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Research Paper

Perspectives on the cultivation in vertical farming of the Sardinian endemics *Hypericum scruglii* Bacch., Brullo & Salmeri

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ABSTRACT

Vertical farming is intended as the indoor cultivation of plants in vertically stacked layers involving the use of artificial lighting, climate control systems, and sensors. *Hypericum scruglii* Bacch., Brullo & Salmeri is a Sardinian exclusive endemism with promising biological properties in the medicinal and cosmetic fields. The use of the plant however at the date is unfeasible due its rarity and conservation status. In order to scaffold a sustainable use of the species, the present work aims at investigating the effects of vertical farming on *H. scruglii* in terms of growth and maintenance of its chemical features and biological properties. To this end *i*) *H. scruglii* plants were cultivated in vertical farming for six months and subjected to controlled temperature, lighting, irrigation and relative humidity; *ii*) two extracts, from plants cultivated in vertical farming and sampled from natural populations were obtained; *iii*) extracts were characterized and compared in terms of total phenolic and flavonoid content, ^{1}H NMR and HPLC-FLD profiling, antidiabetic and antioxidant properties and cytotoxicity. Our results showed that *H. scruglii* cultivated in vertical farming retains its phytochemical profile and key metabolites while exhibiting strong α-glucosidase inhibition, antioxidant activity (evaluated both via ABTS test and MTT assay on Caco-2 cells), and no cytotoxic effects. The present study demonstrates for the first time the feasibility of the cultivation in vertical farming of *H. scruglii*, supporting the potential of vertical farming to enable the sustainable use and the conservation of rare yet valuable medicinal plants.

1. Introduction

Vertical Farming, intended as the cultivation of plants in vertical farms, is a Controlled Environment Agriculture (CEA) practice which implies cultivating in vertically stacked layers within a closed and strictly controlled environment. In vertical farms, light, water and nutrients, heat, and in some cases CO_2 are supplied artificially, optimizing crop production and decoupling it from external environmental conditions (SharathKumar et al., 2020). Vertical farming has been proposed as a viable solution to achieve food safety and security in the near future (Avgoustaki and Xydis, 2020; Oh and Lu, 2023) in response to projected increases in global crop demand of up to 70 % (Avgoustaki and Xydis, 2020; Hunter et al., 2017). Vertical farming in fact, with respect to traditional open-field techniques, could optimize the consumption of soil and other non-renewable resources and reduce the use of pesticides and phytochemicals while ensuring the year-round production of standardized high-quality crops (Avgoustaki and Xydis, 2020; Kaiser et al., 2024; SharathKumar et al., 2020). By contrast, vertical farming energy requirements raise doubts about the extent to which this cultivation method is fully sustainable and profitable with respect to traditional open-field cultivation, passive greenhouses or more high-tech systems, such as greenhouses supplemented with artificial lighting or equipped with computer-assisted regulation of key environmental parameters (Avgoustaki and Xydis, 2020; Kaiser et al., 2024; Stanghellini and Katzin, 2024). Even so, while vertical farming of staple crops is to consider economically unfeasible, this cultivation method is worthy of further investigation if directed to niche crops (Stanghellini and Katzin, 2024), in particular the cultivation of medicinal plants is generating increasing

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interest (Bafort et al., 2022; Bafort and Jijakli, 2024; Dutta et al., 2023). Indeed, CEA of medicinal plants has been described to be profitable in terms of biomass production, extraction yield and content of bioactive compounds, as long as the species-specific cultivation needs are met (Bafort et al., 2022; Bafort and Jijakli, 2024; Dutta et al., 2023). Bioactive compounds are in fact functional metabolites produced by plants to cope with the biotic and abiotic stressors present in their environment (Dixon and Paiva, 1995). By this derives that the *ad-hoc* modulation of growth parameters allowed by vertical farming can be used to trigger a defensive response in the cultivated plants and ideally ameliorate the chemical profiles in the compounds of interest (Oh et al., 2011; Xu et al., 2019).

The vascular flora of Sardinia (Italy), with its 341 endemic taxa (Fois et al., 2022), represents a valuable and still largely unexplored reservoir of unique and structurally diverse phytochemicals for drug development (Sanna et al., 2020). Among these stands out *Hypericum scruglii* Bacch., Brullo & Salmeri (Hypericaceae), an herbaceous plant exclusive of Sardinia (Bacchetta et al., 2010). The plant in fact exhibits several properties of interest in both the medicinal and cosmetic fields: it showed *in-vitro* anti-HIV-1 properties, attributable to a phloroglucinol compound detected for the first time in this species (Sanna et al., 2018); Chiocchio and colleagues (2018) individuated in this plant a strong and selective elastase inhibitor, promising in the development of cosmetics endowed with anti-wrinkle activity; an ethanolic extract of *H. scruglii* formulated in phospholipid vesicles was proposed as treatment for skin diseases and in the formulation of anti-age products (Allaw et al., 2020); the plant was also evaluated in the treatment of fibromyalgia by Kalcev et al. (2021); finally, *H. scruglii* was described featuring the presence of chlorogenic and shikimic acid, hypericin, hyperoside, quercetin, 3-geranyl-1-(2′-methylbutanoyl)-phloroglucinol and 3-geranyl-1-(2′-methylpropanoyl)-phloroglucinol conferring antioxidant and anti-hyperglycaemic properties (Mandrone et al., 2017). However, unlike its well-known congeneric species *H. perforatum* L. (St. John's wort), which is widely used in medicine and health-related applications (Barnes et al., 2001), the use of *H. scruglii* remains, to date, entirely theoretical due to its conservation status. The species is in fact listed as Endangered (EN) in the International Union for Conservation of Nature (IUCN) Red List of the Italian flora (Rossi et al., 2020).

Given this framework, in the present study the possibility of cultivating in vertical farming *H. scruglii* was explored. To this end, we here evaluated the performance of the species cultivated in vertical farming in terms of growth and biomass production. The retention of the key metabolites and of the biological properties characteristics of the plant growing in its natural environment (used as a reference) was evaluated as well. More in detail, the hydroalcoholic extract obtained by *H. scruglii* cultivated in vertical farming was compared with that obtained by the plants growing in their natural environment in terms of content of phenols, flavonoids, and antioxidant properties. The inhibitory activity against α-glucosidase enzyme, a key therapeutic target in type 2 diabetes (T2D) (Floris et al., 2024), was also tested and compared. Finally, ^{1}H NMR based metabolomic and HPLC-FLD profiling were implemented to investigate the presence of the metabolites designated as crucial contributors to the plant's beneficial effects (Allaw et al., 2020; Mandrone et al., 2017; Sanna et al., 2018) and highly responsive to environmental growing conditions (Dixon and Paiva, 1995; Oh et al., 2011; Xu et al., 2019). This approach is useful to track any significant change in plant secondary metabolism linked to varying growing conditions or, as in this case, cultivation methods.

Whitin this work we aim at understanding the extent to which the vertical farming of *H. scruglii* is feasible and we hypothesize that, by adopting a cultivation protocol inspired by the observation of the species in its natural environment, plant's key properties and metabolites would be preserved, allowing a sustainable use of the species.

2. Materials and methods

2.1. Plant cultivation

2.1.1. Sampling of seeds and germination

H. scruglii plants to be cultivated in vertical farming were obtained by seeds collected in August 2023 from a natural population located in Sardinia Island (Italy), in the municipality of Jerzu (Nuoro district), at coordinates 39°45′57.4″N 9°30′41.8″E (Fig. 1A).

