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Yield and antioxidant properties of herb and root of Ashwagandha (*Withania somnifera* L.) grown with permaculture under Subcarpathian conditions

Plonowanie oraz właściwości antyoksydacyjne ziela i korzeni witanii ospałej (*Withania somnifera* L.), uprawianej metodą permakulturową w warunkach Podkarpacia

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Słowa kluczowe: permakultura, witania ospała, ziele, korzeń, plon, ekstrakty, właściwości antyoksydacyjne

Abstract

The aim of this study was to conduct a pilot experiment on the cultivation of ashwagandha (*Withania somnifera* L.) using the permaculture method, to estimate the yield of the herb and roots of this species and to determine their antioxidant properties. The field experiment was conducted in 2024 in the Subcarpathian voivodeships (50°82′ N 23°54′ E), Poland. The experiment was established in raised beds using the permaculture method on lasagne-type perches.

The average herb fresh weight yield was 7.422 kg/m^2. Herb dry matter yield was 2.186 kg/m^2, root fresh weight yield was 1.569 kg/m^2 and root dry matter yield was 0.594 kg/m^2. Both the herb and the roots of ashwagandha hold antioxidant properties; however, the level of antioxidant properties depended on the harvest date. The highest level of antioxidant properties was observed in samples of the herb harvested in August, i.e. on the first harvest date. This value was 33.55% higher compared to the herb sample collected in October (second harvest date). The harvested roots of ashwagandha were characterised by different levels of antioxidant properties in the different harvest dates. In this case, the highest level of antioxidant properties was recorded in root samples collected at the end of the ashwagandha growing season, i.e. at harvest date II. It was also observed that the level of antioxidant properties of the herb and the roots of ashwagandha depended on the type of solvent used to prepare the extracts.

Streszczenie

Celem badań było przeprowadzenie pilotażowego doświadczenia dotyczącego uprawy witanii ospałej (*Withania somnifera* L.) metodą pernakulturową, oszacowanie wielkości plonów ziela i korzeni tego gatunku oraz określenie ich właściwości antyoksydacyjnych. Doświadczenie polowe przeprowadzono w 2024 r. w Polsce na terenie województwa podkarpackiego (50°82′ N 23°54′ E). Eksperyment zrealizowano na podniesionych zagonach metodą permakulturową na grzędach typu lazagne. Średni plon świeżej masy ziela wynosił 7,422 kg/m^2. Plon suchej masy ziela kształtował się na poziomie 2,186 kg/m^2, plon świeżej masy korzeni wynosił 1,569 kg/m^2, zaś plon suchej masy korzeni 0,594 kg/m^2. Zarówno ziele, jak i korzenie witanii ospałej posiadają właściwości antyoksydacyjne, jednakże ich poziom zależał od terminu zbioru. Najwyższy poziom właściwości antyoksydacyjnych zaobserwowano w próbkach ziela zebranego w sierpniu, czyli w I terminie zbioru. Wartość ta była wyższa o 33,55% w porównaniu do próbki ziela zebranego w październiku (II termin zbioru). Zebrane korzenie witanii ospałej cechowały się różnym poziomem właściwości antyoksydacyjnych w zależności od terminu zbioru. Przy czym w tym przypadku najwyższy poziom właściwości antyoksydacyjnych zanotowano w próbkach korzeni zebranych pod koniec okresu wegetacji rośliny, czyli w II terminie zbioru. Zaobserwowano również, że poziom właściwości antyoksydacyjnych ziela oraz korzeni witanii ospałej zależał od rodzaju rozpuszczalnika użytego do przygotowania ekstraktów.

Introduction

The word permaculture was coined from a combination of the words permanent and agriculture, in line with the belief that culture alone could not survive without agriculture and a land use ethics [1, 2]. Permaculture is based on the observation of natural systems, combining the traditions of ancient agricultural systems with the achievements of modern scientific and technological knowledge. Its aim is to replicate systems found in nature, while creating an environment geared towards the production of food for humans and animals. Permaculture systems are, by design, both environmentally friendly and economically efficient.

