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Article

The Agro-Economic Feasibility of Growing the Medicinal Plant *Euphorbia peplus* in a Modified Vertical Hydroponic Shipping Container

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Abstract: Vertical farming is considered as a potential solution to increase yield while decreasing resource use and pesticide impacts compared to conventional agriculture. However, the profitability of cultivating ordinary leafy green crops with low market prices in vertical farming is debated. We studied the agronomic feasibility and viability of growing a medicinal plant—*Euphorbia peplus*—for its ingenol-mebutate content in a modified shipping container farm as an alternative crop cultivation system. The impacts of three hydroponic substrates, three light intensities, three plant localizations and two surface areas on *E. peplus* yield and cost were tested in several scenarios. The optimization of biomass yield and area surface decreased the cultivation cost, with fresh crop cost per kg ranging from €185 to €59. Three ingenol-mebutate extraction methods were tested. The best extraction yields and cheapest method can both be attributed to ethyl acetate at 120 °C, with a yield of 43.8 mg/kg at a cost of €38 per mg. Modeling of the profitability of a pharmaceutical gel based on ingenol-mebutate showed that economic feasibility was difficult to reach, but some factors could rapidly increase the profitability of this production.

Keywords: vertical farming; hydroponics; profitability; biomass yield; ingenol-mebutate; *Euphorbia peplus*

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1. Introduction

In recent years the phenomenon of urbanization has rapidly increased, and more than half of the world's people are now living in cities [1]. The rapid expansion of cities and the increasing population are putting a lot of pressure on food systems. The land area available for agricultural production is predicted to be restrained by urbanization, primarily due to decreased soil fertility as a result of overexploitation and climate change, the deployment of industrial activities and the expansion of cities [2,3].

Indoor farming represents a means of cultivation less dependent on arable land availability and external climate conditions. As pointed out by Agrilyst, "indoor farms" is a generic name encompassing a large range of cultivation systems, including greenhouses, indoor vertical farms, and container farms [4]. Plants suitable for vertical farming are leafy greens, herbs, transplants, and medicinal plants no taller than 30 cm, allowing the maximizing of the indoor space [5]. Vertical farming is seen as a potential solution to increase yield while decreasing resource use and pesticide impacts compared to conventional agriculture [6]. Several authors have indeed reported that vertical farming improves yields as compared to traditional farming, whereas greenhouse farming yields are intermediate [7–10]. Nevertheless, several difficulties have been pointed out for vertical farming, such as a limited number of cultivable varieties, high energy use, high technical requirements for employees, consumer complaints, low market prices for cultivable varieties, start-up costs, property costs in urban areas, and subsequently the profitability of such farming systems [7,11–16].

The profitability of vertical farming is debated. Agrylist reported that only 51% of indoor farming reported operating profitability after 7 years of existence—only 50% of container farms and 27% of indoor vertical farms—and the main crops were leafy greens [4]. The highest reported costs were labor costs, followed by rent, packaging, and energy [4]. In an adapted shipping container, the efficiency of lettuce crop production, measured in several scenarios, was too low to be viable, although improvements in energy consumption and yield efficiency could allow viable crop production [17]. The cultivation of Romaine lettuce and Genovese basil in a modified insulated freight container could not compete with Romaine lettuce and basil in the European market, but improvements in terms of space and plant density in the plant design factory could decrease the production cost for basil from €19/kg to €10/kg [18]. A vertical farm in The Netherlands recently went bankrupt because it was not possible to market their vertically grown vegetables in a financially attractive way [19]. Calculating the profitability of urban farms is challenging, and few studies have been conducted to quantitatively evaluate their viability [15]. On the other hand, vertical farming could be a viable farming solution according to certain models [20,21]. Profitability was linked to the cultivation area, the plant design (using renewable energy and waste valorization), the high unit production yield, and the selling price of the crop [21].

Diversification by cultivating high-added-value plants could be economically less challenging. One-third of the current top 20 drugs on the market are plant derived [22,23]. According the World Health Organization, the percentage of the population which has used plant-based medicine at least once is 48% in Australia, 31% in Belgium, 42% in the United States of America, and 70% in Canada [24]. Among medicinal plants, the Euphorbiaceae family includes more than 2000 species, generally characterized by the production of an irritating latex in the stems and leaves [25]. *Euphorbia peplus*, more commonly known as milkweed or petty spurge, is an annual herbaceous weed that develops in temperate or hot climates, and is an interesting candidate for vertical cultivation due to its small size and rapid growth [24]. *E. peplus* is found in gardens, ornamental groves or fields, and its latex is rich in alkaloids, terpenoids, and cardenolides, which gives it a defensive role against attacks by pathogens or herbivorous insects, since those compounds are toxic [26]. *E. peplus* is a long-day plant with a C3 photosynthetic mechanism [27]. Its scientific interest soared in the early 2000s, when ingenol mebutate, a diterpene ester present in the sap of *E. peplus*, was discovered to be efficient against actinic keratosis, a precancerous skin disease [25,28].

Indoor farming in a controlled environment allows the user to control important factors in terms of biomass and secondary metabolite yield. Light intensity, the photoperiod, and the light spectrum regulate plant photosynthesis, growth, and secondary metabolite accumulation [29–32]. For optimal light quality and intensity, there is not a single answer: it is specific to the plant species, the plant growth stage, the specific secondary metabolites, and the environmental practices [31].

In soilless cultivation, substrate cultures employs substrate media to provide support to plants and provide for plant root and shoot growth [33]. The main role of the substrate is to supply the plant with water and oxygen, for its growth through the root system. Common soilless plant growth substrates include rockwool, vermiculite, perlite, clay beads, and coconut fiber. They have specific water and oxygen retention capacities [34,35]. Soilless plant substrates can affect nutrient availability, physiological processes, plant growth, yield quantity, and quality [36–39].

The goal of this work was to investigate the agronomic feasibility and economic viability of growing a medicinal plant in a modified shipping container farm as an alternative crop cultivation system by analyzing the cost requirements and the resulting crop yield.

2. Materials and Methods

2.1. Chemicals and Reagents

Potassium hydroxide and sulfuric acid were purchased from VWR International (Leuven, Belgium). Rockwool cubes (25 × 25 × 40 mm) for the germination of seeds were purchased from Grodan (Roermond, The Netherlands). Cultivation substrates were Grodan Delta Rockwool® blocks (75 mm × 75 mm), coconut fiber, and GROROX® clay beads from Terra Aquatica (Fleurance, France). Nutrient solutions were ready-to-use solutions designed for hydroponic culture from Mills Nutrients (Aalsmeer, The Netherlands) and from Plagron (Weert, The Netherlands). Plagron Hydro A has an NPK of (3-0-1) with 4.2% Ca, and Hydro B has an NPK of (1-3-6) with 1.4% MgO. Mills Nutrient Basis A has an NPK of (3-0-1) with 4% Ca and Mills Nutrient Basis B has an NPK of (0-4-3) with 1% Mg. Mills Nutrient Basis A/B was used in the trial 2 of the substrate experiment. Plagron Hydro A/B was used in all others experiments.

2.2. Vertical Hydroponic Container

The experiments took place in a 30 m^2 vertical environment-controlled horticultural ready-to-use production unit from Urban Crop Solutions (Waregem, Belgium) (Figure 1).