The population selected for the sampling of seed material was chosen based on the presence of several hundred *H. scruglii* plants, ensuring that seed collection would pose no threat to the conservation of the species. Moreover, the same population was studied and characterized in its chemical profile and biological activity in previous studies (Chiocchio et al., 2018; Mandrone et al., 2017; Sanna et al., 2018). Around one hundred fruits were collected from several randomly selected plants, ensuring to capture inter-individual diversity.

Once in the laboratory, seeds were separated from the fruits (Fig. 1B) and preserved at room temperature in dark and dry conditions until sowing. Seeds were sown on agar medium during October 2023 following the protocol reported in Porceddu et al. (2020). Briefly, 1 % agar (Sigma) solution was sterilized and poured in Petri dishes. Once the agar medium cooled down and solidified, seeds were carefully placed on the top of it (Fig. 1C), the Petri dishes were then closed and incubated at room temperature (ca 24 °C). Petri dishes were monitored to follow seed germination and the development of plantlets. Seeds were checked weekly and catalogued into three categories: "unmodified seeds", "germinating seeds" (intended as those starting to protrude their main root as in Fig. 2A), and "plantlets" (principal root and cotyledons clearly visible, seed tegument completely lost, Fig. 2B). Seed germination data were collected on 3 Petri dishes containing 200 seeds each and reported as % of developing seeds with respect to total sown seeds ± standard deviation (SD).

2.1.2. Monitoring plants' development

Upon reaching approximately 1 cm in length (Fig. 1D), plantlets were transferred from Petri dishes to peat soil seedling trays (Eazy Plug starter plug tray, HGA Garden B.V., Goirle, Netherlands) and after that, plants (ca 2 cm of height) were transferred to 10 cm × 10 cm pots (Fig. 1E) on a 50/50 (v/v) mix of perlite and standard peat soil presenting the following characteristics pH 7.5, conductivity 0.4 dS/m, apparent density (dry) 180 Kg/m^3, total porosity (v/v) 87 %. The ratio between organic (peat) and inorganic (perlite) components was determined to resemble the physical characteristics of the natural substrate where *H. scruglii* occurs, which is typically light and well drained due to a relevant mineral fraction. The organic component also exhibits good drainage properties, moreover it was intentionally chosen with standard and moderate chemical properties (pH and conductivity within typical ranges) to provide a neutral substrate suitable for further modifications when required. Seed germination and plant development took place under a couple of 40 Watts Cosmorrow COP4065 LED strips (Secret Jardin – Agomoon SRL, Manage, Belgium) held at ca 30 cm above plants (Fig. 1E). LED lighting provided white light (6500 °K) at a mean photosynthetic photon flux (PPF) of 108 μmol s^{-1} (measured at 20 cm). Photoperiod was set at 14 h of light, for a daily light integral (DLI) of ca 7.5 mol m^{-2} day^{-1}, intended as the amount of photosynthetically active radiation (PAR, expressed as moles of photons) reaching a square meter over a 24-hour time period. After 5 months of growth in the laboratory, 150 plants were transferred within the vertical farm for the starting of the experiment in March 2024 (Fig. 1F). Plants' development was monitored as regard: plant height, intended as the height of the main stem from its base to its apex; the plant diameter, intended as the

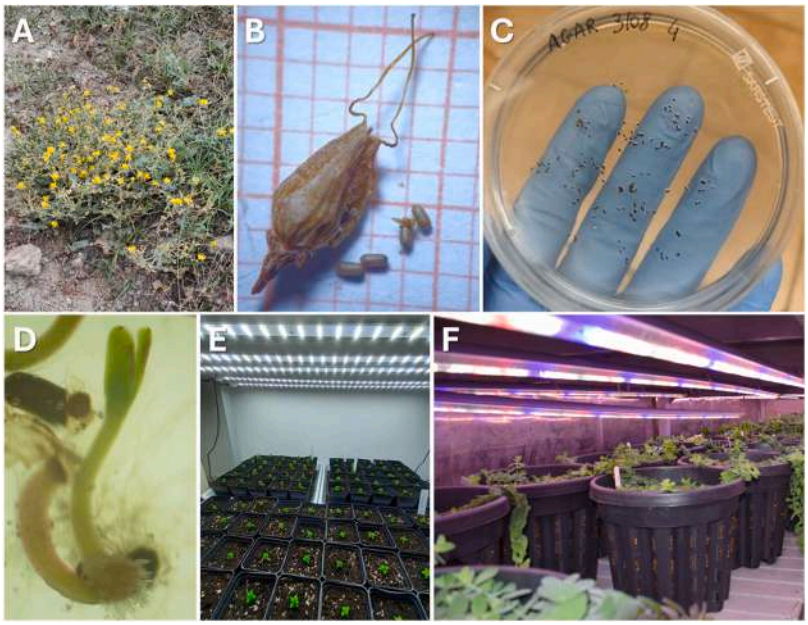

Fig. 1. Workflow of *H. scruglii* propagation. From panel A to F: natural population of *H. scruglii*; Fruit and seeds; seeds on agar medium; germinated seed observed at the microscope; *H. scruglii* plantlets; *H. scruglii* once transferred in the vertical farm.

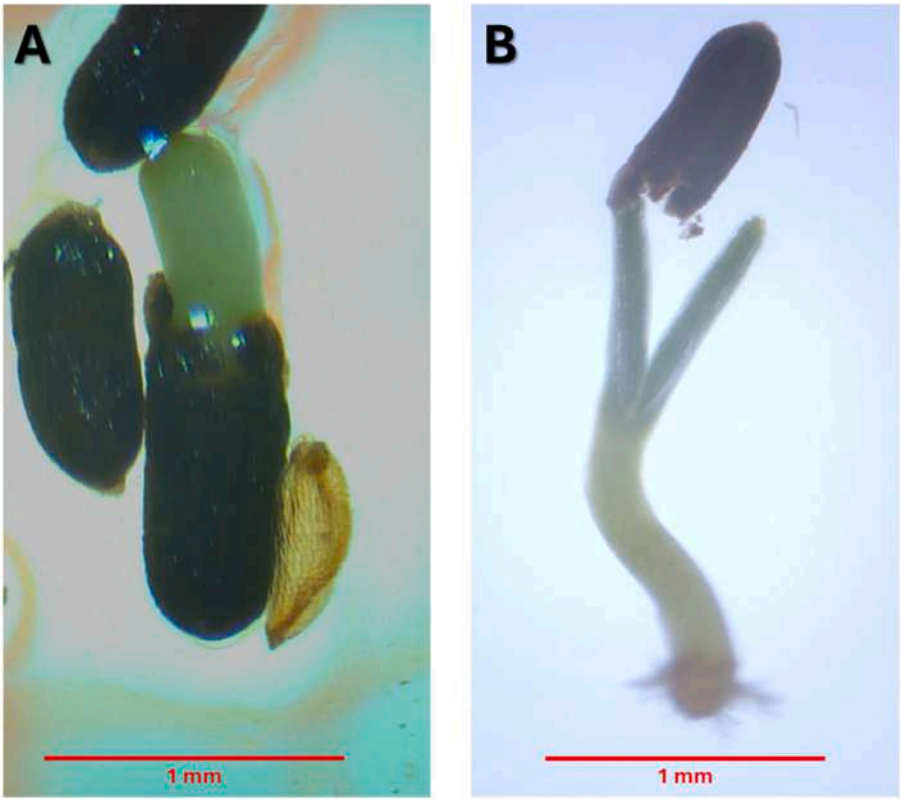

Fig. 2. Development of *H. scruglii* seeds. In panel A a germinating seed protruding its main root; in panel B a plantlet where the main root and the cotyledon are clearly visible and the seed tegument is lost.

maximum diameter reached by the plant growing at the soil level; and finally, the number of basal shoots, intended as the new shoots being produced from the base of the plant (excluding the ones being produced along the main stem). Plant measurements started in the laboratory and continued during the first weeks of cultivation within the vertical farm. Moreover, to monitor any flection in the growth parameters, 20 plants were kept within the laboratory and used as a control to monitor the growth trends of plants once placed in the vertical farm environment.

