteone in hairy root cultures of pigeon pea (*Cajanus cajan*) co-treated with multiple elicitors. Plants 11: 834.

Ghimire, B. K., Thiruvengadam, M. and Chung, I. M. 2019. Identification of elicitors enhances the polyphenolic compounds and pharmacological potential in hairy root cultures of *Aster scaber*. South African Journal of Botany 125: 92-101.

Häkkinen, S. T., Cankar, K., Nohynek, L., van Arkel, J., Laurel, M., Oksman-Caldentey, K. M. and Van Droogenbroeck, B. 2023. *Cichorium intybus* L. hairy roots as a platform for antimicrobial activity. Pharmaceuticals 16(2): 140.

Imam, K. M. S. U., Xie, Y., Liu, Y., Wang, F. and Xin, F. 2019. Cytotoxicity of *Cichorium intybus* L. metabolites. Oncology Report 42: 2196-2212.

Irfan, A., Imran, M., Khalid, M., Ullah, M. S., Khalid, N., Assiri, M. A., … and Shahzad, M. 2021. Phenolic and flavonoid contents in *Malva sylvestris* and exploration of active drugs as antioxidant and anti-COVID19 by quantum chemical and molecular docking studies. Journal of Saudi Chemical Society 25: 101277.

Janda, K., Gutowska, I., Geszke-Moritz, M. and Jakubczyk, K. 2021. The common cichory (*Cichorium intybus* L.) as a source of extracts with health-promoting properties - A review. Molecules 26(6): 1814.

Joo, T., Sowndhararajan, K., Hong, S., Lee, J., Park, S. Y., Kim, S. and Jhoo, J. W. 2014. Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 cells by stem bark of *Ulmus pumila* L. Saudi Journal of Biology Science 21: 427-435.

Laezza, C., Imbimbo, P., D'Amelia, V., Marzocchi, A., Monti, D. M., Loria, A. … and Rigano, M. M. 2024. Use of yeast extract to elicit a pulp-derived callus cultures from Annurca apple and potentiate its biological activity. Journal of Functional Foods 112: 105988.

Le, T. V. A., Nga, T. P. M., Nguyen, P. N. and Nguyen, T. K. O. 2022. Genotypic and phenotypic diversity of endemic golden camellias collected from the North of Vietnam. Chemistry and Biodiversity 19: e2202200843.

Lee, D., Park, J., Choi, J., Jang, H. and Seol, J. 2020. Anti-inflammatory effects of natural flavonoid diosmetin in IL-4 and LPS-induced macrophage activation and atopic dermatitis model. International Immunopharmacology 89: 107046.

Lee, Y. S., Ju, H. K., Kim, Y. J., Lim, T. G., Uddin, M. R., Kim, Y. B., … and Yang, T. J. 2013. Enhancement of anti-inflammatory activity of aloe vera adventitious root extracts through the alteration of primary and secondary metabolites *via* salicylic acid elicitation. PLoS One 8: e82479.

Lehmann, S., Serrano, M., L'Haridon, F., Tjamos, S. E. and Metraux, J. P. 2015. Reactive oxygen species and plant resistance to fungal pathogens. Phytochemistry 112: 54-62.

Mai, N. T. P., Le, T. V. A., Nguyen, B. C., Le, N. H. T. and Do, Q. M. 2022. Carrot hairy roots (*Daucus carota* L.) characterisation and optimisation for high β-carotene extraction. Indonesian Journal of Biotechnology 27: 171-178.

Matvieieva, N., Bessarabov, V., Khainakova, O., Duplij, V., Bohdanovych, T., Ratushnyak, Y., … and Kobylinska, N. 2023. *Cichorium intybus* L. "hairy" roots as a rich source of antioxidants and anti-inflammatory compounds. Heliyon 9: e14516.

Mohebodini, M., Fathi, R. and Mehri, N. 2017. Optimization of hairy root induction in chicory (*Cichorium intybus* L.) and effects of nanoparticles on secondary metabolites accumulation. Iranian Journal of Genetics and Plant breeding 6(2): 60-68.

Papetti, A., Mascherpa, D., Carazzone, C., Stauder, M., Spratt, D. A., Wilson, M., … and Gazzani, G. 2013. Identification of organic acids in *Cichorium intybus* inhibiting virulence-related properties of oral pathogenic bacteria. Food Chemistry 138: 1706-1712.

Phuyal, N., Jha, P. K., Raturi, P. P. and Rajbhandary, S. 2020. Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. Scientific World Journal 2020: 8780704.

Rao, Y. K., Fang, S. H. and Tzeng, Y. M. 2007. Evaluation of the anti-inflammatory and anti-proliferation tumoral cells activities of *Antrodia camphorata*, *Cordyceps sinensis*, and *Cinnamomum osmophloeum* bark extracts. Journal of Ethnopharmacology 114: 78-85.

Riyadi, P. H., Susanto, E., Anggo, A. D., Arifin, M. H. and Rizki, L. 2023. Effect of methanol solvent concentration on the extraction of bioactive compounds using ultrasonic-assisted extraction (UAE) from *Spirulina platensis*. Food Research 7: 59-66.

Rizvi, W., Fayazuddin, M., Shariq, S., Singh, O., Moin, S., Akhtar, K. and Kumar, A. 2014. Anti-inflammatory activity of roots of *Cichorium intybus* due to its inhibitory effect on various cytokines and antioxidant activity. Ancient Science of Life 34: 44-49.

Sevón, N. and Oksman-Caldentey, K. M. 2002. Agrobacterium rhizogenes-mediated transformation: Root cultures as a source of alkaloids. Planta Medica 68(10): 859-868.

Shah, M., Jan, H., Drouet, S., Tungmunnithum, D., Shirazi, J. H., Hano, C. and Abbasi, B. H. 2021. Chitosan elicitation impacts flavonolignan

biosynthesis in *Silybum marianum* (L.) Gaertn. cell suspension and enhances antioxidant and anti-inflammatory activities of cell extracts. Molecules 26: 791.

Smetanska, I., Oksana Tonkha, T. P., Hunaefi, D., Mamdouh, D., Patyka, M., Bukin, A., … and Omelian, A. 2021. The influence of yeast extract and jasmonic acid on phenolic acids content of *in vitro* hairy root cultures of *Orthosiphon aristatus*. Slovak Journal Food Science 15: 1-8.

Street, R. A., Sidana, J. and Prinsloo, G. 2013. *Cichorium intybus*: Traditional uses, phytochemistry, pharmacology, and toxicology. Evidence-Based Complementary and Alternative Medicine 2013: 579319.

Tabatabaee, S., Sanjarian, F., Lohrasebi, T. and Karimi, M. 2021. Enhanced inulin production by hairy root cultures of *Cichorium intybus* in response to Pi and Fe starvation. Molecular Biology Research Communications 10(2): 85-91.

Takahashi, A., Yamamoto, N. and Murakami, A. 2011. Cardamonin suppresses nitric oxide production *via* blocking the IFN-γ/STAT pathway in endotoxin-challenged peritoneal macrophages of ICR mice. Life Science 89: 337-342.

Wang, Z., Guan, Y., Yang, R., Li J., Wang, J. and Jia, A.Q. 2020. Anti-inflammatory activity of 3-cinnamoyltribuloside and its metabolomic analysis in LPS-activated RAW 264.7 cells. BMC Complementary Medicine and Therapies 20: 1-13.

Zhao, J., Davis, L. C. and Verpoorte, R. 2005. Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnology Advance 23: 283-333.