Satisfying the needs of oneself and of the nearest people and beings, without polluting or over-exploiting the environment, is the goal of every permaculturist [2, 3, and 4]. Permaculture involves leaving the garden or crop to nature, with very little or no interference by the user in the processes taking place. Permaculture gardens and crops make use of natural processes occurring in nature, for example in the soil under the influence of soil organisms or the interaction between plants and animals. For this reason, permaculture proponents dispense with digging, weeding and watering. They also do not remove organic residues which, once decomposed, enrich the soil and contribute to water retention [5, 6].

They do not use chemical treatments. Ornamental plants or vegetables can be grown in boxes, which allow more demanding species of plants to be grown in a weak site and improve garden design. They make it easier to control the soil pH and fertilise the site. Permaculture strives for self-sufficiency, creates healthy ecosystems, cares for biodiversity and efficient use of resources while also making work easier as there is no need to bend down [5, 7].

Although there is an increasing amount of information on this cultivation system, there is still a lack of scientific reports on yield effects and ready instructions on permaculture cultivation technology. Therefore, the aim of the present study was to conduct a pilot experiment using the permaculture method with the herb ashwagandha (*Withania somnifera* L.), to estimate the yield of the herb and roots of this species and to determine the antioxidant properties of the obtained raw material.

Material and Methods

Plant Material

A field experiment on the cultivation of ashwagandha was conducted in 2024 at the Experimental Field of the State University of Applied Sciences in Krosno, located in Subcarpathian Voivodship 50°82′ N 23°54′ E), Poland. The experiment was established in raised beds using permaculture method on lasagne-type perches. For this purpose, wooden boxes measuring 1.00 x 0.75 x 5.75 m were prepared.

The boxes were then divided into 1 x 1m^2 experimental fields. The boxes were directly set on the grass. They were filled from underneath with cardboard boxes (without adhesive tape, foil, varnish, etc.). The next layer consisted of shredded dry branches, sticks and old rotten planks (which will turn into rich organic matter over time and under the action of microorganisms).

The wood layer was covered with straw and a layer of compost mixed with soil. The last layer was the compost itself, on which the ashwagandha seedlings were planted. The experiment was repeated in 4 replications. The seedlings came from seeds purchased from a garden shop in Krosno territory.

Before sowing, the seeds were soaked in tap water for 24h, then sown shallowly in a mixture of soil (garden and organic soil) and compost (50:50) in multi pots and covered with perlite. The multicups were kept in the greenhouse at temperatures above 10^{o}C. After a week, the first emergence took place. When the seedlings were 7 cm long, they were transplanted into P9 production pots filled with a 50:50 soil/compost mixture. The plants were watered every three days.

Approximately, after 5 weeks since sowing, the seedlings were planted out into a permanent place at a spacing of 30x25 cm. Plant care during the growing season consisted of hand weeding, soil loosening and irrigation as needed. In order to carry out tests for the evaluation of antioxidant properties, harvesting of the herb and roots was done twice during the vegetation of ashwagandha i.e.: 1st date – 20 August, 2nd date – 30 September.

After harvesting, the raw material was washed, crushed into small pieces, frozen at –25°C and then lyophilised (0.37 mBa) for 48h, in a FreeZone 12L lyophiliser, from Labconco (Labconco Corporation Kansas City, MO, USA). The resulting freeze-dried samples were stored at 5°C in dark glass containers, out of light. The final harvest of ashwagandha was carried out by hand on the 5th of October at harvest maturity. Immediately after harvesting, the fresh weight yield of the herb and roots was determined and, after drying at 35°C, the air-dry weight yield of the herb and roots of ashwagandha was calculated.

Photo 1. Ashwagandha during vegetation.

Source: photography by the Author.

