Posterbeitrag zum Themenkreis: Geschützter Anbau

Rhodiola rosea: Potenzial des Anbaus in einem vertikalen Indoor Farming System zur Verbesserung der Produktion bioaktiver Substanzen

Rhodiola rosea *potential of cultivation in an indoor vertical farming system enhancing the production of bioactive substance*

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Zusammenfassung

Rhodiola rosea ist eine mehrjährige Heilpflanze, deren Wurzeln für die Pharmaindustrie bedeutsame Wirkstoffe enthält. Im Rahmen des Projekts “Aufbau eines Forschungsschwerpunkts Indoor Farming“ forschen die Fachbereiche Gartenbau und Biotechnologie der Hochschule Weihenstephan-Triesdorf gemeinsam an einer optimierten Kultivierung und der Nutzung des pflanzlichen Rohstoffes. Dabei steht insbesondere die Inkulturnahme von Rhodiola rosea in das hydroponische Kulturverfahren einer Indoor Vertical Farm im Vordergrund. Die erfolgreiche Inkulturnahme von *Rhodiola rosea* in den zwei unterschiedlichen Bewässerungssystemen, Deep Water und Aeroponik, lieferte erste Erkenntnisse hinsichtlich potenzieller Erträge in Indoor Vertical Farmen und ermöglicht fortlaufende Untersuchungen zur Identifikation einer angepassten Kulturführung. Für die chemische Analyse der Sekundärmetaboliten erfolgte eine Anpassung der Extraktionsmethode für Salidrosid, Rosavine und Tyrosol. Die Untersuchungen zu den Gehalten an Sekundärmetaboliten aus dem Feldanbau wurden an Modell-Pflanzenmaterial unterschiedlichen Alters durchgeführt. Hierfür wurden sowohl oberirdische als auch unterirdische Pflanzenteile miteinbezogen. Im mehrlagigen Indoor Farming System lag die Trockenmasse der jungen Rhodiola-Pflanzen nach 63 Tagen Kulturzeit bei 1,54 ± 0,77 g/Pflanze im Deep Water System und bei 0,91 ± 0,35 g/Pflanze im Aeroponischen System. In zukünftigen Versuchen sollen sowohl das Aeroponische System als auch die Einflüsse einer Indoor Vertical Farm auf die Produktion von Sekundärmetaboliten näher betrachtet werden. Diese und folgende Ergebnisse bilden die Basis für die Optimierung der Herstellung von *Rhodiola rosea* Produkten.

Keywords: *Rhodiola rosea*, Indoor Vertical Farming, Sekundärmetabolite, Gartenbau, Biotechnologie, Salidrosid, Rosavine.

Abstract

Rhodiola rosea is a perennial medicinal plant whose roots contain active ingredients that are of special interest to the pharmaceutical industry. As part of the project "Establishment of a research focus on indoor farming", horticulturists and biotechnologists at the University of Applied Sciences Weihenstephan-Triesdorf conduct joint research with an overall aim to optimize plant cultivation and a

holistic use of all plant raw materials. In particular, the focus is on the incultivation of Rhodiola rosea in the hydroponic cultivation systems of an indoor vertical farm. The successful cultivation of *Rhodiola rosea* in deep water and aeroponic as hydroponic systems, provided first insights regarding potential yields that can be reached in an indoor vertical farm as well as valuable data for the ongoing investigations to identify an adapted culture management. For the chemical analysis of secondary metabolites, an existing extraction method was adjusted for salidroside, rosavine and tyrosol. The investigations on the contents of secondary metabolites from field cultivation were carried out on model plant material. For this purpose, both above-ground and below-ground plant parts were included. In the multilayer indoor farming system, the dry weight of the *Rhodiola rosea* plants was 1.54 ± 0.77 g/plant in the deep water system and 0.91 ± 0.35 g/plant in the aeroponic system after a cultivation time of 63 days. In future trials, both the aeroponic system and the influences of an indoor vertical farm on secondary metabolite production will be considered in more detail. These and the following results will form the basis for optimizing the production of *Rhodiola rosea* products.

Keywords: *Rhodiola rosea*, indoor vertical farming, secondary metabolites, horticulture, biotechnology, salidroside, rosavins.