Figure 1. Modified vertical hydroponic shipping container.

The container had the following characteristics:

- Dimensions: 12,192 mm × 2438 mm × 2896 mm
- Germination area: Eight PE food-grade plastic trays (1220 mm × 560 mm) at 4 levels equipped with separate manual irrigation valves and LED lights adjustable in intensity allowing a maximum of 2400 seeds to germinate.
- Cultivation area: Thirty-six food-grade plastic trays (1220 mm × 560 mm) with 2 irrigation systems and at 3 levels, each 600 mm high, equipped with automatic irrigation valves and LED lights adjustable in intensity. Each cultivation tray had a capacity of 24 plants (Figure 2).
- Irrigation system: A deep-water irrigation system supplied irrigation for 1 min every 5 min, with water recirculation to the water reserve. The irrigation system included a water reserve (800 L), a connection to the reserve of concentrated nutrient solutions, peristaltic pumps, the piping assembly sized according to the required flow rates, the flow control valve, filters (UV and physical filters) for water recirculation, and a control station connected to the LED control circuit and to various measuring instruments—including a pH-meter, an EC-meter, a kWh meter, and a CO_2 concentration sensor.
- LED lighting: For the substrate experiment (see Section 2.4), LEDs with an irradiance of 150 $\mu mol\ m^{-2}\ s^{-1}$ at a distance of 30 cm were used. The LED spectrum was composed of 35% blue (450 nm) and 65% red (660 nm). For the light intensity experiment (see Section 2.5), LEDs adjustable up to 500 $\mu mol\ m^{-2}\ s^{-1}$ were used. The spectrum was composed of 20.8% blue, 22.7% green, 52.5% red, and 4% far red.

Figure 2. Cultivation area inside the modified vertical hydroponic shipping container. Thirty-six cultivation trays were placed on either side of the alley. Each cultivation tray could hold 24 plants distributed in bands of 8 plants in the cultivation tray as follows: along the wall, in the center of the tray, and along the alley.

The container was controlled using a remote monitoring application that allowed the user to program culture conditions and to have an overview of the environmental parameters. Apart from the air conditioning unit, all the components were located inside the container.

2.3. *Euphorbia peplus Growing Conditions*

Seeds were obtained from greenhouse cultures of *E. peplus* until flowering and seed formation, after 90 days of culture. *E. peplus* seeds were sown in rockwool and irrigated every four days to remain moistened. The plants at the two-leaf stage were transplanted to the hydroponic substrates. The total duration of the experiment was 15–20 days for germination and 47–50 days of cultivation.

2.4. *Substrate Experiment*

Three hydroponic substrates were tested: rockwool, coconut fiber and clay beads, under 150 μmol m^{-2} s^{-1} irradiation. The culture parameters were set as follows: pH = 6.2, temperature = 23 °C day and 18 °C night $+/-1$ °C, relative humidity = 80% $+/-$ 10%, CO_2 concentration = 400 μmolmol^{-1}, and a photoperiod of 18 h day/6 h night. The electro conductivity was set to 0.16 Sm^{-1}. Two (trial 1) or six (trial 2) cultivation tray replicates were prepared for each substrate, corresponding to a total of 48 or 144 plants per substrate. Two independent experiments were performed.

2.5. *Light and Localization Experiment*

Two light intensities were investigated to evaluate the effects of different PPFDs on growth. The light intensity was adjusted to obtain 250 and 500 μmol m^{-2} s^{-1}. The culture parameters were identical to those of the substrate experiment except for the electro conductivity which was set to 0.2 Sm^{-1}. The culture substrate was rockwool. Six or 5 cultivation tray replicates were prepared for each light intensity, and a total of 120 or 144 plants per light intensity was tested. The effect of plant localization was measured by dividing the cultivation tray into subunits of 8 plants according to their localization: along the wall, in the center of the tray, or along the alley (see Figure 2). Two independent experiments were performed.

2.6. Biomass Accumulation Measurements

Fresh and dried biomass measurements were performed using an electronic scale (precision = 0.01 g). The drying of the vegetative system was realized in an oven at 40 °C for 5 days.

2.7. Apical Growth Measurements

The height of the fresh harvested plants was measured using a graduated slat from the base of their stem to their apex.

2.8. Total Ingenol Measurements

To evaluate the impacts of the culture parameters (substrate, light, localization) on the production of ingenol by the plants, a total ingenol quantification method was internally developed by Celabor (Herve, Belgium) based on the protocol reported by [40], excluding the methanolysis step to be able to specifically identify ingenol mebutate and ingenol. Other ingenol esters were not quantified, as they were found in insignificant amounts in comparison of the 2 main compounds.

A specific extraction protocol to obtained UPLC-quality grade samples was developed. After grinding the dried plants with a 250 µm sieve, 100 mg of accurately weighed sample was placed in a 2.0 mL volumetric flask, 1.5 mL of methanol was added, and the flask was placed in an ultrasound bath for 3 cycles of 5 min each, with vortexing in-between. Then, the flask was completed to 2 mL with methanol and filtrated with PTFE 0.22 µm (Millipore) into a UPLC vial and injected for analysis.

A UPLC-DAD-MS/MS system (Waters SA) was employed, equipped with an Acquity UPLC device including a quaternary pump, autosample and column thermostats, an Acquity PDA UV/Visible Detector, and a Xevo mass spectrometer. UPLC separation was performed by injecting 2 µL via the autosampler on Waters Acquity UPLC BEH C18 (100 × 2.1 mm i.d. and 1.7 µm particle size) at a flow rate of 0.5 mL min^{-1}. The mobile phase was composed by (0.6% formic acid + 2 mM ammonium formate) aqueous buffer at pH 2.4 (A) and acetonitrile (B) with the following gradient: from 80% to 20% of A for 6 min, then from 20% to 0% of A for 0.5 min, an isocratic mode at 0% of A for 2.5 min, and finally a linear gradient to 80% of A for 2 min, reconditioning for 3 min (total time of 14 min). The temperature of the oven column was programmed at 40 °C.

Stock solutions of ingenol (1.0 mg/2 mL) and ingenol mebutate (1.0 mg/2 mL) from Bio-Connect (Huissen, The Netherlands) were prepared in methanol, and were diluted with methanol to prepare a series of standard working solutions.

2.9. Extraction Procedures

Three extraction procedures were carried on the base of a selection work done during "Tropical Plant Factory" research program, focusing on the environmental impact of the solvents used. Ethyl acetate is usually reported to have lower environmental risks than methanol [41] and is also reported to be purification solvent for isolation of ingenol mebutate [42]. Moreover, ethyl acetate extracts of euphorbiacea are reported to have strong antiviral and antitumoral activities [43,44]. In addition, in order to reduce the use of organic solvent, supercritical CO2 was also considered as cosolvent, as it is already reported to efficiently extract diterpenes from coffee [45].

The aerial parts of the *E. peplus* biomass produced during the experiments described above were collected and dried at 40 °C. The resulting dry material was milled using a 4-mm sieve with a SM200 Retsch cutting mill. In order to have enough material for the extraction processes trials on the same sample, the biomasses obtained from every experiment were pooled and homogenized. The global dry content was estimated to be 11% of the fresh biomass.