Soil Conditions

Before setting up the field experiment, soil samples were taken from the layer of compost mixed with soil according to the standard [8] in order to determine the abundance of soil in macro- and micro-elements and soil pH in 1 M KCL. These tests were performed by the District Chemical and Agricultural Station in Rzeszów.

Table 1. Physicochemical Properties of the Soil.

Content of macro- and micro-nutrients and organic matter	macronutrients (mg/100g)			Corg. (%)	Organic matter (%)	pH (KCl)	micronutrients (mg/kg)			
	P205	K20	Mg				Cu	Mn	Zn	Fe
	29.5	41.3	15.8	1,62	2.79	6.3	5.2	177.5	13.5	2193

Source: Data based on the results obtained by the District Chemical and Agricultural Station in Rzeszów 2024.

The abundance of bioavailable phosphorus, potassium and magnesium was very high, and that of copper, manganese, iron and zinc was medium. The organic matter content of the arable layer was high at 2.79% (Table 1). The results of the study allowed the soil to be classified in the medium agronomic category [9]. The soil was slightly acidic (pH 6.3 in 1M KCl).

Meteorological Conditions

Table 2. Weather Conditions During the Vegetation of Ashwagandha in 2024, According to the Meteorological Station in Dukla

Year	Months						Average
	IV	V	VI	VII	VIII	IX	IV-IX
Rainfall [mm]							
2024	23.3	35.7	52.9	11.7	15.3	22	27.78
The average sum of long-term (1989-2014)	55.9	95.6	100.9	116.5	30.1	53.1	79.8
Air temperature [°C]							
2024	10.03	12,37	17.13	20.37	21.33	15.53	16.2
Long-term average (1989-2014)	9.2	13.6	16.4	19	19.4	14.02	15.52
Hydrothermal coefficient							
2024	2.3	2.9	3.1	0.6	0.7	1.4	1.9

Ranges of hydrothermal coefficient values according to Sielianinov: k ≤0.4 – extremely dry month; 0.4<K ≤0.7 – very dry; 0.7<K≤1.0 – dry; fairly dry – 1.0< K ≤1.3; optimal – 1.3< K ≤1.6; moderately humid – 1.6<K ≤2.0; humid, 2.0<K≤2.5; very humid 2.5<K≤3.0; extremely humid K>3.0.

The course of weather during the growing season of ashwagandha was variable, as shown in Table 2. In the analysed period from April to September 2024, there were large differences in the amount of rainfall per month compared to the multi-year average (1989-2014). The year 2024 was classified as moderately humid, as evidenced by the Sielianinov hydrothermal coefficient values (Table 2).

However, there was a significant variation in the hydrothermal index values between the different months of the ashwagandha growing season. Average rainfall in 2024 reached 27.78 mm per month, but was still far below the multi-year norm. Average air temperatures in 2024 appeared to be higher than the multi-year norm, except for May, which could favour the vegetation of ashwagandha, which prefers warm conditions (Table 2).

Sample Preparation

Extracts of the herb and roots of ashwagandha were prepared by crushing 0.75g of freeze-dried material in a mortar with 80% methanol (Sigma-Aldrich, St. Louis, MO, USA), topping it up in portions of a total volume of 25 ml. The extraction time was 4 hours. Each sample obtained was aspirated through a funnel with a sintered disk into a 25 ml volumetric flask. The extraction process was carried out at 22°C with limited light. The extracts were stored in the dark at 5°C for one week [10]. Extraction was carried out using four different solvents, i.e. water, 30%, 50% and 70% ethanol.

Measurement of Antioxidant Properties

The antioxidant properties were measured using the stable DPPH radical. Measurements were performed using spectrophotometric methods with a Genesys 150 UV-Vis spectrophotometer (Thermo Scientific) and wavelengths are given in the method descriptions.

Measurement of Antioxidant Potential Using the DPPH Radical

One of the more commonly used methods for the measurement of antioxidant activity is that using a DPPH solution. DPPH (2.2-diphenyl-1-picrylhydrazyl) is a free radical with a relatively high persistence and can therefore be easily prepared for testing [11]. The 2.2-diphenyl-1-picrylhydrazyl radical is a dark purple crystalline solid, well soluble in organic solvents and insoluble in water. In alcohol solution, it is dark violet in colour with an absorbance maximum with a wavelength of 517 nm.