Introduction

Vertical farms are capable of cultivating crops on multiple layers and achieving high crop productivity and uniformity, without the need of crop protection chemicals (Carotti et al., 2021). Indoor vertical farming permits plant production in a completely closed environment that is isolated from external weather conditions, including sunlight. Instead of sunlight, artificial lights provide the energy needed for plant-photosynthesis (Mempel et al., 2021). This cultivation system allows the possibility to achieve high yield with a very small cultivation area while recreating the optimal growth conditions for the specific plant characteristics. The advantage of constant and controllable weather conditions in an indoor vertical farming system is to avoid negative effects of possible harmful climatic events, which are very common in open field systems, on the harvest and profit. Moreover, indoor vertical farming offers great opportunities for a consistent and sustainable production of plant raw materials all year round for the pharmaceutical, cosmetic and food industry (Wittmann et al., 2020). The main constraints of this system are the high costs that derive from the energy usage related to the level of technology adopted. One of the ways to make indoor vertical farming profitable is by cultivating high value, high yield plants. *Rhodiola rosea,* also known as “golden root” or “roseroot” naturally stores salidroside and rosavins in its roots that are of great interest for the pharmaceutical industry. Roseroot is a perennial plant that can reach a height of 36-70 cm and usually produces yellow blossoms (Brown et al., 2002). In its natural habitat, roseroot grows on poor soil and on rocks covered with a very thin layer of soil (Galambosi et al., 2014). This aspect combined with the plant morphology makes *Rhodiola rosea* very appealing for a soilless cultivation in an indoor vertical farming system. It has always been used as a medicinal plant but only in the last decades with newly developed methods of analysis, it was demonstrated that *Rhodiola rosea* root contains three cinnamyl alcohol-vicianosides (rosavin, rosin and rosarin) that are specific to this species (Dubichev et al., 1991). These secondary metabolites give *Rhodiola rosea* the property of having beneficial effects against pain, headache, haemorrhoids, an anti-inflammatory effect and also the possibility to be used as a stimulant to improve concentration (Panossian et al., 2010). Because of these characteristics, the presence of *Rhodiola rosea* in its natural environment has been threatened by wild harvesting and for this reason it entered in the Red List of endangered species in many countries (Howes et al., 2020). The plant grows naturally in Russia, Scandinavia, European Alpine and Carpathian Mountains, and the main techniques for cultivating *Rhodiola rosea* have been reviewed (Galambosi et

al., 2014). Table 1 reassumed the main research done on the cultivation systems adopted in Europe and the relative results of the secondary metabolites, salidroside and total rosavins content (in % of dry mass). From the table it is deducted that the main cultivation system adopted in Europe is the open field cultivation. The cultivation areas in all European countries remain small, estimated totally about 8–12 (Galambosi et al., 2014). A study done in Norway documented a main influence of the chemical composition in *Rhodiola rosea* by genetic factors, temperature and day length (Thomsen et al., 2011). Another study demonstrated noticeable differences in the salidroside and total rosavin content of Rhodiola rosea cultivated in ten different habitats of one area of between 0.46 to 2.61 % and 0.67 to 2.7 % (György et al., 2013). The identification of optimal growing condition for *Rhodiola rosea* in an indoor vertical farm would allow to continuously ensure the optimal concentrations of secondary metabolites. In addition, *Rhodiola rosea* roots being consistently damaged from the harvest represent a consistent loss after several years of cultivation and a critical issue of open field production (Galambosi et al., 2007). The challenge of commercially cultivating this plant at significantly higher levels in order to meet the existing global demand (Brinckmann et al., 2021), could be overcome by adopting indoor vertical farming systems.

The aim of our study was to investigate the effect of a soilless, hydroponic cultivation on *Rhodiola rosea* in an indoor vertical farm system. Furthermore the extraction method of secondary metabolites was adjusted for later trials by using model plant materials from field cultivation. This study results will be used during the project to further investigate and increase secondary metabolites of *Rhodiola rosea* in indoor vertical farming hydroponic cultivation.