Supercritical CO_2 extraction was done on a 100 mL SFE lab-scale device: 15 g of dried biomass were exposed to a mixture of CO_2 + 5% ethyl acetate (*w*/*w*) at 60 °C under 350 bar for 15 min, at a flow rate of 50 g/min. The remaining ethyl acetate was evaporated under reduced pressure.

Hot ethyl acetate extraction was done on a Thermo-Dionex ASE350 device: 5 g of dried biomass were transferred into an 11-mL extraction cell. The material was extracted by ethyl acetate at 120 °C under 100 bar by applying 2 cycles of 15 min. Then, ethyl acetate was evaporated under reduced pressure.

As a comparison, dried biomass was also extracted by ethyl acetate at room temperature. In brief, 10 g of dried material were suspended in 100 mL of ethyl acetate in a 250-mL Erlenmeyer flask. The resulting solution was mixed using a magnetic stirrer at room temperature (25 °C) for 60 min. After filtration, ethyl acetate was evaporated under reduced pressure.

Mass yields of the extraction processes were determined gravimetrically after solvent evaporation and ingenol mebutate was quantified in the dry extracts after solubilization in methanol according to the protocol described in Section 2.8.

2.10. Economical Evaluation of *E. peplus* Production

Cultivation and extraction were monitored from an operational point of view, and integrated into a budget to assess profitability. All the investments and actions related to the culture were listed to monitor them and assess profitability by assessing the price of the final product.

2.11. Data Analysis

For the substrate experiment, a one-way analysis of variance (ANOVA) was performed to test the significance of differences caused by "substrate" in measured values (height, fresh biomass, dried biomass, and total ingenol content). In the light and localization experiment, a two-way analysis of variance (ANOVA) was performed to test the significance of differences in the factors "light" and "localization" and their interactions with measured values. Prior to this analysis, the homogeneity of variance was tested using the Levene test, and the normality of data was tested using Anderson–Darling and Ryan–Joiner tests. ANOVA results with p under 0.05 were considered as significant.

To identify the means or medians that contributed to the ANOVA effect, a Student t-test or Wilcoxon test on data not normally distributed was performed. An adjustment for multiple comparison was applied with Holm corrections for multiple testing. Differences at $p < 0.05$ were considered significant.

All of these calculations were conducted using R version 4.0.2. and Minitab® 19.2020.1 (64-bit) software.

3. Results and Discussion

3.1. Substrate Experiment

Three hydroponic substrates were tested, and fresh biomass, dried biomass, height, and total ingenol were measured. Results are showed in Figure 3 and Table 1.

Table 1. Mean and standard deviation of total ingenol (mg/kg) measured in aliquots of 24 plants in two independent hydroponic cultivations of *E. peplus* in three substrates (rockwool, coco fiber, and clay beads). Student's comparison of means was applied with 95% confidence; means that do not share a letter are significantly different.

	Trial 1		**Trial 2**	
Substrate	**Number of Aliquots**	**Total Ingenol**	**Number of Aliquots**	**Total Ingenol**
Clay beads	2	61.8 ± 1.57 ^{a}	6	60.0 ± 12 ^{a}
Coco Fiber	2	59.9 ± 3.84 ^{a}	6	63.5 ± 8.84 ^{a}
Rockwool	2	61.7 ± 1.41 ^{a}	6	61.8 ± 5.17 ^{a}

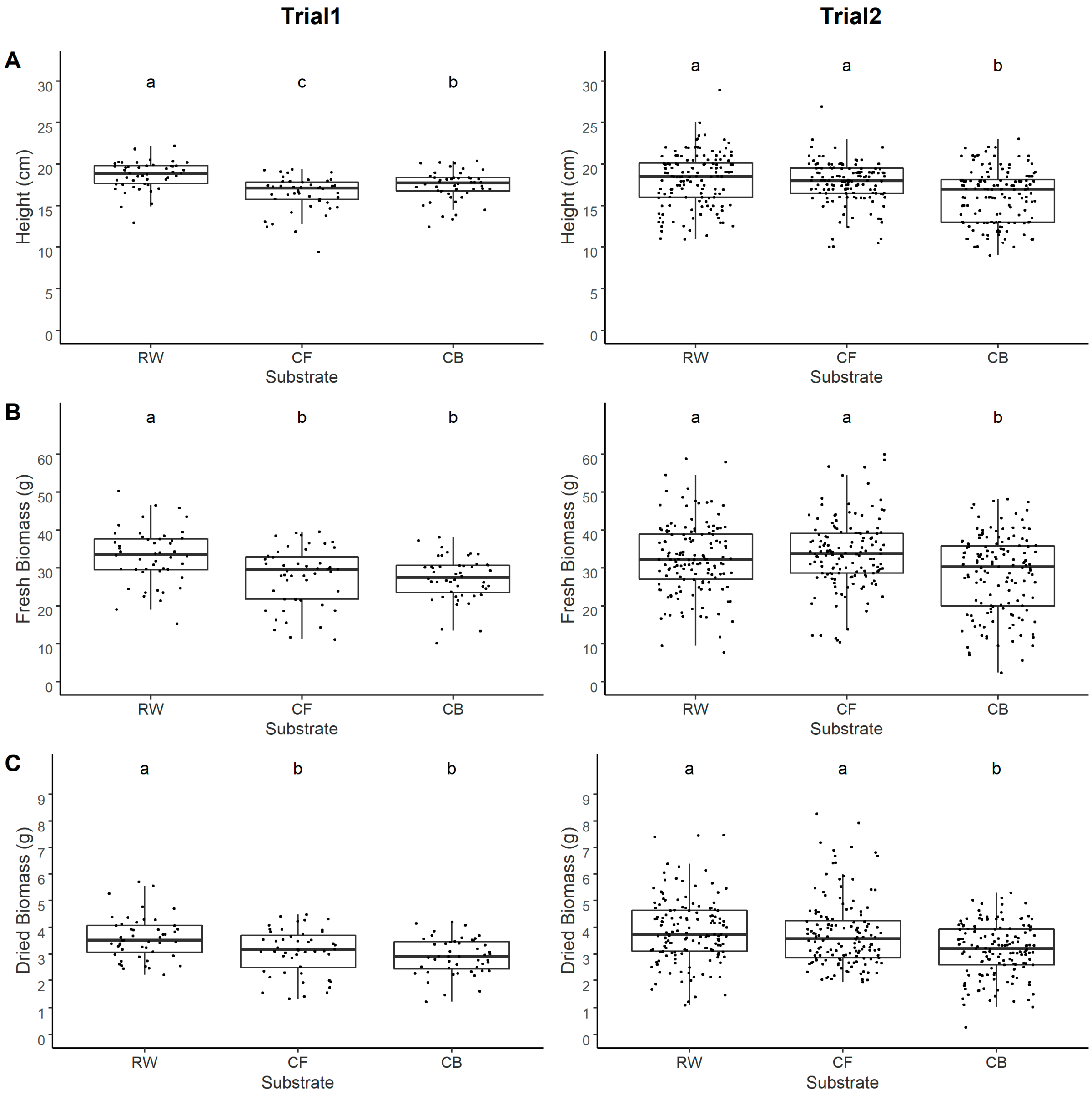

Figure 3. Boxplot of *Euphorbia peplus* height (**A**), fresh biomass (**B**), and dried biomass (**C**) according to the substrate (RW: rockwool; CB: clay beads; CF: coco fiber) in two independent experiments. Student or Wilcoxon's comparison test was applied with 95% confidence; means or medians that do not share a letter are significantly different. Trial 1: *N* = 48 plants. Trial 2: *N* = 144 plants.