Measurement results are usually reported as the number of equivalents of the reference substance or as the radical scavenging rate expressed in %:

$$\text{DPPH radical reduction } [\%] = \frac{A_0 - A_t}{A_0} \cdot 100$$

where:

A_0 – absorbance of the control sample

A_t – absorbance of the test sample after the specified time (t = 10 min).

Statistical Analysis

Statistical analysis of the tested samples was performed using Statistica 13.3 (StatSoft, Visual Basic, TIBCO Software Inc., PL). Herb and root yield and antioxidant property scores were analysed using ANOVA test. Results were expressed as mean ± standard deviation and were considered statistically significant at $p<0.05$.

Results and discussion

Yield of Fresh and Dry Weight of Herb and Roots

Based on the permaculture ashwagandha cultivation study conducted, the average fresh herb weight yield harvested in October at harvest maturity was 7.422 kg/m^2.

Plant dry matter yield per 1 m^2 was 2.186 kg/m^2, root fresh matter yield was 1.569 kg/m^2 and root dry matter yield was 0.594 kg/m^2 (Table 3). These results can be considered high compared to the study of Obidowska et al. [12], who, in their study of a trial of ashwagandha cultivation in Poland under container growing conditions without covers, obtained a fresh herb weight yield of 351.21 g per plant.

Table 3. Fresh and Dry Weight Yield of Herb and Root of Ashwagandha

Feature	yield kg/m^2	Min	Max	Yield per 1 plant kg/m^2
fresh herb mass	7.422±1,40a	5.95	9.85	0.742
herb dry matter	2.186±0,43b	1.77	2.87	0.218
fresh root mass	1.569±0.21c,b	1.24	1.92	0.156
dry root mass	0.594±0.08c	0.44	0.68	0.059

± Standard deviation

Explanation: a-c average values denoted by different letters in the rows are statistically significant different at p≤0.05

The high fresh weight yield of herb and root of ashwagandha in our study may have been due, among other things, to the very high content of elements, i.e. phosphorus, potassium and magnesium, in the soil (Table 1). According to Williamson et al. [13] permaculture cultivation in raised beds is characterised by significantly higher yields and better raw material quality compared to conventional practices.

According to these authors, permaculture methods focus on imitating the natural recycling of the ecosystem through the addition of organic matter, minimal soil disturbance and species diversity of the plants grown. They are featuring the promotion of microbial activity and plant synergy. All these activities contribute to a significantly higher carbon, nutrient and organic matter content compared to conventionally cultivated soil.

Similar yield results of above and below ground parts of ashwagandha under raised bed conditions were also obtained by Prajapati et al. [14], Pandey and Shukla [15] and Arand et al. [16]. On the other hand, Noworolnik [17] in his study on the effect of soil quality on the yield of aboveground parts of plants describes that, among many habitat-agronomic factors, soil conditions and especially their nutrient abundance (besides nitrogen fertilisation), have the strongest effect on yield.

According to Helfenstain et al. [18], zinc is a very important nutrient for ensuring the correct course of vital processes in the plant. According to the studies of these authors, zinc participates in nitrogen metabolism, thanks to which the plant rapidly absorbs this element and produces a higher yield of green mass. In our study, the soil zinc content was at an average level of 13.5 mg/kg (Table 1).

According to Ukalska-Jaruga et al. [19], the yield of both above- and below-ground parts of the plant is strongly influenced by the content of organic matter in the soil, which, by influencing the physical, chemical and biological properties of soils, affects their productive function. In addition, research by Księżak [20] reports that the organic matter available in the soil is a source of nutrients for plants and readily available carbon for many groups of microorganisms, and also has a retention function.