Tab. 1. zeigt Ergebnisse aus der Literatur zum Anbau von Rhodiola rosea; (g) = Trockenmasse in Gramm/Pflanze, (%) = Gehalt in der Trockenmasse in Prozent, ns= nicht untersucht, // = keine Unterscheidung zwischen Rhizom- und Wurzelmasse

Tab. 1. *shows results from the literature of Rhodiola rosea cultivation; (g) = grams of dry mass/plant, (%) = content in the dry mass as percentage, ns = not studied, // = no distinction between rhizome and roots mass*

Cult. system	**Time**	**Country**	**Rhizome dry mass (g)**	**Roots dry mass (g)**	**Salidroside (%)**	**Total rosavins (%)**	**Literature**
Natural env.	Diff. age	Finland	ns	51.37	1.73	2.02	(Galambosi et al., 2007)
field	5 years		ns	165.37	0.8	1.61	
field	4 years	Finland	147.9	63.71	0.35	1.28	(Galambosi et al., 2009)
field	3 years		58.05	32	0.866	2.24	
field	2 years	Norway	ns	ns	ns	4.78	(Thomsen et al., 2011)
Natural env.	Diff. age	Norway	ns	ns	1.54	1.69	(György et al., 2013)
field	5 years	Italy	ns	132.5	1.10	1.03	(Scartezzini et al., 2011)
field	5 years		ns	84	0.83	1.03	
field	3 years	Italy	ns	ns	0.558	1.65	(Egger et al., 2007)
field	5 years	Poland	120.4	//	0.462	3.61	(Węglarz et al., 2008)
field	3 years		27.3	//	0.259	3.03	

field	Diff. age	Poland	ns	ns	0.141	3.65	(Altantsetseg et al., 2007)
field	2 years	Bulgaria	ns	ns	0.85	ns	(Bozhilova et al., 2010)
field	3 years		ns	ns	1.10	ns	
field	3 years	Bulgaria	71.35	7.95	2.15	ns	(Platikanov et al., 2008)
Natural env.	Diff. age	Mongolia	17.64	//	0.469	1.00	(Magsar et al., 2011)
photo culture	135 days	Russia	2.3	ns	0.5	ns	(Kovaleva et al., 2003)
	245 days		10.5	ns	1.2	ns	
Natural env.	Diff. age	Russia	62.7	//	1.44	2.50	(Galambosi et al., 2015)
field	6 years		112.7	ns	1.24	2.22	
field	10 years		174.5	ns	1.30	1.44	

Material and methods

Cultivation trial in the indoor vertical farm

The plant material was taken from six months old *Rhodiola rosea* plants. At the Applied Science Centre for Smart Indoor Farming, the plants were washed and a random sample of 144 seedlings was chosen for the trial. The trial took place in a climate chamber equipped with two levels. On the top level an aeroponic irrigation system and on the level below a deep-water irrigation system was used (Fig. 1). Three euro-stacking boxes (0.6 x 0.4 x 0.22 m) were adjusted for each irrigation system and a plant density of 100 plants per m^2 was used. The nutrient solution was taken from a previously plant trial with Rhodiola rosea in a closed system (Jüttner et al. 2023), which presented the following amounts of nutrients: 11.75 mmol l^{-1} $N\text{-}NO^{3-}$, 1,25 mmol l^{-1} P, 6.5 mmol l^{-1} K, 1 mmol l^{-1} Mg, 2.75 mmol l^{-1} Ca, 1.5 mmol l^{-1} S, 20 µmol l^{-1} Fe, 10 µmol l^{-1} Mn, 20 µmol l^{-1} B, 0.5 µmol l^{-1} Mo, 0.5 µmol l^{-1} Cu and 1 µmol l^{-1} Zn. The electrical conductivity (EC) and the pH was checked every week in order to reach a target EC of 1.5 mS/cm and a pH of 5.5 - 6. The average air temperature of the system during the trial was 20.5 ± 0.6 °C, with a relative humidity of 78.7 ± 8.8 %. As light source 5 LED modules (L-series NS12, Valoya, Sweden) per level with a light intensity of 190 ± 50 µmol m^{-2} s^{-1} and a photoperiode of 16 h was used. Three different plants holding systems (cone collars) were used instead of a substrate. They differed from each other in material (neoprene and foam) and shape (circular or clips). Before the cultivation started a sample of young plants at a similar morphological stage was randomly chosen and fresh mass of the whole plant weighed respectively. The average fresh weight of 1.15 ± 0.42 g was used as comparison for total plant weight increase. The cultivation in the indoor vertical farm was successful and lasted for 63 days in total. A sample of 5 plants in each box was harvested and averaged, which served as one repetition. In total 15 plants per treatment were analysed in 3 repetitions. Every sample plant was analysed regarding fresh mass and dry mass of stems, rhizome and roots. The drying process was done in a drying oven (Memmert UN750plus), set at a temperature of 60 °C for 4 days.