The fresh and dried biomass increased when *E. peplus* was grown on rockwool, was intermediate on coco fiber, and decreased on expanded clay beads. Rockwool has very favorable aeration properties, but a low water buffering capacity and hydraulic conductivity characteristics that may lead to insufficient water and nutrient uptake, with the development of water stress symptoms in case of insufficient irrigation [46,47]. In our experimental conditions, the limited water buffering capacity of rockwool was bypassed by constant water availability.

Although coconut fiber is described as having a high water-holding capacity, and good drainage and aeration properties [48], the biomass was lower than or similar to that of rockwool, with a variability between the two trials, which could be linked to the change of nutritive solution and high degradation of coco fiber. Coconut fiber posed a filtering problem due to greater degradation of the substrate, and hence more labor was needed to clean the filtering system very regularly.

While clay beads have good aeration characteristics and can be reused, they have a low water-holding capacity [46]. The yield was lower and the crop had lower aerial part development, and exhibited fresh biomass decreases of 18% and of 13% compared to rockwool in trials 1 and 2, respectively.

The total ingenol content was not influenced by the hydroponic substrate in our experimental conditions. Rockwool was the most appropriate hydroponic substrate for growing *E. peplus* under deep-water irrigation with enhanced yield, which is an important factor for reaching economic viability.

3.2. Lighting and Localization Experiment

The effects of light intensity and plant localization on plant height, fresh biomass, and dried biomass were tested in two independent trials. Both factors were statistically significant ($p < 0.001$). The interaction between the two factors was also statistically significant, showing that each factor cannot be interpreted independently. Results are shown in Figure 4.

In the field, plant localization has been showed to have an impact on yield. Compared to mild-field yields, winter wheat yields in various edges decreased from 7.5% to 17.5% depending on the type of edges [49]. The lower yields at the field borders are explained by several factors, such as limited fertilizer inputs, soil compaction, reduced chemical inputs, or competition for water and nutrients by forest borders and hedgerows, but the main factor is lower solar irradiance in those edge regions due to shading [49,50].

In container cultivation, air conditioning provides continuous homogenous ventilation, and nutrient and temperature levels are homogenous across the surface area. However, shifts in PAR intensity may occur depending on the plant localization inside a tray. Moreover, the plants close to the alley have more space to develop, and shading from surrounding plants is reduced.

In cultivation under 500 PAR, better growth was observed in the middle of the trays, and reduced growth at the edges. The mean decrease in fresh biomass at the edges was 8.2% in trial 1 and 14.6% in trial 2. A mean PAR loss of 12.5% was observed in the alley-localized plants, and it was 9.2% in the wall-localized plants, as compared to the center-localized plants. The wall acted as a light reflector that reduced the decrease in PAR.

Under 250 PAR, the pattern was different. Growth was better along the alley, followed by a 35% yield reduction along the wall and 14% in the centers of the trays in the first trial, and a 21% yield reduction along the wall and 13% in the centers of the trays in the second trial. A loss of PAR was also observed at the edges, but to a lesser extent (3.3% along the wall and 7.3% along the alley). Increased space availability reduces interplant shading, allows better red/far-red light ratios within the canopy, and decreases competition from other plants, so that more energy is allocated to developing biomass [51,52]. The plants along the alley benefited from a spectrum likely to slightly differ from the spectrum received by the plants located at the centers of the trays and along the wall, which may have favored better growth at lower PPFD [53].

In less favorable locations compared to favorable ones (center or alley), a decreased PAR had a greater impact on fresh biomass yield losses, with a mean loss of 21% under 250 PAR compared to 11.4% under 500 PAR: higher light intensity allowed the plants located in less favorable locations to better catch up with their growth delays. The plants combining 250 PAR and a localization near the wall had more difficulties in growing than the plants in all other combinations. Distinct apical growth was observed between both trials. Part of the variability linked to the nutritive solution cannot be excluded, as the solely nutritive parameter measured continuously was electroconductivity.

In the substrate trial with an intensity of 150 PAR, the mean fresh weight of euphorbia cultivated in rockwool was 32.7 g. With an extended spectrum and light intensity, the mean fresh weights reached 69.1 g (250 PAR) and 102 g (500 PAR), which represent increases in mean shoot fresh weight by 111% and 212%, respectively, compared to the 150 PAR experiment. The same trend was observed for dry biomass. In addition to the effect of increasing of light intensity, the addition of green light at 250 and 500 PAR might increase photosynthesis in the lower plant canopy and increase total plant yield because the green wavelength better penetrates through the deeper plant canopy [54–56].

The total ingenol content was measured in the aerial parts of the plants (Table 2). No difference was measured under different light intensities or based on plant localization. The mean content was 70.6 mg/kg in trial 1 and 61.7 mg/kg in trial 2. The production of bioactive substances can be stimulated in response to environmental stress [57]. In particular, higher temperature results in increased terpenoid yield [58–63]. Salt stress also induces changes in terpene production [64,65]. Such factors should be further investigated to increase the ingenol content of *E. peplus*.