In our research, the value of this property was 2.79 % p.s.m. and can be considered high. According to Trawczyński [21] and Janowiak and Spychaj-Fabisiak [22], one of the main measures of soil fertility is the content of organic carbon in the soil, which at the same time is a basic determinant of soil productive capacity.

In our study, the organic carbon content of the soil was at an average level of 1.62 %. According to Neina [23] and Widłak [24], soil pH also plays a major role in plant yield. The value of this trait in our study was 7.1 pH and was defined as neutral. According to Kloc-Szatnik [25], most horticultural plants grow and develop best at a pH of 6.2-7. *Withania somnifera* belongs to the group of plants that yield noticeably better in neutral soil.

Similar reports in Rajeswara's work [26] state that ashwagandha grows well in well-drained sandy, sandy and loam soils with light texture and pH of 7.0-8.0. According to Srivastava et al. [27], the high yield of herb and roots of ashwagandha is also influenced by meteorological conditions and especially air temperature, which should be between 20°C and 31°C.

This is confirmed by authors' own research, where air temperatures in 2024 were higher than the multi-year average except in May, which may have favoured the vegetation of ashwagandha, which prefers warm weather conditions during growth and development.

Antioxidant Properties of the Herb and Root

The DPPH reagent method is widely used to measure the antioxidant capacity of natural raw materials: fruit, juices, plant extracts, food. It is especially often used in the determination of the antioxidant properties of phenolic compounds [28]. It is fast and accurate, and the results obtained are reproducible and comparable with values obtained from other tests using free radical scavenging capacity [29].

Analysing the data obtained after the DPPH radical test on the antioxidant properties of the extracts of the herb and the root of ashwagandha, it can be concluded that both the herb and the roots of ashwagandha hold antioxidant properties, however, a higher value of the tested trait was obtained in the roots of ashwagandha. It was also observed that the degree of DPPH radical reduction varied between harvesting dates for both the herb and roots of this species (Figure 1).

The highest level of antioxidant properties was observed in the samples of herb harvested in August, i.e. on the first harvest date. This value was 33.55% higher compared to the herb sample collected in October (second harvest date) (Figure 1). The harvested roots of ashwagandha had different levels of antioxidant properties in the different harvest dates.