2.1.3. *Vertical farm features and growing conditions*

The experiment took place in a vertical farm consisting of a container at the end of its service life repurposed as a controlled environment

agriculture module (Demetra 4 Vertical Farming module, GREATIT Company, Rezzato, Italy) and powered by solar energy, hosted by the EAS-TIBIA s.r.l. Agricola Company in the Municipality of Milis (Oristano district, Sardinia, Italy) (Fig. 3).

The container used in the experiment presents the following characteristics:

- Dimension (internal size):
  12 × 2.29 × 2.25 m (length, width, height);
- Cultivation area:
  67.2 m^2 distributed in two shelves of four cultivating surfaces each (0.8 × 10.5 m), vertically spaced of 0.4 m;
- Irrigation system: ebb-and-flow fertigation system monitoring in real time values of pH and EC (electrical conductivity) of the irrigating solution;
- Lighting:
  LED lighting within the vertical farm was supplied with a spectral distribution of 23.9 % of blue, 25.9 % of green, 49.2 % of red and 1 % of far red, at a photosynthetic photon flux density (PPFD) ranging from of 175 to 268 μmol m^{-2} s^{-1}, depending on the relative position on the shelf;
- Temperature: temperature was kept at the required values by a built-in heating ventilation and air conditioning (HVAC) system;
- Relative humidity: humidity was controlled by a dehumidification unit coupled with an automatic humidification system specifically designed for the chamber dimensions (water-vapor production capacity of 1.5 L/h);
- Control and monitoring:
  A specific built-in remote-control application allows to program and modify culture conditions in real time, while a monitoring station (OPI - Decision Support System, EVJA s.r.l., Naples, Italy) offers an overview of environmental parameters within the vertical farm (temperature, relative humidity, dew point, potential transpiration, light intensity, soil temperature and water content);
- Energy requirements:
  The module exhibits an average daily energy demand of approximately 70 kWh, which varies according to the specific growing conditions adopted (temperature, light duration and intensity, number shelves in use).

The growing conditions applied in the experimental phase of this study have been selected based on the scientific literature addressing the autecology of the species (Bacchetta et al. 2010) and after visiting, characterizing and monitoring natural populations of *H. scruglii* (see Section 2.2 and Table 2). However, the obtained parameters were applied with slight modifications since natural conditions are not fully reproducible within the vertical farm module or can result far too extreme if implemented indoor.

Growing conditions were set as follows: irrigation took place twice a day for 15 min and the irrigating solution was maintained at pH levels of 6.5 – 7, while EC was maintained lower than 1 mS/cm. Photoperiod was set at 14 h of light (keeping the DLI around 11.16 mol m^{-2} day^{-1}), providing to periodically reposition plants to ensure uniform lighting to induvial plants. Day/night temperature was kept respectively at 23 and 18 °C while day/night air relative water content was kept at 50 % and 65 % respectively. Table 1 reports the growing parameters measured within the vertical farm during the experiment. Data are reported as mean ± SD of 96 daily measurements (one each 15 min) collected for one month. Any discrepancy between the set values and those measured within the vertical farm (Table 1) should be attributed to the expected range of variation associated with the operation of the vertical farming system.

2.2. Collection of plant material from natural population and from the vertical farm

Plant material was sampled from *H. scruglii* growing in its natural environment (WHS hereafter, standing for wild *H. scruglii*) and cultivated in vertical farming (VFHS from now on, standing for vertical farm

Table 1
Parameters measured within the vertical farm.

Parameter and sensor position		Day	Night
Temperature (°C)		20.45 ± 2.66	17.72 ± 2.27
Relative humidity (%)		65.06 ± 17.06	74.25 ± 16.49
Dew point (°C)		13.09 ± 4.17	12.62 ± 4.05
Potential transpiration (hPa)		8.75 ± 4.90	5.38 ± 3.71
Radiation (W/m^2)	Soil level	18.36 ± 1.15	0.00 ± 0.03
	Shelf level	9.28 ± 2.09	0.01 ± 0.04
Soil temperature (°C, pot)	Top	22.22 ± 1.58	18.79 ± 1.60
	Middle	20.75 ± 1.74	18.42 ± 1.77
	Bottom	21.05 ± 0.61	18.81 ± 0.59
Water content (%, pot)	Top	5.10 ± 5.99	5.52 ± 6.90
	Middle	36.29 ± 1.77	36.79 ± 0.61
	Bottom	52.43 ± 2.65	52.85 ± 0.36

Data are reported as the mean ± SD, of 96 daily measurements collected for one month. Temperature, relative humidity, dew point and potential transpiration refer to the vertical farm environment. Radiation was measured at the shelf level and at the soil level. Soil temperature and water content were measured at three different levels in pots (top, middle and bottom level).

Fig. 3. The vertical farm module used in the experiment.

H. scruglii). Since the aim of this study was to detect differences in phytochemical composition and biological properties between the two growing contexts examined (*i.e.*, wild populations and vertical farming), a particularly large sampling size was adopted, comprising approximately 100 individuals. This extensive sampling effort was implemented to minimize the influence of interindividual variability – not the primary focus of the present study – and ensure a robust and representative characterization of *H. scruglii* in relation to the two growing conditions.

WHS was sampled from the same natural population from which the seeds to obtain the plants to be used in the experiment were initially collected. By doing this, the genetic uniformity of the plants included in the experiment was ensured and any interpopulation variability could be excluded from confounding experimental results. Plant material from WHS was collected during June 2024, corresponding to the blooming stage. In Table 2, temperature and light radiation data measured in the collection site of WHS are reported. Data are reported as mean value ± SD of 24 daily measurements (one every 60 min) collected during the two months preceding the sampling of the plant material. Temperature and light radiation measurements were registered by a HOBO Pendant Temperature/Light 64 K Data Logger (HOBO Data Loggers, Bourne, MA). To be noted that the great variability of light radiation measured in the field (SD exceeding the mean) is to be attributed to the inherent variability of incident sunlight throughout the day from dawn to sunset and due to cloud cover, transient shading and changing weather conditions.

VFHS was collected during August 2024 after six months of cultivation. The sampling of plant material from the vertical farm started as soon as some plants started to produce flowers, when plants reached a mature phenological status comparable with wild plants (in Fig. 4, plants prior sampling).