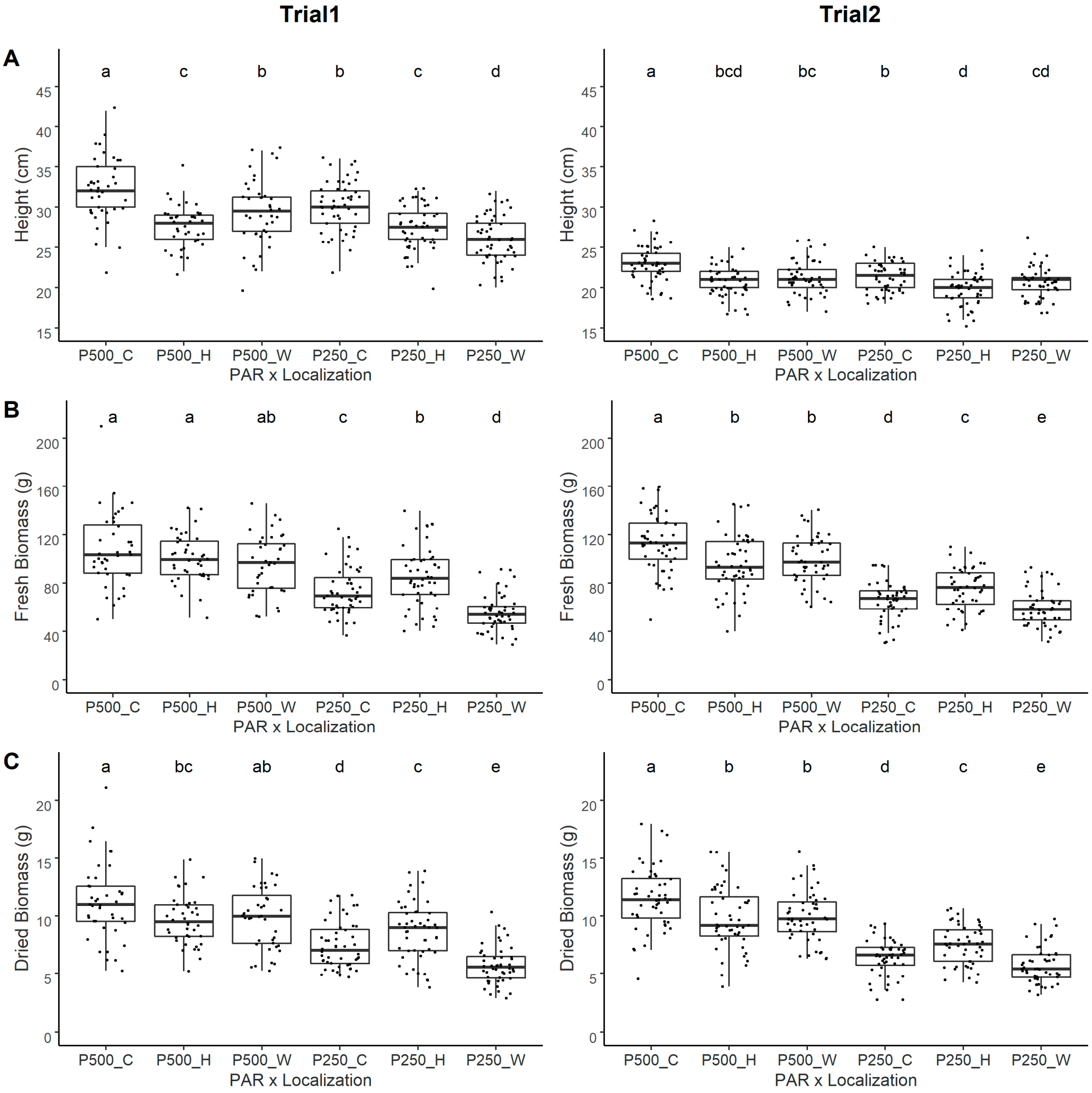

Figure 4. Boxplot of *Euphorbia peplus* height (**A**), fresh biomass (**B**), and dried biomass (**C**) under the interaction of light intensity (P250: 250 PAR; P500: 500 PAR) and the plant localization in the cultivation tray (W: wall; C: center; H: alley) in two independent experiments. Wilcoxon's comparison of medians was applied with 95% confidence; medians that do not share a letter are significantly different. Trial 1: PAR 250: *N* = 48 plants per localization; PAR 500: *N* = 40 plants per localization. Trial 2: *N* = 48 plants per localization and light intensity.

Table 2. Total ingenol mean content and standard deviation in the aerial parts of *E. peplus* as a function of light intensity or plant localization in two independent experiments. Total ingenol was measured in aliquots of 8 plants. Student's comparison of means was applied with 95% confidence; means that do not share a letter are significantly different. Light intensity: Trial 1: PAR 250: N = 18 aliquots; PAR 500: N = 15 aliquots; trial 2: N = 18 aliquots. Localization: Trial 1: N = 11 aliquots; trial 2: N= 12 aliquots.

		Trial 1			**Trial 2**		
Light Intensity	(µmolm^{-2} s^{-1})	**250**	**500**		**250**	**500**	
Total Ingenol	(mg/kg)	74.7 ± 25.4 ^{a}	65.7 ± 19.6 ^{a}		60.9 ± 2.61 ^{a}	62.4 ± 3.08 ^{a}	
Localization		**Center**	**Alley**	**Wall**	**Center**	**Alley**	**Wall**
Total Ingenol	(mg/kg)	71.5 ± 24.6 ^{a}	72.4 ± 24.1 ^{a}	68.0 ± 22.3 ^{a}	62.8 ± 1.81 ^{a}	61.3 ± 3.63 ^{a}	60.9 ± 2.88 ^{a}

3.3. Economic Evaluation of E. peplus Production

The cost price is an economic term that refers to all the costs supported by a company to produce a goods/a service. It includes direct costs and indirect costs. Indirect costs are expenses not directly linked to the production of the product/service (advertising, rental of premises, salaries, etc.). Different calculation approaches exist: variable cost price, direct cost price, coefficient method, and activity-based costing [66]. Therefore, a company supplying different products and services has to choose the right analysis in order to understand how much a service or a product costs.

In this study, all the costs are directly related to the production activity.

The study will be useful to forecast an economical evaluation of (i) raw chemical production, and (ii) pharmaceutical production based on ingenol-mebutate. The forecast calculation for the pharmaceutical market is based on assumptions and general costs. The objective is to verify the economic viability of this type of model.

3.3.1. Economic Comparison of *E. peplus* and Romaine Lettuce Production

We compared the cultivation cost prices of *E. peplus* and Romaine lettuce in a modified shipping container. Results are showed in Table 3.

The economic feasibility of medicinal *E. peplus* production was calculated based on (i) the substrate test under light conditions of 150 PAR and (ii) the light trial under 500 PAR. The results are expressed in Table 3 in the "R&D Container" columns. Those results were also projected on a 10-sqm larger "R&D+ Container" with one more shelf above the top one. The romaine lettuce results are projected according to the technical possibilities offered by a commercial container optimized for smaller plant production with an extra space of 10 m^2 from the same supplier, similarly to the R&D containers in terms of layout (a germination corner, a cultivation corner, and a central alley). The output represents the fresh biomass produced in one year per container, including 5% quality loss.

Capital expenditure (capex) represents major long-term expenses. The "R&D container" capex is the price of a research container with an LED light of 150 PAR, or adapted with the cost of replacing initial lighting by modular lighting up to 500 PAR. The capex of the commercial container was obtained from Urban Crop Solutions.

Operating expenses (opex) represent the day-to-day expenses to keep a company running. They include staff costs and daily costs necessary to generate outputs such as electricity, water, substrates, and fertilizers. The opex range per year for *E. peplus* cultivation varied from €28,597 for the 150-PAR R&D container to €36,505 for the 500 PAR-R&D+ container: the opex increased by 27% when the size of the cultivation area was increased. In parallel, *E. peplus* production yield varied from 192 to 776 kg per year, i.e., a 304% increase. This highlights that optimizing the cultivation area and growing conditions is significant for the output of a crop and the calculation of its economic feasibility.

Table 3. Cultivation costs of *Euphorbia peplus* and Romaine lettuce in several scenarios according to the light intensity and growing surface, generating fresh biomass, output, capex, and opex. The cost per kg of fresh biomass includes capex and opex; the contribution of each particular cost to the total cost was calculated as a percentage. The values mentioned under the "R&D" container are experimental results, and the values mentioned under the "R&D+" container and "Commercial container" are projected results.