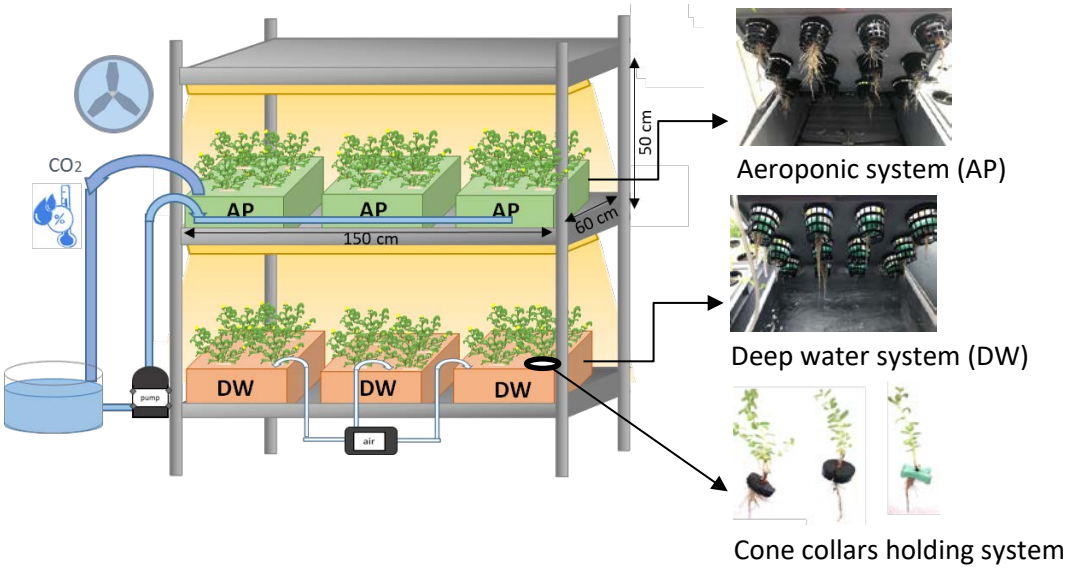

Abb. 1. Links eine Darstellung des technischen Aufbaus der vertikalen Indoor Vertical Farm für den Anbau von *Rhodiola rosea*. Rechts von oben das aeroponische Bewässerungssystem, das Deep Water System sowie die drei Haltesysteme (cone collars)

Fig. 1. *On the left a representation of the indoor vertical farm´s technical set up adopted for Rhodiola rosea cultivation. On the right from above a picture of the aeroponic irrigation system, the deep water irrigation system as well as the holding support system (cone collars)*

Extraction of secondary metabolites from *Rhodiola rosea* plants

Preparation of the plants for the extraction

The model plants which were analysed came from field cultivation of a farmer specialised in *Rhodiola rosea* production in Thuringia. The plants were harvested in summer 2021 and came from different cultivation years, here described as cultivation year 1 to 4. The youngest plants were harvested after one year of cultivation (year 1) and the oldest plants were harvested after four years of cultivation (year 4). First, the harvested plants were cleaned of excess soil and washed with tap water. Then the plants were divided into their individual components: leaf and stem, rhizome, rhizome shell, roots and fine roots. The fractions were dried in a convection dryer at 60 °C for 61 h, followed by additional 28 h at 40 °C (Fig 2).

Abb. 2. links) Pflanze aufgeteilt in Blätter, Knospen, Rhizom, Wurzeln, Feinwurzeln sowie Rhizomschalen. Rechts) Pflanzenteile im Konvektionstrockner

Fig 2. *Left) Plant organs leaves, buds, rhizome, roots, fine roots and rhizome shells. Right) Plant parts in convection dryer*

The dried plant parts were shrink-wrapped and stored at 7 °C in a refrigerator, until further processing. For the subsequent determination of the active ingredient content, the plant parts were grinded for 20 seconds at 30 Hz using a ball mill (Retsch GmbH MM 400). The obtained powder was stored in the refrigerator at approximately 7 °C until extraction. For the extraction two sample plants per year were used. The fresh mass of the plants are shown in table 2.