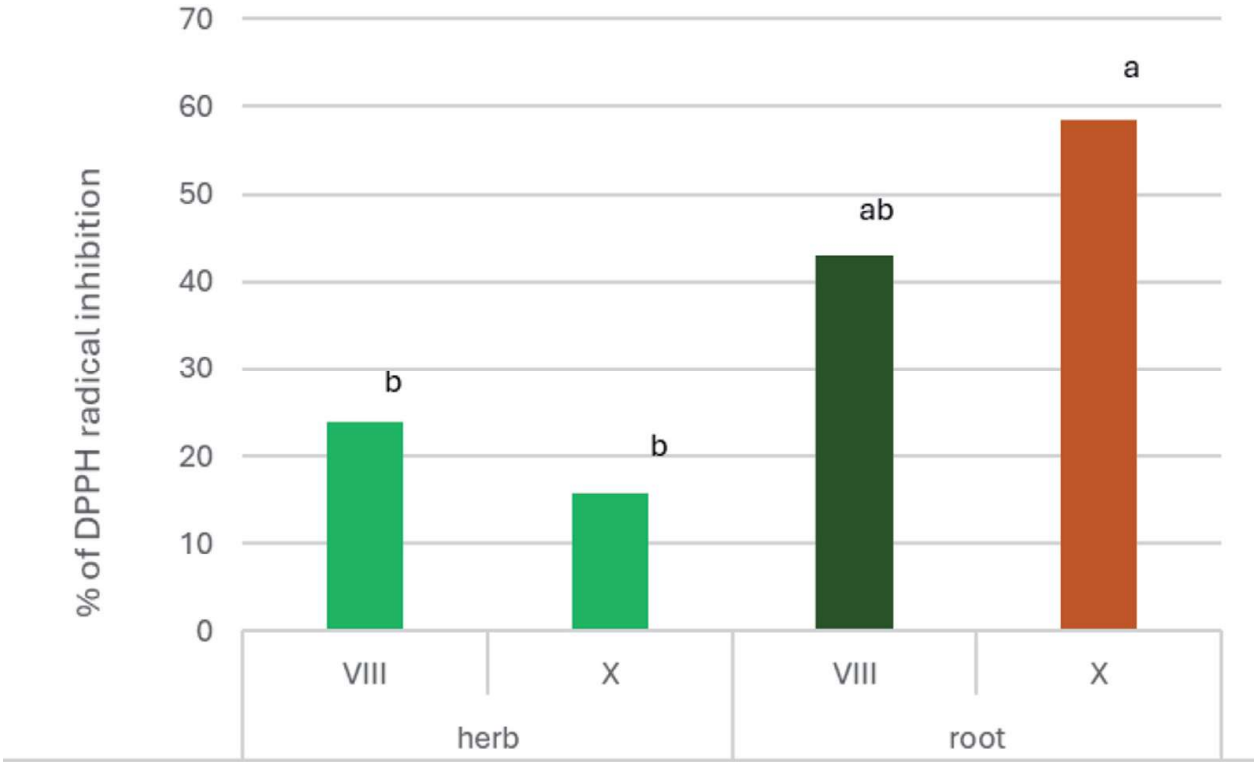

Figure 1: DPPH test for comparison of antioxidant properties in the herb and in the root at different harvest dates.
Explanation: a-b mean values denoted by different letters in the rows are statistically significant different at $p \leq 0.05$

Whereas, in this case, the highest level of antioxidant properties was observed in root samples collected at the end of the ashwagandha growing season, i.e. at harvest date II. The degree of DPPH radical reduction for these samples ranged from 38.10-78.11% and was 36% higher than root extract samples harvested in August (harvest date I). Jezierska and Sykuła [30] obtained values ranging from 22.22±0.069 to 33.03±0.074 in ashwagandha herb extracts.

Lower values than the above discussed in the extracts from ashwagandha herb were obtained by Jezierska and Sykuła [31], which ranged from 22.22±0.069 to 33.03±0.074. According to many authors, the prevailing climatic conditions during the growing season shape the intensity of abiotic stress factors (UV radiation, temperature, precipitation) being the main factor modelling the synthesis of secondary metabolites, the level of which depends on the vegetative part of the plant and its developmental stage [32, 33].

According to Gupta et al. [34], exposure to sunlight can affect photosynthesis and the production of secondary metabolites, which often have antioxidant effects. According to our study, the level of antioxidant properties of the ashwagandha herb may have been affected by weather conditions, as there were high air temperatures and there was a drought from July to August (Table 1).

The research results of Fernando et al. [35], reported that extracts from the above-ground parts of ashwagandha showed significant variation in the levels of antioxidant properties at different growth stages of this species. In the study of these authors, the highest total free radical scavenging capacity was achieved by extracts from the herb harvested just after the flowering stage. This is confirmed by the results of our own study, where the highest level of free radical scavenging was observed for extracts from the herb harvested in August, i.e. just after flowering. Sharma et al. [36, 37] in their study prove that the DPPH radical scavenging potential may also depend on the location of the plantation where the ashwagandha is grown.

In the authors' study, it was observed that the level of antioxidant properties of the herb and roots of ashwagandha depended not only on the date of harvesting but also on the type of solvent used to prepare the extracts.

The weakest free radical scavenging capacity was observed in aqueous extracts compared to ethanol extracts, with alcohol extracts prepared with 70% ethanol holding the highest free radical scavenging capacity, with values ranging from 19.80-78.11%. The aqueous extracts had the weakest oxidative properties. The degree of DPPH radical reduction for these samples ranged from 8.5-38.10%. In a study by Dhanani et al. [38], the aqueous extracts of *W. somnifera* also obtained the lowest free radical scavenging activity compared to the alcoholic extracts. The results of our study are also consistent with the study of Ezaez et al. [39],

who observed the highest DPPH scavenging potential in methanolic extracts (81.98 ± 0.49%), followed by acetone extracts (62.57 ± 1.14%) and the lowest in aqueous extracts (53.72 ± 1.32%).