Crop		***Euphorbia peplus***								**Romaine Lettuce**	
Light	(μmolm^{-2} s^{-1})	150				500				150	
Fresh Biomass per crop	g	32.7				102				102	
		R&D container		R&D+ container		R&D container		R&D+ container		Commercial container	
Total Growing surface (sqm)	sqm	30		40		30		40		50	
Fresh Biomass (incl. 5% quality loss)	(kg/yr/sqm)	6.4		6.34		19.97		19.39		34.04	
OUTPUT	(kg/yr)	192		254		599		776		1702	
CAPEX	(EUR/sqm)	3500		3000		3833.33		3375		3100	
CAPEX (15-yr depreciation)	(EUR/yr)	7000		8000		7667		9000		10,333	
OPEX											
Technical Staff at €210/day	(EUR/yr)	12,023		13,395		12,023		13,395		19,467	
Engineer staff at €310/day	(EUR/yr)	4437		4394		4437		4394		1465	
Director staff at €600/day	(EUR/yr)	2147		2126		2147		2126		709	
Electricity at €0.2/kW	(EUR/yr)	6650		8845		9177		12,206		10,964	
Water at €4.94/m^3	(EUR/yr)	33		45		34		45		50	
Seeds	(EUR/yr)	0		0		0		0		75	
Fertilizer	(EUR/yr)	1001		1319		1001		1319		810	
Substrates (rockwool)	(EUR/yr)	1255		1657		1255		1657		934	
pH adjustors	(EUR/yr)	47		62		47		62		76	
Container maintenance	(EUR/yr)	1001		1301		1001		1301		1626	
TOTAL	(EUR/yr)	**28,597**		**33,144**		**31,124**		**36,505**		**36,176**	
COST per kg of fresh biomass											
CAPEX (15-yr depreciation)	(EUR/kg)	36.44	20%	31.54	19%	12.80	20%	11.60	20%	6.07	22%
Labor Technical staff	(EUR/kg)	62.59	34%	52.81	34%	20.07	31%	17.27	29%	11.44	42%
Labor Eng. staff	(EUR/kg)	23.10	12%	17.32	11%	7.41	11%	5.67	10%	0.86	3%
Labor Director staff	(EUR/kg)	11.18	6%	8.38	5%	3.58	6%	2.74	5%	0.42	2%
Electricity	(EUR/kg)	34.62	19%	34.87	21%	15.32	24%	15.74	27%	6.44	24%
Water	(EUR/kg)	0.18	0%	0.18	0%	0.06	0%	0.06	0%	0.03	0%
Seeds	(EUR/kg)	-	0%	-	0%	-	0%	-	0%	0.04	0%
Fertilizer	(EUR/kg)	5.21	3%	5.20	3%	1.67	3%	1.70	3%	0.48	2%
Substrates (rockwool)	(EUR/kg)	6.53	4%	6.53	4%	2.09	3%	2.14	4%	0.55	2%
pH adjustors	(EUR/kg)	0.24	0%	0.24	0%	0.08	0%	0.08	0%	0.04	0%
Container maintenance	(EUR/kg)	5.21	3%	5.13	3%	1.67	3%	1.68	3%	0.96	3%
TOTAL	**(EUR/kg)**	**185.31**		**162.22**		**64.74**		**58.67**		**27.33**	

When calculating the cost per kg of produced *E. peplus*, the most expensive scenario was the R&D container with low-power LEDs because productivity was lowest. The container can be used 7.1 times a year, accounting for container cleaning and harvest. A higher capacity requires more labor and electricity. The cultivation of a plant for producing a pharmaceutical drug requires regular quality monitoring by qualified labor. Labor contributed from 52% to 44% of the total crop cost, followed by capex (19–20%) and electricity (19–27%). The enhanced productivity in the 500-PAR R&D and R&D+ containers drastically decreased the crop cost per kg by 65% and 68%, respectively. As a consequence, the total production cost per kg of fresh *E. peplus* ranged from €59 to €185.

We simulated the cultivation of a common vegetable in the container to compare the production cost of a medicinal crop with that of a traditional leafy-green crop in vertical container farming. Romaine lettuce was grown in short hydroponic culture, and harvested after 25–30 days of cultivation, at a mean biomass of 102 gr. The container can be used 11.6 times per year, accounting for container shutoff for harvest, cleaning, and re-planting. Projections showed that productivity could reach almost 2 tons per year due to a shorter cultivation time and greater utilization of space with an enhanced surface area of 50 m^2. Therefore, the biomass per crop, the surface area, and the culture cycle are important factors when considering productivity. Labor needs were greater owing to a quick turnover and pre- or post-cultivation work such as seeding, harvesting, and packaging. On the other hand, the need for qualified labor was lower. The total production cost of 1 kg of romaine lettuce in the commercial container was estimated to be €27.33, including 47% for labor, followed by capex (22%) and electricity (24%). Table 4 shows the retail sales and purchase prices of Romaine lettuce in several cities. The retail price of Romaine lettuce in Belgium is about five times lower than in Singapore or New York, which makes the Belgian market difficult to access. The purchase price per kg at 50% gross margin in Singapore is still about two times lower than the production cost. Those actual production costs make it very difficult to compete against traditional growing methods and confirm that profitability of vertically grown traditional leafy greens is difficult to reach with the actual design of the modified shipping container. The next steps for leafy-green vertical farming would be continuing improvements in the factory engineering and design, to reduce capex and opex and reach affordable food costs [18].

Table 4. Retail sales prices and retail purchase prices at 50% gross margin for Romaine lettuce [18].

		Retail Sales Prices	**Retail Purchase Prices (at 50% Gross Margin)**
Europe-Belgium	(EUR/kg)	5.00	2.50
USA-New York	(EUR/kg)	22.00	11.00
Asia-Singapore	(EUR/kg)	26.00	13.0

3.3.2. Economic Evaluation of the Production of Ingenol-Mebutate as a Raw Material

The cost of ingenol-mebutate extraction from *E. peplus* was evaluated following three methods: ethyl acetate at 120 °C, ethyl acetate at room temperature, and supercritical CO_2. The costs were estimated based on the biomass generated during the cultivation process, the extraction yields, and the capex and opex costs. Results are showed in Table 5.

Extraction started with the drying of the plant. Eleven percent of residual biomass was obtained from fresh biomass after drying, corresponding to 66 to 85 kg per year under the 500 PAR scenario. Those outputs represent a very low load for industrial drying, grinding, extraction, and purification devices, which can handle much more biomass. To take the low level of occupation of the devices into account, the occupation rate of the devices was set to 10% for the 500 PAR scenario. The best extraction yields were obtained with ethyl acetate at 120 °C, followed by supercritical CO_2 and then ethyl acetate, both at room temperature. In the CAPEX, the extraction and purification devices represented the highest costs. Opex were higher with extraction at 120 °C due to higher needs in operators and electricity, followed by extraction with supercritical CO_2, which also induced high costs in operators and electricity. Extraction yield with ethyl acetate at room temperature and with supercritical CO_2 were decreased by 45.31% and 26.3% compared to ethyl acetate at 120 °C, respectively.

Table 5. Extraction costs of ingenol-mebutate from *Euphorbia peplus* following three extraction methods (ethyl acetate at 120 °C, ethyl acetate at room temperature, supercritical CO_2), and according to the biomass generated in the container R&D and R&D+ under 500 PAR. Extraction yields are experimental results.