Tab. 2. Frischmasse der Pflanzenteile in Gramm aus den 4 Anbaujahren

Tab. 2. *Fresh mass in gram of the plant parts in 4 years of field cultivation*

	fresh mass (g) year 1	**fresh mass (g) year 2**	**fresh mass (g) year 3**	**fresh mass (g) year 4**
roots	7.41	26.59	24.04	37.08
fine roots	11.95	15.20	18.62	15.10
rhizom	11.53	14.39	20.08	29.06
rhizom shells	10.14	12.94	21.80	19.81
leafs and buds	8.76	9.58	16.74	13.91

Extraction and measurement of the active ingredients with UV-HPLC

The method used was adapted from Ganzera et al. (2001). The detected substances were: salidroside, tyrosol, rosarin, rosavin and rosin. 150 mg of the grinded plant parts (by the measurement of the particle-size distribution an average median value of x_{50} = 557.5 ± 10.6 µm was obtained) were added to an Erlenmeyer flask with 450 µl of methanol (analytical grade), which was then placed in an ultrasonic bath (frequency: 35 kHz) for 10 minutes. After subsequent transfer to a suitable centrifuge tube, the mixture was centrifuged at 3500 rpm (781 G) for 5 minutes. For this the centrifuge from Heraeus Sepatech Biofuge A was used. The supernatant was removed and transferred to a 25 ml volumetric flask. The residue was again mixed with 450 µl methanol. This procedure was performed three times. Then 25 ml volumetric flask was filled with methanol up to the calibration mark. The solution was measured using an HPLC with UV detector from VWR Hitachi Chromaster at a wavelength of 205 nm, a high pressure double piston pump from VWR Hitachi Chromaster and a column from Agilent (ZORBAX Eclipse XDB-C18; 4.6 x 150 mm) with a pore size of 5 µm and a suitable precolumn. The flow rate used was 1.0 ml/min and the injection volume was 10 µl (Meyer et al., 2009). As a reference a standard solution with the 5 compounds (salidroside, tyrosol, rosarin, rosavine and rosin) from PhytoLab GmbH & Co. KG was used (7.9 µg/ml salidroside, 7.6 µg/ml tyrosol, 6.1 µg/ml rosarin, 6.4 µg/ml rosavin, 6.2 µg/ml rosin). For the extraction a representative sample of 150 mg was taken by using a Cross plate division. The extract was measured 3 times with the HPLC.

Results and discussion

Cultivation results from the indoor vertical farm

The average final fresh weight in the aeroponic system was 10.53 ± 5.68 g/plant, meanwhile in the deep water system it was slightly higher at 11.37 ± 7.18 g/plant. The weight distribution between the plants organs was similar in the two systems except for the rhizome that, on average, developed more in the deep water system reaching 1.98 g/plant compared to 0.94 g/plant in the aeroponic system (see Fig. 3). This difference can be explained by the intrinsic properties of the aeroponic system, since during this trial the plants were hanging without any substrate, just held by cone collars, and so they have been subjected to a greater force of gravity with respect to the plants in the deep water system (Gopinath et al., 2017).

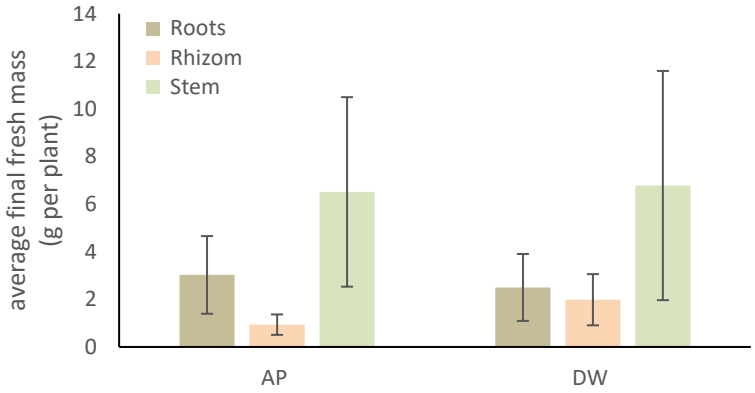

Abb 3. Frischmasse (g/Pflanze) von *Rhodiola rosea* Pflanzen, aufgeteilt in ihre Organe nach 63 Tagen Anbau in einem vertikalen Anbausystem mit verschiedenen Bewässerungsverfahren Aeroponik (AP) und Deep Water (DW)