In both WHS and VFHS, leaves and stems were randomly collected from 100 healthy individuals, then pooled to obtain 140 *g* of fresh plant material for each growing condition. WHS and VFHS biomasses were then oven-dried at 40 °C up to constant weight and vacuum sealed until extraction.

2.3. Preparation of H. scruglii extracts

A quantity of 43 g and 28 g of grounded dried plant material from WHS and VFHS, respectively (resulting by the dehydration of the same quantity of fresh plant material), was extracted three times with 80 % ethanol following the protocol described in Sanna et al. (2023). The two extracts were deprived of the ethanol by rotary evaporation at 40 °C, and the residual aqueous phase was freeze dried.

2.4. Determination of total phenolic and flavonoid content

The total phenolic content (TPC) was measured using the Folin-Ciocalteu method (Singleton and Rossi, 1965) with slight modifications. WHS and VFHS extracts were mixed with Folin-Ciocalteu reagent, distilled water, and sodium carbonate, then incubated in the dark. Absorbance was read at 750 nm, and results were expressed as mg gallic acid equivalents (GAE) per g dry weight (DW).

Total flavonoids content (TFC) was determined using the aluminium nitrate colorimetric method (Zhishen et al., 1999), with modifications. Extracts were mixed with aluminium nitrate, sodium acetate, and ethanol, incubated for 40 min, and the absorbance measured at 415 nm. Results were expressed as mg quercetin equivalents (QE) per g DW.

Table 2
Temperature and light intensity measured at the growing site of the wild population of *H. scruglii*.

Parameter	Day			Night		
	Mean ± SD	Max.	Min.	Mean ± SD	Max.	Min.
Temperature (°C)	26.20 ± 6.61	43.60	12.21	18.68 ± 3.33	29.15	11.62
Radiation (W/m^2)	523.46 ± 635.64	2438.13	9.94			

Mean values are reported as the mean ± SD, based on 24 daily measurements collected for two months. Maximum and minimum temperatures are also reported. Both temperature and light intensity were measured at the soil level.

2.5. ABTS radical scavenging activity

ABTS·$^+$ radical-scavenging activity was assessed following Delogu et al. (2016), using Trolox as a standard. ABTS·$^+$ was generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate and incubating in the dark for 24 h. The mixture was diluted to an absorbance of 0.700 ± 0.05 at 734 nm. WHS and VFHS extracts were added to 1 mL of ABTS·$^+$, and absorbance was measured after 1 min. Results were expressed as EC_{50}, the concentration required to reduce the absorbance by 50 %.

2.6. α-Glucosidase inhibition assay

To evaluate α-glucosidase inhibitory activity, a reaction mixture was prepared containing 120 μL of 0.1 M phosphate buffer (pH 6.8), 40 μL of a *Saccharomyces cerevisiae* enzyme solution (0.125 U/mL), and 20 μL of WHS or VFHS extract at varying concentrations. The mixture was incubated at 37 °C for 15 min. Subsequently, 20 μL of the substrate p-nitrophenyl α-D-glucopyranoside (pNPG), dissolved at 5 mM in phosphate buffer, was added. After incubation at 37 °C for 15 min, the reaction was stopped by introducing 50 μL of 0.2 M sodium carbonate and the release of *p*-nitrophenol was measured at 405 nm using a microplate reader (Delogu et al., 2021). Acarbose, the reference α-glucosidase inhibitor, was used as a positive control. The extracts' inhibitory effects were reported both as a percentage inhibition at a concentration of 100 μg/mL and as IC_{50} values (μg/mL), indicating the extract concentration required to inhibit 50 % of enzymatic activity.

2.7. Effects on cell viability and intracellular ROS levels

Cell viability was evaluated using the MTT assay on Caco-2 cells. Cells were cultured under standard conditions: 5 % CO_2 atmosphere, 95 % relative humidity, and temperature of 37 °C. Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10 % fetal bovine serum (Gibco, Grand Island, NY) and 1 % penicillin/streptomycin (Euroclone, Milan, Italy) was used for maintenance. For the assay, cells were seeded in 96-well plates at a density of 5×10^3 cells per well and exposed to varying concentrations of WHS or VFHS extract (ranging from 0 to 100 μg/mL). After a 24-hour incubation period, an MTT solution (0.5 mg/mL) was added and plates were incubated for additional 3 h at 37 °C. The resulting formazan crystals were solubilized in DMSO, and absorbance was recorded at 590 nm to quantify cell viability.

Intracellular reactive oxygen species (ROS) levels were quantified using the 2′,7′-dichlorofluorescein diacetate (DCFH-DA) assay to assess the antioxidant potential of the extracts (Sanna et al., 2023). Caco-2 cells were exposed to varying extract concentrations (0 – 100 μg/mL) for 24 h. After treatment, cells were incubated with 10 μM DCFH-DA at 37 °C for 30 min. Hydrogen peroxide (H_2O_2, 2 mM) was subsequently added to induce oxidative stress. The resulting fluorescence from oxidized DCF was measured with excitation at 485 nm and emission at 530 nm. Fluorescence readings were collected over a 60-minute period to evaluate the extracts' ability to mitigate ROS production.

2.8. ^{1}H NMR profiling and HPLC-FLD

10 mg of VFHS and WHS extract were dissolved in 1 mL of mixture (1:1) of phosphate buffer (90 mM; pH 6.0) in H_2O-d_2 (containing 0.1 % TMSP) and MeOH-d_4, then 700 μL of supernatant were transferred into

Fig. 4. *H. scruglii* after six months of cultivation within the vertical farm. Notice the presence of flowering plants.

NMR tubes. For each extract, three different samples were prepared to test reproducibility. ^{1}H NMR spectra were recorded at 25 °C on a Varian Inova instrument (equipped with a reverse triple resonance probe). For ^{1}H NMR profiling the instrument was operating at ^{1}H NMR frequency of 600.13 MHz, and MeOH-d_4 was used as internal lock. Each ^{1}H NMR spectrum consisted of 8 scans (corresponding to 18 min) with the relaxation delay (RD) of 120 s; acquisition time 4 s; block size of 2; 45-degree pulse, and spectral width of 9595.8 Hz (corresponding to δ 16.0). The spectra were manually phased and baseline corrected, and calibrated to the internal (TMSP) at δ 0.0 using Mestrenova software (Mestrelab Research, Santiago de Compostela, Spain). Metabolites were identified on the basis of literature data (Mandrone et al., 2017; Sanna et al., 2018), and in-house database. Values of metabolite concentration (calculated by quantitative analysis q-NMR) were expressed as mean ± SD of three replicates as mg/g (of extract). The calculation was based on the integrated signal area (*Ix*) in the spectrum, which is directly proportional to the number of nuclei (N*x*) responsible for the corresponding resonance. The molar ratio (M*x*/M*y*) between each metabolite *x* and the internal standard *y*, was determined using the following equation (Bharti and Roy, 2012): $\mathbf{Mx / My = (Ix / Iy) \times (Ny / Nx)}$

HPLC-FLD analysis of the extracts was carried out using C18 column (Gemini C18, 3 μm, 110 Å, 4.6 × 100 mm, Phenomenex Italy, Castelmaggiore, Italy). The analysis was performed according to Gadzovska et al. (2005) with some modifications. Mobile phase A was ammonium acetate buffer (0.01 M) at pH 8.0, and phase B was a mixture of methanol and acetonitrile (5:4, v/v). The step gradient was the following: starting conditions 40 % A and 60 % B (0–5 min), reaching 92 % B (5–10 min), reaching 100 % B (10–15 min), 100 % B was kept from min 15 to min 18, then starting condition were restored in 2 min. The flow rate was 1 mL/min with a 10 μL of injected volume. Total run time was 28 min. The FLD detector recorded simultaneous fluorescence (315/590 nm, excitation/emission). Retention time value (t_R) for hypericin was 16 min. Samples were injected at a concentration of 5 mg/mL in 50 % phase A and 50 % phase B. Standard curve for the quantification was built using six different concentrations (from 0.1 to 3.25 μg/mL) of commercial standard of hypericin.