EXTRACTION COSTS				
		Extraction Method		
		Ethyl Acetate 120 °C	**Ethyl Acetate Room Temp.**	**Supercritical CO_2**
OUTPUT				
Dried biomass R&D+ container	(kg/yr)	85	85	85
Dried biomass R&D container	(kg/yr)	66	66	66
Ingenol-mebutate per dried kg	(mg/kg)	43.76	23.94	32.17
CAPEX				
Drying equipment	(EUR)	15,000	15,000	15,000
Grinding equipment	(EUR)	20,000	20,000	20,000
Extraction pilot	(EUR)	500,000	100,000	800,000
Evaporation equipment	(EUR)	30,000	30,000	30,000
Purification equipment	(EUR)	500,000	500,000	500,000
Occupation Rate	(%)	10	10	10
TOTAL CAPEX (20-yr depreciation)	(EUR/yr)	5325	3325	6835
OPEX				
Technical Staff at €210/day	(EUR/batch)	840	840	840
Engineer staff at €310/day	(EUR/batch)	620	310	620
Director staff at €600/day	(EUR/batch)	600	300	600
Electricity at €0.2/kW	(EUR/batch)	4000	800	4000
Water at €4.94/m^3	(EUR/batch)	49.4	49.4	0
Solvent (CO_2, EtOAc)	(EUR/batch)	228.10	228.10	199.3
Filtration/Evaporation/Concentration	(EUR/batch)	2500	3500	500
Purification consumables	(EUR/batch)	1500	1500	1500
Purification solvents	(EUR/batch)	1500	1500	1500
Equipment maintenance	(EUR/batch)	200	200	500
Total OPEX costs/batch	(EUR/batch)	12,037.5	9,227.5	10,259.30
Total OPEX costs/year	(EUR/yr)	90,281.25	69,206.22	76,944.75
CAPEX + OPEX	(EUR/yr)	95,606.25	72,531.22	83,769.75

The cost of 1 mg of ingenol-mebutate was calculated (Table 6). The low extraction yield of ethyl acetate at room temperature was not compensated by its reduced cost: the production cost per mg was the highest. The cheapest method was extraction using ethyl acetate at 120 °C: the production cost per mg was €37.80. The cost price was calculated by adding flat fees to the production cost. The flat fees, including commercial works, administrative works, and bottling were evaluated at 2 to 3 euros per mg, hence the cost price ranging from 40 to 73 euros per mg.

When comparing cost price obtained with the ethyl acetate at 120 °C extraction method, with the market price of ingenol-mebutate as a raw chemical product (Table 7), we should note that the price of units of ingenol-mebutate from suppliers of laboratory products varies greatly. As a consequence, the potential gross margin per year and per R&D or R&D+ container showed a wide range from 5162 to 311,557 EUR for the 1 and 5 mg units, the market price for 10 mg being too low to generate a gross margin.

The selection of the appropriate extraction method allowed us to increase the extraction yield. However, the plant content in ingenol-mebutate was low, so that the extraction yield remained low too. Previous extraction works on *E. peplus* showed a low yield of about 1.1 mg/kg [67]. Other ways of generating ingenol-mebutate have been explored. Semi-chemical synthesis of ingenol mebutate from ingenol has also been developed, at a greater yield of ~250 mg/kg [68,69]. Total chemical synthesis of ingenol was proposed in the early

2000s. The isolation procedure was complex and costly (37 to 46 steps), and yields ranged from ca. 0.1% overall yield to 80% average yield per step [70–72]. Simplified synthesis of ingenol-mebutate in 14 steps has been developed, but no information on yield and cost has been provided [73].

Table 6. Production cost and cost price of ingenol-mebutate extracted from vertically cultivated *E. peplus* according to three methods (ethyl acetate at 120 °C, ethyl acetate at room temperature and supercritical CO2), calculated from the cultivation cost and extraction cost, and according the biomass generated in the "R&D" and "R&D+" containers with luminosity of 500 PAR. Cost price are production cost plus flat fees.

		Extraction Method		
		Ethyl Acetate 120 °C	**Ethyl Acetate Room Temp.**	**Supercritical CO_2**
OUTPUT				
Ingenol-mebutate per year in R&D container	(g/yr)	2.88	1.58	2.12
Ingenol-mebutate per year in R&D+ container	(g/yr)	3.73	2.04	2.75
CULTIVATION COST				
R&D container	(EUR/yr)	38,790	38,790	38,790
R&D+ container	(EUR/yr)	45,505	45,505	45,505
EXTRACTION COST	(EUR/yr)	95,606	72,531	83,770
CULTIVATION & EXTRACTION COST				
R&D container	(EUR/yr)	134,397	111,322	122,560
R&D+ container	(EUR/yr)	141,111	118,036	129,275
Production cost of Ingenol-mebutate				
R&D container	(EUR/mg)	46.6	70.5	57.8
R&D+ container	(EUR/mg)	37.8	57.8	47
Cost price of Ingenol-mebutate				
R&D container	(EUR/mg)	49	73	60
R&D+ container	(EUR/mg)	40	60	50

Table 7. Market price per unit of ingenol-mebutate from laboratory suppliers. Calculation of the potential gross margin per year and per container by comparison of the cost price in "R&D" and "R&D+" containers with luminosity of 500 PAR and the ethyl acetate at 120 °C extraction method, for each selling unit (1 mg; 5 mg; 10 mg). Supplier 1: MedChemExpress; supplier 2: MyBiosource.

	Market Price/Unit			**Potential Gross Margin (EUR/yr/Container)**					
				R&D Container			**R&D+ Container**		
	1 mg	**5 mg**	**10 mg**	**1 mg**	**5 mg**	**10 mg**	**1 mg**	**5 mg**	**10 mg**
Supplier 1	67	213	352	51,913	5162	-	100,803	48,535	-
Supplier 2	123.4	246.8	-	214,720	-	-	311,557	174,689	-

3.3.3. Economic Evaluation of Ingenol-Mebutate Production for Pharmaceutical Purposes

The feasibility of producing a medicine based on ingenol-mebutate was calculated following projective hypotheses and experimental results. The cultivation and extraction costs were based on experimental results. The development, gel production (formulation), and flat fees costs were hypotheses based on the literature and consultation. The economic feasibility of producing an ingenol-mebutate pharmaceutical product was calculated with Picato® gel, a prescription medicine containing ingenol mebutate and used to treat skin actinic keratosis. Two different dosages of the gel have been approved for use on the face and the scalp (0.015%) or the trunk and extremities (0.05%). Picato® gel was authorized for use in the EU in November 2012, but was withdrawn in June 2020 because the risks in actinic keratosis might outweigh the benefits. Further research should be led to develop new products based on metabolites of interest present in the latex of *E. peplus*. Moreover, although the medicine is not produced anymore, the present study showed the methodology and key elements for calculating the economic feasibility of producing a medicinal plant and could be transposed to other cases.

The economic evaluation of pharmaceutical production based on ingenol-mebutate Picato® gel is shown in Table 8.

Table 8. Production cost of pharmaceutical ingenol-mebutate from *Euphorbia peplus* according to the market characteristics of Picato® gel and the cultivation yields. The cultivation and extraction yields generated output, capex, and opex. Three scenarios according to the type of Picato® gel produced (0.015%, 0.05%, and a mix of the two items) were simulated. The return on investment was calculated according to the scenarios and to the annual biomass generated by cultivation in the R&D and R&D+ containers under two light intensities. A simulation with the best annual biomass multiplied by 10 (meaning running 10 shipping containers) is also presented. IngMeb: ingenol-mebutate.