Fig 3. *Final fresh mass (g/plant) of* Rhodiola rosea *plants divided in their organs after 63 days of cultivation in a vertical farming setup. Two irrigation systems were adopted: the aeroponic (AP) and deep water (DW)*

Subsequently, the average total final dry mass per plant in the aeroponic system was 0.91 ± 0.35 g/plant and in the deep water system was 1.54 ± 0.77 g/plant. In our case the average dry rhizome weight after 63 cultivation days was 0.20 ± 0.9 g/plant in the aeroponic system and 0.67 ± 0.33 g/plant in the deep water system (see Fig. 4). The generally lower values obtained with the aeroponic system can be explained with the same reasoning as before and with the higher complexity that the usage of this system implies. These results are in line with another study done on Rhodiola rosea in a closed system regarding the overall growth of the plants (Kovaleva et al., 2003).

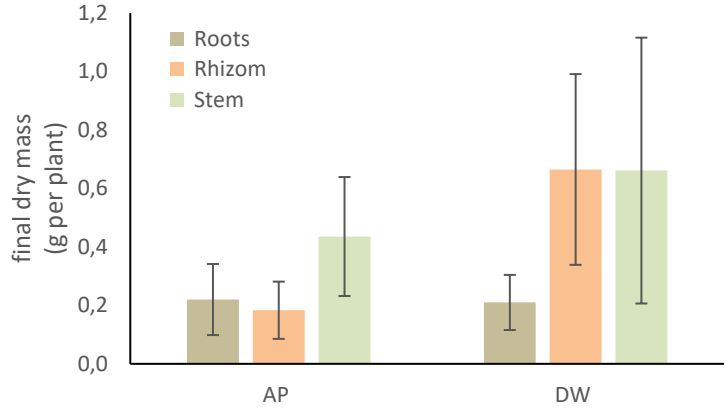

Abb. 4. Trockenmasse (g/Pflanze) von *Rhodiola rosea* Pflanzen, aufgeteilt in ihre Organe nach 63 Tagen Anbau in einem vertikalen Anbausystem mit verschiedenen Bewässerungsverfahren Aeroponik (AP) und Deep Water (DW)

Fig. 4. *Final dry mass (g/plant) of* Rhodiola rosea *plants divided into their organs after 63 days of cultivation in a vertical farming setup. Two irrigation systems were tested: the aeroponic (AP) and deep water (DW)*

Rhodiola rosea *yield potential in indoor vertical farm*

According to a statement of a specialist for *Rhodiola rosea* cultivation in the open field in Germany, indoor vertical farming production would need to reach a yield increase of 10 to 40 times compared to his current yield of 3-5 t/ha (dry weight of the roots) to be an alternative ready for application. In our experiment an annual yield of 37.45 g/m^2, based on the yield obtained after 63 days, was calculated. Even though these results are still low, according to the literature the usage of indoor vertical farming can increase the yield of plants per square meter compared to field production (van Delden et al., 2021). This could be done by an optimization of climatization conditions, higher light intensities, the use

of hydroponic and by having the possibility to cultivate year-round with optimal conditions. Accordingly, indoor vertical farming can reduce the cultivation time of several crops (van Delden et al., 2021). Based on this, a potential yield of 1 t/ha could be reached after 12 weeks at a plant density of 50 plants/m² in indoor vertical farming (Jüttner et al., 2023). Further research is needed to develop climate strategies which increase yields per square meter while reducing the cultivation time. Furthermore, the quality of secondary metabolites must be continuously monitored. In this regard, applying specifically adjusted light strategies can influence secondary metabolites accumulation, as verified for different plants (Loconsole et al., 2021). Based on this, the potential to reach a yield increase of up to 10 to 40 times per square meter seems feasible. Consequently, the use of a multilayer system in indoor vertical farming can significantly increase yield potential further.