Regarding the chemicals used in this section, deuterium oxide (D_2O, 99.90 % D), CD_3OD (99.80 % D) were purchased from Eurisotop (Cambridge Isotope Laboratories, Inc, France). Standard 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid sodium salt (TMSP), sodium phosphate dibasic anhydrous and sodium phosphate monobasic anhydrous and all the other chemicals, standards and solvents were purchased from Sigma-Aldrich Co. (St. Louis, MO).

2.9. Statistical analysis

Evaluation of radical scavenging activity via ABTS assay was conducted in three independent assays and the results reported as mean value ± SD. Total phenolic and flavonoid content, as well as ^{1}H NMR and HPLC-FLD analysis were conducted in three readings and the results expressed as mean value ± SD. Since both WHS and VFHS extracts were obtained by pooling and extracting a substantial number of individual plants, this approach was considered appropriate to have a representative chemical profile of *H. scruglii* grown under vertical farming conditions or deriving from natural populations. Total phenolic and flavonoid content, as well as ^{1}H NMR and HPLC-FLD results were also expressed as fold changes (VFHS vs WHS). Fold changes above 2 and below 0.5 were considered relevant and indicating, respectively, upregulation or downregulation of VFHS with respect to WHS, following commonly adopted thresholds in metabolomics (Deng et al., 2023; Liu et al., 2024).

The experiments to evaluate biological activities of the extracts (α-Glucosidase inhibition, cell viability and intracellular ROS), were conducted in three replicates (three reaction mixtures or cellular cultures treated with the same extract), and the results reported as mean value ± SD (α-Glucosidase inhibition) or by bar plots (cell viability and intracellular ROS). GraphPad Prism software version 9 (San Diego, CA, USA) was used to evaluate statistical significance of differences, determined by two-way ANOVA (p-values below 0.05).

3. Results

3.1. Seed germination and plant growth

In accordance with Porceddu and colleagues (2020), we observed good germinability of seeds as soon as a week from sowing, while after 13 days several plantlets were clearly distinguishable. Details on seed germination are provided in Table 3.

Plants' development following the transfer on soil is reported in the time series plots in Fig. 5. The graphs clearly show how the growth trends were positive and constant throughout the monitoring period. It is also possible to notice how the transfer from the laboratory to the vertical farm did not result in any marked deviation in the growth rates.

3.2. Plant water content and extract yield

Water content of WHS and VFHS varied markedly, in fact the obtained dried plant material constituted the 30.71 % and the 19.86 % of the fresh plant material, respectively. However, extract yield varied less markedly, resulting in the 22 % and 28 % for WHS and VFHS, respectively (expressed as w/w %).

3.3. Antioxidant activity, TPC and TFC

The radical scavenging activity, evaluated by ABTS assay, revealed comparable good antioxidant capacity of extracts with EC_{50} values close to that of Trolox, used as a standard. More precisely, WHS and VFHS showcased EC_{50} values of 7.2 ± 2.7 and 8.3 ± 0.1, respectively (expressed as μg/mL) while Trolox exhibited EC_{50} values of 3.4 ± 0.3. EC_{50} values indicate that WHS is featured by slightly higher antioxidants properties. WHS also resulted featured by slightly higher phenolics and relevant higher flavonoid content with respect to VFHS. Detailed results are reported in Table 4.

3.4. α-Glucosidase inhibitory activity

As shown in Table 5, both the extracts exhibited strong inhibitory activity against α-glucosidase, their IC_{50} values being lower than that of acarbose. More precisely, WHS and VFHS are respectively 34 and 13 times more effective than the commercial drug. Moreover, WHS and VFHS at 100 μg/mL, were able to inhibit the enzyme activity by 100 % and 89 %, respectively. These results confirm that the promising activity of *H. scruglii* as inhibitor of α-glucosidase is maintained, even if slightly lower, when the plant is cultivated in vertical farming.

3.5. Cell viability and intracellular ROS levels

Cytotoxicity of the extracts was evaluated using Caco-2 cells exposed for 24 h to five different concentrations of the extracts (1, 10, 25, 50 and 100 μg/mL) and then examined by MTT test. The results (Fig. 6) showed that the treatment with the extracts did not result in significant reduction in cell viability at concentrations up to 50 μg/mL. At 100 μg/mL, both extracts caused a slight but statistically significant decrease in cell viability compared to non-treated (NT) cells. However, viability remained relatively high (around 80 %), indicating that the extracts are not cytotoxic at this concentration.

Since ABTS assay documented good antioxidant activity of the extracts and since oxidative stress is one of the detrimental consequences of diabetes, the potential of WHS and VFHS extracts to inhibit H_2O_2-induced ROS levels in a cellular model was evaluated. Fig. 7 shows that, as expected, ROS levels increased in H_2O_2 treated cells. However, the treatment with WHS and VFHS extracts determined a dose-dependent inhibition of ROS levels. A statistically significant reduction in ROS levels was observed at 50 μg/mL for WHS extract and at 100 μg/mL for both WHS and VFHS extracts. Notably, treatment with both extracts at 100 μg/mL reduced ROS levels to values comparable to those observed NT. These results suggest a potential role of *H. scruglii* extracts in reducing ROS formation in cells.

Table 3
Seed germination data.

	8 days	13 days	18 days	35 days
Germinated seeds (%)[a]	16.93 ± 15.48	43.76 ± 14.39	57.02 ± 16.24	
Plantlets (%)[b]		4.19 ± 3.24	32.16 ± 22.40	
Transferred plantlets (%)[c]				21.98 ± 16.34

Values refer to the mean value ± SD of the counts performed on three capsules each containing 200 seeds. Percent refers to the initial number of seeds. [a] refers to seeds protruding their main root and includes plantlets; [b] are those plants presenting well developed cotyledons and no tegument; [c] are those finally transferred on soil medium.

3.6. Phytochemical analyses

The experimental results discussed so far confirmed that biological properties of *H. scruglii* are largely preserved when the plant is cultivated in vertical farming. Following this, the quantitative q-^{1}H NMR profiling of WHS and VFHS extracts was performed to observe at a finer scale any change in the metabolome of the plant in relation to the two growing conditions. While the metabolites fingerprint resulted completely preserved in VFHS, WHS extract is featured by higher levels of chlorogenic acid, 3-geranyl-1-(2′-methylbutanoyl)-phloroglucinol, quercitrin and sucrose (Fig. 8 and Table 6). In the case of hypericin, since it was not detectable by ^{1}H NMR profiling for its content being below the detection threshold (Mandrone et al., 2017), it was analyzed by HPLC-FLD, resulting in content of 0.3 mg/g and 0.1 mg/g in WHS and VFHS, respectively (fold changes of 0.33).