Characteristics of Picato® gel						
A: Distributor Price/unit	(EUR/3 gel)	36.9				
B: Distributor Price/unit	(EUR/2 gel)	36.9				
Volume per gel tube	(g)	0.47				
A: 0.015% IngMeb	(μg IngMeb/gel)	70.4				
B: 0.05% IngMeb	(μg IngMeb/gel)	235				
Production cost of Picato® gel		**R&D container**	**R&D+ container**	**R&D container**	**R&D+ container**	**(R&D+ container): 10 units**
Cultivation yield (fresh biomass)	(kg/yr)	192	254	599	776	7756.4
Extraction yield at 0.0043758%	(g IngMeb/yr)	0.92	1.22	2.88	3.73	37.3
OUTPUT						
A: 0.015% IngMeb	(gel/yr)	13,064	17,336	40,954	53,015	530,152
B: 0.05% IngMeb	(gel/yr)	3934	5195	12,273	15,887	158,871
100% A- 3 gels/unit	(EUR/yr)	161,492	213,232	503,737	652,087	6,520,870
100% B- 2 gels/unit	(EUR/yr)	72,592	95,849	226,432	293,117	2,931,166
75% A-25%B	(EUR/yr)	139,267	183,887	434,411	562,344	5,623,444
CAPEX						
GMP gel production	(EUR)	200,000	200,000	200,000	200,000	400,000
Capex (30 yr depreciation)	(EUR/yr)	6667	6667	6667	6667	13,333
OPEX						
Development costs	(EUR)	300,000,000	300,000,000	300,000,000	300,000,000	300,000,000
Development cost (20-yr depreciation)	(EUR/yr)	15,000,000	15,000,000	15,000,000	15,000,000	15,000,000
Cultivation cot	(EUR/yr)	35,597	41,144	38,790	45,505	455,050
Extraction cost—EtOAc 120 °C	(EUR/yr)	92,944	92,944	95,606	95,606	143,531
Gel production cost (75% A-25%B)	(EUR/yr)	8123	10,725	25,338	32,800	327,999
Flat fees	(EUR/yr)	45,000	45,000	90,000	90,000	300,000
CAPEX + OPEX	(EUR/yr)	15,188,330	15,196,480	15,256,401	15,270,577	16,239,914
Return on investment—100% A	(yr)	94	71	30	23	3
Return on investment—100% B	(yr)	209	158	67	52	5
Return on investment—75%A-25%B	(yr)	109	83	35	27	3

The economic feasibility of producing a medicinal molecule is calculated from annual biomass production and the ingenol-mebutate extraction yield from the plant. These two values will fix the quantity of gel tubes that can be produced and the output. The price of Picato® gel in drugstores was €71.96 per packet-unit of three tubes at 0.015% or two tubes at 0.05%. Taking into account the gross margin of distributors and drugstores at 1.3 and 1.5, respectively, the sales price by the manufacturer can be estimated as €36.90. The output is linked to the productivity of cultivation and the efficacy of ingenol-mebutate extraction. It will generate from €161,492 to €652,087 per year if the sales are 100% of the gel at 0.015%. The enhanced productivity in the 40 sqm container at 500 PAR increased the output by 304%. If we consider that the sales are represented by 100% of the gel at 0.05%, the output depletes to the range of €72,592 to €293,117 per year. The constant price of the more concentrated gel is not compensated for by the lower number of tubes. In fact, the increase in ingenol-mebutate content causes a sharp decrease in the quantity of tubes produced and generates a lower turnover due to too low a price compared to the gel at

0.015%. This projection is not the most profitable one for the manufacturer. Supposing mixed production composed of 75% of gel at 0.015% and 25% of gel at 0.05%, the turnover would range from €139,267 to €562,344 per year.

The capex of the pharmaceutical company is represented by the tube production facilities. It can be estimated at a relatively low price due to the low number of tubes produced annually (53,000 maximum), taking into account that a pharma-GMP label is required.

The opex can be estimated based on the research and development, cultivation, extraction, gel production costs, and flat fees. The extraction costs for the research containers under 150 PAR and 500 PAR were estimated at occupation rates of 5% and 10%, respectively. The extraction cost with 10 units of R&D+ container under 500 PAR was estimated at an occupation rate of 100%. The gel production cost was estimated at a unit price of 0.75 EUR per tube. Flat fees include operational costs, such as renting a building, electricity and heating costs, and staff costs (administrative and commercial teams). They were estimated to be 300,000 EUR in the 10 R&D+/500 PAR scenario, in which the number of annually produced tubes reached 437,000 (75%A–25%B). In the other scenarios, the volume of annually produced tubes ranged from 11,000 to 43,700 (75%A–25%B); these are small quantities that do not require a 100% occupation rate for flat fees. We converted this low volume into an occupational rate of 15% for the R&D and R&D+ containers under 150 PAR, versus 30% under 500 PAR. The development costs of a drug include pre-clinical and clinical studies. The estimation of the average cost of drug development is difficult. It largely varies according to the studies that have to be carried out, from USD 92.0 million to USD 884 million, or even to USD 1395 million [74–76]. Moreover, the clinical costs of drug development vary depending on the treatment category. They range from USD 312 million for analgesics/anesthetics to USD 448 million for anti-infective drugs [74]. As Picato® is a topical product, an intermediate value was hypothesized for the development cost. Although we hypothesized a relatively optimistic development cost and allocated its cost over the term of a patent, this item represented the main cost, and all the other costs appeared as a very low load. Due to very high development costs, the return on investment would be about 100 years at the lowest productivity level, compared to around 30 years at the best productivity level. It would be necessary to multiply production by 10—for example, by having 10 highly productive containers, to reach a return on investment within less than 5 years, while hypothesizing that the market is sufficiently developed to absorb the number of tubes produced each year—about 152,000 units of 3 (75%) and 2 (25%) tubes.

Although the simulation of the profitability of Picato® gel showed that economic feasibility would be difficult to reach, certain factors could rapidly increase the profitability of ingenol production. The improvement in the ingenol content in the plant by a more specific and adapted cultivation process would increase the extraction yield rapidly. The doubling of the extraction yield by increasing the ingenol content through abiotic factors would reduce the return on investment time to 14 years. Furthermore, upcoming new plant factory designs with increased growing surfaces and planting densities will reduce the capex and the cost per mg of vegetable, and profitability will be less challenging [18].

4. Conclusions

The economic feasibility of producing a metabolite for pharmaceutical purposes is closely linked to the biomass yield, the concentration of the metabolite in the plant, and the extraction yield. A low biomass yield, a low phytomolecule content, and a low extraction yield complicate the economic feasibility of the process and should carefully be checked to assess profitability. Considering all vertical plant production, the biomass yield depends on the cultivation surface area, the length of growing cycle, the growing density of the plant, its biomass, and abiotic cultivation factors, such as light, temperature, substrate, and CO_2 content. Considering *E. peplus* production in the R&D+ container with enhanced light, we succeeded in increasing fresh shoot biomass by 200% by choosing the appropriate substrate and the appropriate light, and by increasing the surface area. The content in a specific metabolite is also an important factor for reaching economic viability. A low content in a specific metabolite negatively impacts the extraction yield and the final output of a medicinal product. In the specific case of ingenol-mebutate in *E. peplus*, its content is low. Therefore, testing abiotic factors to maximize the metabolite content is important for profitability, such as temperature and salt stresses in the particular case of ingenol-mebutate. The size of the cultivation plant is also an important factor, as we have showed that increasing the surface area by the use of several containers allows access to significant return on investment. Finally, the therapeutic dose of the phytomolecule in the drug and the selling price of the drug directly influence the potential turnover of the pharmaceutical company and return on investment. In our particular case, it was established that a complete return on investment might be reached between 3 and 5 years in case of high investments funds enable to acquire 10 containers.

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