Content of secondary metabolites in Rhodiola rosea *coming from the field Results of the active ingredient ratio inside the different plants organs*

The plants were evaluated in regards of how the active ingredient content changes over the time of cultivation and in the different plant parts. The individual active ingredients salidroside, tyrosol and the rosavins (rosarin, rosavin, rosin) were analyzed. In Figure 5, the proportions of the total active ingredients extracted are presented in relation to the dry mass of the individual plant parts are plotted in % over the 4 years of cultivation. There was a tendence that the content of salidroside increases over the cultivation years, while the content of rosavins tends to decrease over the cultivation years (data not shown).

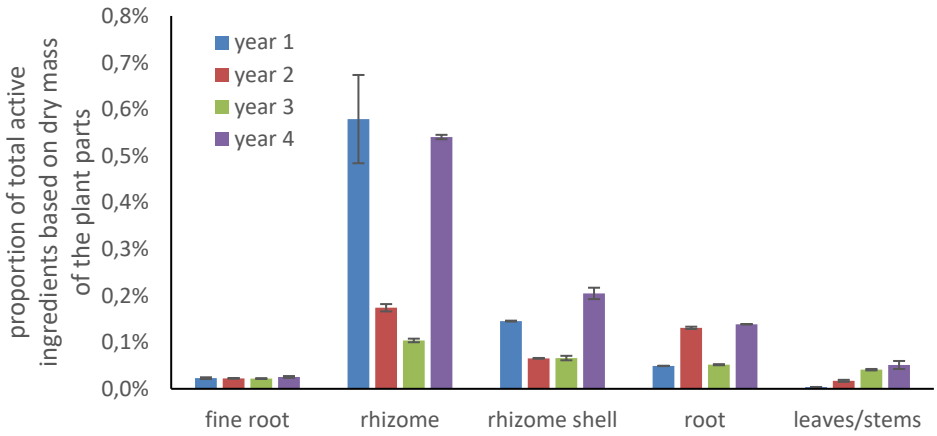

Abb. 5. Anteil der Gesamtwirkstoffe bezogen auf die Trockenmasse der Pflanzenteile in % über 4 Jahre Feldanbau, Wiederholung der HPLC-UV-Messung n=3., 2 Pflanzen

Fig. 5. *Proportion of total active ingredients based on dry mass of the plant parts in % over 4 years of field cultivation, repeat of the hplc-uv-determination n=3, 2 plants*

Within the plant components, active compounds were found in all parts, but mostly in the rhizome, followed by the root on a dry weight basis. This indicates that the entire plant can be used for active ingredient extraction. In order to have more consistent data more trials will be carried out.

Conclusion and future further steps

Critical initial steps have been achieved regarding *Rhodiola rosea* cultivation in an indoor vertical farm and a defined methodology for the secondary metabolite extraction has been tested and delineated. The method will be used in the next trials on plants cultivated in the indoor vertical farm. In particular, the fact that a high concentration of secondary metabolites, from *Rhodiola rosea* cultivation on the field, has been found already in the first year makes it possible to suppose that the life cycle of this

perennial plant can be optimized in a shorter period. In the next steps, we will also consider the legal requirements for pharmaceutical production that at the moment would prevent the market production in indoor vertical farming systems. However, in the future new production systems will be necessary to fulfil the demand of pharmaceutical material and at the same time prevent the wild harvesting. Indoor vertical farming constitutes the optimal system to experiment different environmental conditions that can stimulate the active ingredients development in the plant organs. Regarding that, the focus on the optimal growth of roots and rhizome is going to be investigated further, considering different specific parameters like root temperature, a new sustainable cultivation system and also the possibility to provide beneficial bacteria to the underground part of the plant (Lee et al., 2015). Alongside, the influence of different cultivation parameters will be checked to identify how they act as stressors for increasing secondary metabolites accumulation. At the same time, the currently used aeroponic irrigation systems will be used again and optimized regarding the type of nozzles and the time of irrigation. On the other hand, the deep water system confirmed, to have high potential for the *Rhodiola rosea* cultivation.

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