4. Discussion

Currently, the great majority of vertical farming production consists of leafy vegetables, followed by tomatoes, herbs, flowers, and microgreens (Wong et al., 2020), hence, the opportunities offered by the cultivation in vertical farming of medicinal plants are still largely underexplored and, by so, underexploited. Nevertheless, it is in the cultivation of niche, high-value crops such as medicinal plants that vertical farming may deliver its most significant advantages (Stanghellini and Katzin, 2024). In vertical farming, in fact, plant physiology can be strategically influenced by modulating growing conditions within cultivation modules triggering the production of crops with enhanced biological and organoleptic properties through the induction of beneficial specialized metabolites (Bafort et al., 2022; Wong et al., 2020). Within this framework, the feasibility of the cultivation in vertical farming of *H. scruglii* was assessed especially with regards to the preservation of the plant's biological activity.

From a morphological point of view, the prostrate growth habitus of *H. scruglii* resulted perfectly adequate to fit the shelves of a vertical farm where space is exploited vertically. Moreover, the performance of the cultivation in terms of biomass production resulted satisfactory since more than one hundred individuals were obtained from seeds and grown without any major issue in less than a year (Fig. 4). These two elements proved the suitability within the spaces of a vertical farm and the rapid biomass production indicated by Bafort et al. (2022) as key requirements for the vertical farming of medicinal plants. The biomass obtained in vertical farming was featured by higher water content with respect to wild plants, possibly because of the constant water supply set to simulate the features of the natural environment of *H. scruglii* during its vegetative season (when the plant thrives on water-saturated soils). However, once dehydrated and extracted, WHS and VFHS yielded comparable amounts of extract (VFHS yielding a 6 % more than WHS).

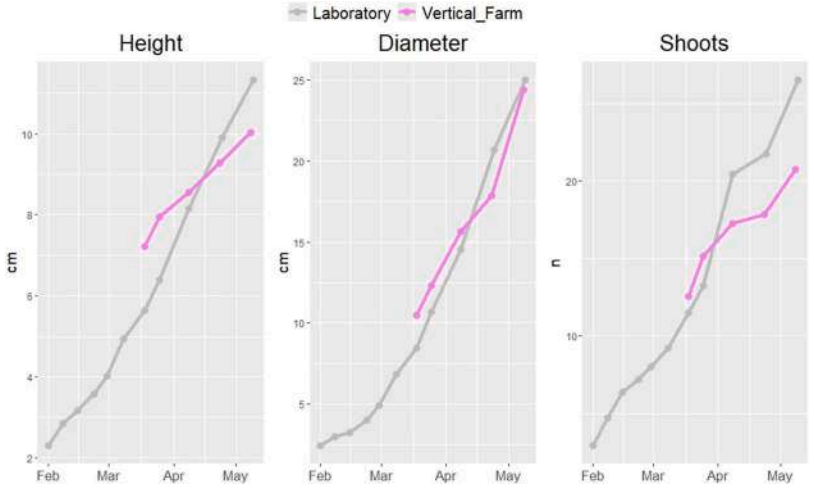

Fig. 5. Time series plots reporting plant development as regards height, diameter and number of basal shoots. Measurements started as soon as plants were placed on soil pots (February 2024) and ended on May 2024. Different line colours indicate different growing conditions (*i.e.*, grey, laboratory; violet, vertical farm module).

Table 4
Total phenolic (TPC) and total flavonoid content (TFC) measured in the extracts obtained from WHS (wild *H. scruglii*) and VFHS (vertical farming *H. scruglii*).

	WHS	VFHS	Fold changes
TPC (mg GAE/g)	369.4 ± 14.1	276.8 ± 12.9	0.74
TFC* (mg QE/g)	155.5 ± 9.3	42.9 ± 5.2	0.27

Results are expressed as the mean value of three technical replicates ± SD. Fold changes between VFHS and WHS were calculated for each parameter and the ones labeled by an asterisk (*) are considered downregulated.

Table 5
Inhibitory activity of WHS (wild *H. scruglii*) and VFHS (vertical farming *H. scruglii*) extracts against α-glucosidase.

		WHS	VFHS	Acarbose
α-Glucosidase	**IC_{50} (μg/mL)**	2.7 ± 0.1	6.9 ± 0.2	90.0 ± 7.3
	Inhibition % (100 μg/mL)	100 ± 0.4	89 ± 0.7	

Results are expressed as the mean value of three independent assays ± SD. Difference between WHS and VFHS IC_{50} is statistically significant (p-value < 0.0001, f-value 1158).

Nevertheless, the higher water content of VFHS should be considered when evaluating the feasibility of scaling up the cultivation approach here described as it would imply higher energy demand for biomass processing, particularly during the dehydration step prior extraction.

Another key performance indicator to consider when evaluating the vertical farming of a medicinal plant is the concentration of the phytomolecules of interest within the biomass and the retention of plant's biological activity (Bafort et al., 2022). While exploratory spectrophotometric assays revealed WHS to be characterized by higher antioxidant properties and higher phenols and flavonoids than VFHS, biological activity of *H. scruglii* was not hampered by vertical farming, supporting our initial hypothesis. Extracts from both sources in fact exhibited comparable α-glucosidase inhibitory activity, antioxidant capacity, and no cytotoxicity, indicating that vertical farming did not compromised the extract's efficacy and safety. Phytochemical analyses also confirmed that vertical farming preserves all of the signature metabolites of *H. scruglii* including hypericin (Mandrone et al., 2017; Sanna et al., 2018). From the quantitative viewpoint, however, differences between the extracts can be observed involving the relevant specialized metabolites chlorogenic acid, geranyl-butanoylphloroglucinol, quercitrin and hypericin (more abundant in WHS than in VFHS). On the contrary, catechin, an important health-promoting compound known for its antioxidant activity (Zanwar et al., 2014), was found at comparable levels in WHS and VFHS.

Diversity and variations in plants' chemical profiles are determined by genetic factors but, as in the present study case, can also arise from contrasting environmental factors (*e.g.*, light intensity and spectral composition, temperature, water and nutrients availability) acting on the same genetics (Ding et al., 2023; Oh et al., 2011; Xu et al., 2019). In the present study the favourable growing conditions applied within the vertical farm may have provided *H. scruglii* plenty of energy and carbon substrates for the production of greater amounts of primary metabolites with respect to wild plants copying with more scant resources and a harsher environment. Conversely, phenols, flavonoids and relevant protective metabolites peaked in wild *H. scruglii*. Since these metabolites counteract the effects of adverse environmental conditions in nature, the downregulation of these metabolites in VFHS should be interpreted as a result of the growing conditions applied within the vertical farm, lacking those stressors which characterize natural environments. Most notably, ultraviolet (UV) wavelengths (100 to 400 nm range) were absent from the vertical farm lighting setup. UV-B light, known for its detrimental effects towards cell membranes, proteins and DNA (Hollósy, 2002; Nawkar et al., 2013), after being perceived by plants thanks to the photoreceptor UVR8 (UV-B resistance 8), initiate a stress response cascade involving, among the others, the biosynthesis of flavonoids (Dixon and Paiva, 1995; Wong et al., 2020; Xu et al., 2019). Once synthesized flavonoids act screening photosynthetic centres and chemically quenching ROS deriving from photooxidation (Dixon and Paiva, 1995; Xu et al., 2019). By this, derives that the modulation of environmental growing conditions (first and foremost light intensity, duration and spectral composition) is a key factor in CEA to maintain crops yield, quality, biological values and functional metabolites' content (Ding et al., 2023; Wong et al., 2020). Chiocchio et al. (2022) integrated specific light wavelengths in smart greenhouses equipped with LED lighting hosting *Taxus baccata* L., obtaining improved biomass, plant structure, and content of sucrose, aromatic compounds and metabolites; treatment of red and blue light improved the content of taxane in *Amentotaxus yunnanensis* H.L.Li. (ex *Taxus yunnanensis* W.C.Cheng & L. K.Fu) (Su et al., 2012); Bafort et al. (2022) forecast profitable cultivation in vertical farming of *Euphorbia peplus* L. given an optimal setup of the

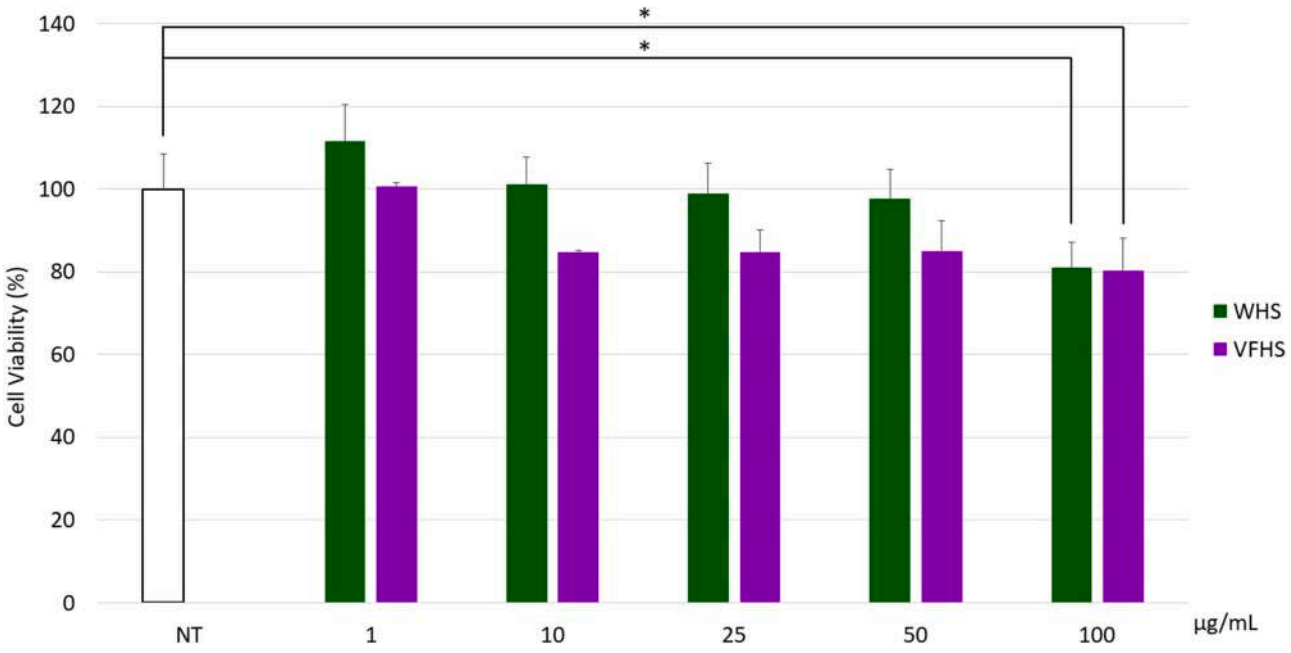

Fig. 6. Effect of WHS (wild *H. scruglii*) and VFHS (vertical farming *H. scruglii*) extracts on Caco-2 cell viability. Cells were exposed for 24 h to five extract concentrations from 1 µg/mL to 100 µg/mL. Asterisks indicate values statistically different from NT, non-treated cells (* p-value < 0.05; NT vs WHS at 100 µg/mL f-value = 9.7; NT vs VFHS at 100 µg/mL f-value = 8.6).

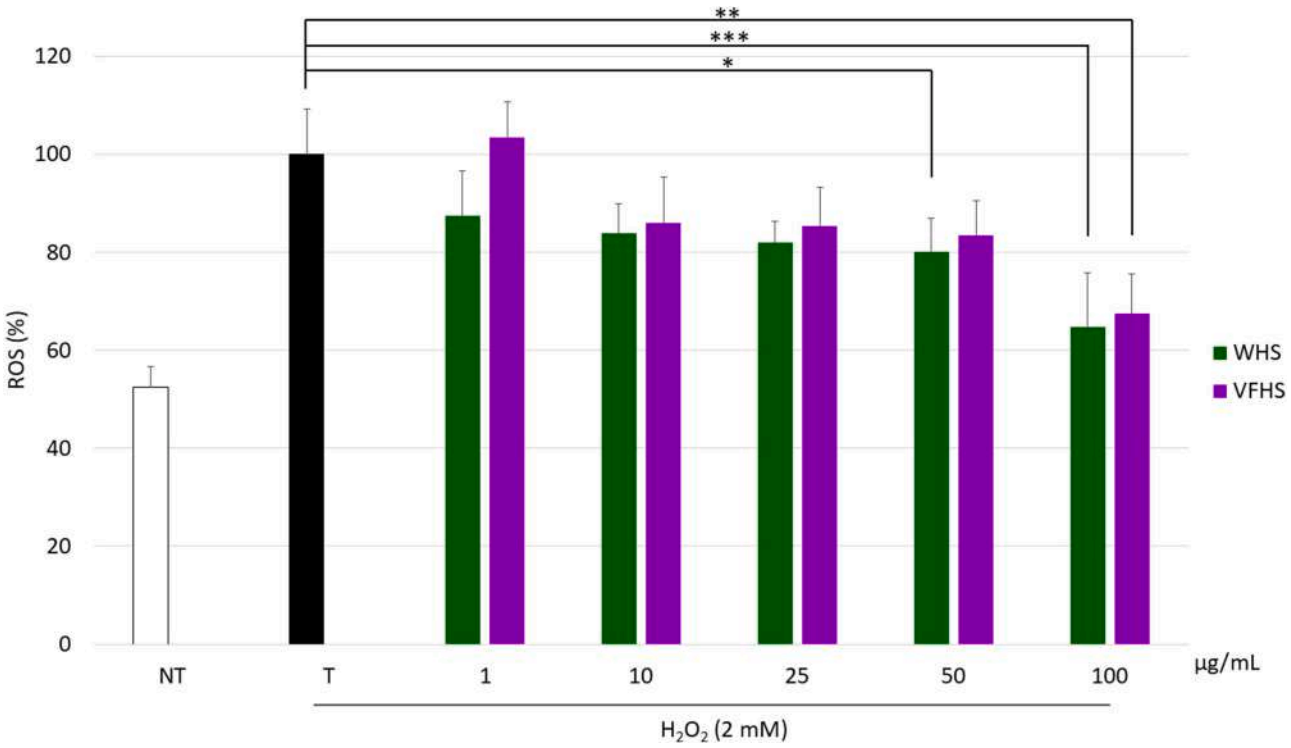

Fig. 7. Reduction of H_2O_2-induced ROS in Caco-2 cells by different doses of WHS (wild *H. scruglii*) and VFHS (vertical farming *H. scruglii*) extracts. NT, non-treated cells; T, cells treated with only H_2O_2 (2 mM for 1 h incubation). Statistically significant differences in ROS content compared to H_2O_2 treated cells, are indicated by asterisks (* p-value < 0.05, ** p-value < 0.001, *** p-value < 0.0001; T vs WHS at 50 µg/mL f-value = 8.9; T vs WHS at 100 µg/mL f-value = 29.9; T vs VFHS at 100 µg/mL f-value = 22.9). All H_2O_2-treated samples, with the exceptions of the extracts at concentrations of 100 µg/mL, were statistically different from NT.

cultivation in terms of substrate, light intensity and plant distribution within the vertical farm; modulating light quality and intensity will result in enhanced precursors of anticancer vinblastine and vincristine in *Catharanthus roseus* (L.) G.Don (Fukuyama et al., 2017, 2013) as well as in improved biomass and alkaloids content (Molchan et al., 2017); Wong et al. (2020) observed how antinutrients in leafy greens were related to low-light vertical farming environments.

Notably, in the present study, the comparable biological activity observed between WHS and VFHS is achieved despite a marked reduction in key specialized metabolites in VFHS. In this sense extract's

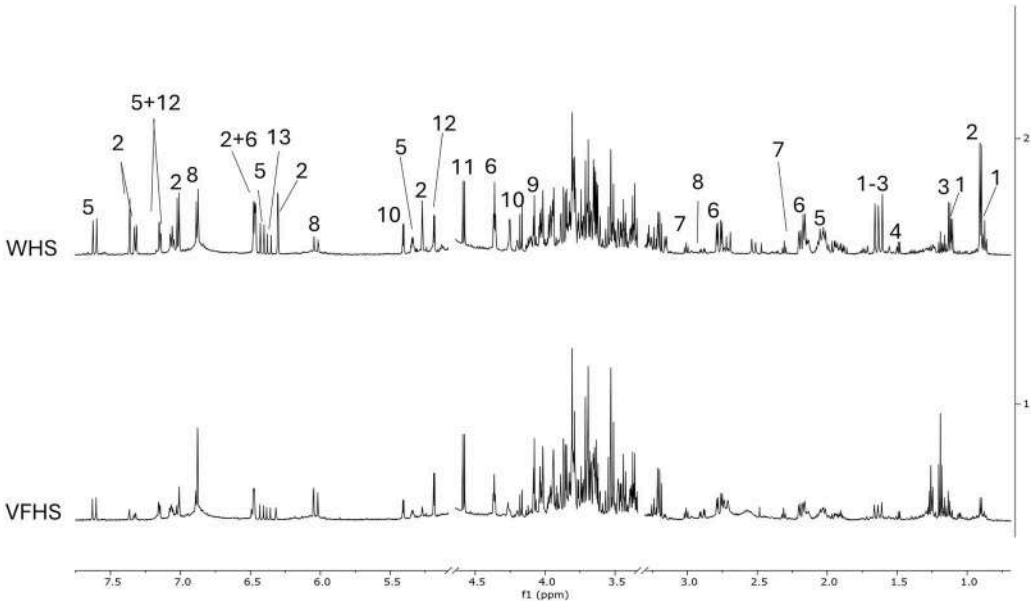

Fig. 8. ^{1}H NMR profiles of WHS (wild *H. scruglii*) and VFHS (vertical farming *H. scruglii*) extracts. 1 = 3-geranyl-1-(2′-methylbutanoyl)-phloroglucinol; 2 = quercitrin; 3 = 3-geranyl-1-(2′-methylpropanoyl)-phloroglucinol; 4 = alanine; 5 = chlorogenic acid; 6 = shikimic acid; 7 = GABA; 8 = catechin/epicatechin; 9 = fructose; 10 = sucrose; 11 = β-glucose; 12 = α-glucose; 13= protocatechuic acid.

Table 6
Concentration of the metabolites in WHS (wild *H. scruglii*) and VFHS (vertical farming *H. scruglii*) extracts.

Metabolite	NMR signal (δ, multiplicity)	WHS (mg/g)	VFHS (mg/g)	Fold changes
alanine	1.49, d	2.07 ± 0.12	1.81 ± 0.24	0.87
catechin	6.04, d	26.12 ± 4.77	33.28 ± 11.32	1.27
chlorogenic acid*	7.6, d	85.36 ± 5.82	40.62 ± 2.18	0.47
fructose	3.94, m	129.42 ± 2.96	159.62 ± 5.73	1.23
GABA	3.00, t	3.59 ± 2.12	6.44 ± 0.85	1.79
3-geranyl-1-(2′-methylbutanoyl)-phloroglucinol*	1.11, d	17.43 ± 4.27	8.04 ± 2.37	0.46
3-geranyl-1-(2′-methylpropanoyl)-phloroglucinol	1.13, d	23.8 ± 5.91	14.63 ± 3.87	0.61
glucose	4.6, d; 5.2, d	100.92 ± 8.87	140.66 ± 3.70	1.39
protocatechuic acid	6.37, d	14.99 ± 2.21	8.05 ± 0.63	0.53
quercitrin*	7.36, d	96.96 ± 11.96	18.87 ± 0.92	0.19
shikimic acid	6.52, m	94.02 ± 0.71	57.12 ± 3.57	0.6
sucrose*	5.4, d	80.97 ± 18.97	36.76 ± 0.00	0.45

Multiplicity and chemical shift δ of the signal used for the quantification are reported. Metabolite concentration (mean ± SD of three independent measurements) is expressed as mg/g of extract DW. Fold changes between VFHS and WHS were calculated for each metabolite and the ones labeled by an asterisk (*) are considered downregulated.

efficacy seems to be granted even by minimal amounts of such bioactive compounds. These findings raise important questions about whether the biological efficacy of an extract depends on the concentration of individual compounds, or instead on synergistic effects deriving from the interactions of functional metabolites forming part, even in small amounts, of plant's phytochemical profile.

In conclusion, in the present study we proved for the first time the feasibility of the cultivation in vertical farming of *H. scruglii* which, given the growing conditions applied during the experiment, produced abundant plant biomass featured by higher extraction yield and comparable biological activity with respect to the plant growing in its natural environment. The obtained results, although preliminary, can be considered promising since proved the possibility of a sustainable exploitation of *H. scruglii*, which use, at the date, is completely precluded. Future perspectives of the present research should address the modulation of the cultivation protocol, especially concerning the integration of UV light, to leverage on plant protective mechanisms to further improve the content of the compounds of interest in the plant. Moreover, a focus on the energy requirements of the cultivation would allow a more complete evaluation of the actual feasibility of vertical farming *H. scruglii*.

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CRediT authorship contribution statement

Antonio De Agostini: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ilaria Chiocchio:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Sonia Floris:** Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation. **Anna Maria Cicilloni:** Writing – review & editing, Methodology. **Ferruccio Poli:** Writing – review & editing. **Manuela Mandrone:** Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation. **Francesca Pintus:** Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation. **Cinzia Sanna:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Antonio De Agostini reports financial support and equipment, drugs, or supplies were provided by EAS-TIBIA s.r.l. Agricola. Anna Maria Cicilloni reports a relationship with EAS-TIBIA s.r.l. Agricola that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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