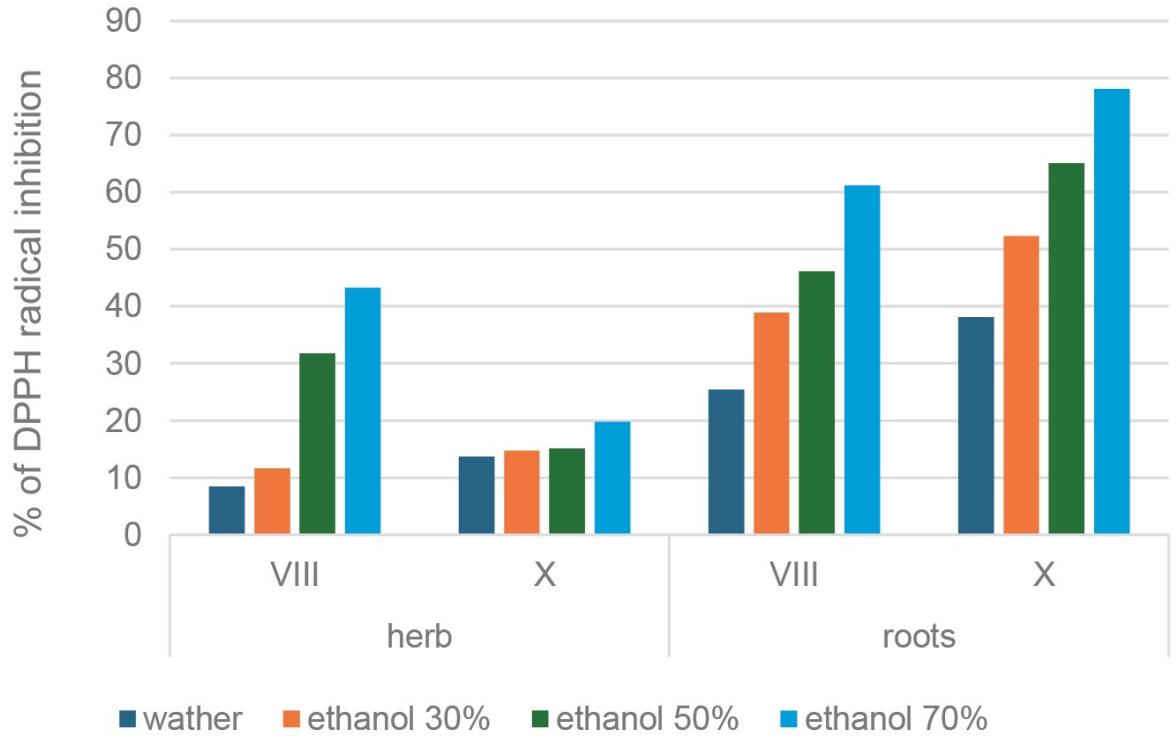

Figure 2: Effect of aqueous and alcoholic extracts on the antioxidant properties of the herb and roots of ashwagandha at different harvest dates.

Conclusion

In conclusion, the results of the pilot study on the cultivation of ashwagandha on raised beds using the permaculture method under the conditions of the Subcarpathian Voivodship, it can be stated that this method developed favourable soil conditions for the cultivation of this species, which brought tangible benefits in the form of high herb and root yield. The herbs, as well as the roots of ashwagandha, hold antioxidant properties, and their level depended on the harvest date. The highest level of antioxidant properties was observed in samples of the herb harvested in August, i.e. on the first harvest date. The collected roots of ashwagandha were also characterised by different levels of antioxidant properties in the different harvest dates.

Notably, the highest level of antioxidant properties was recorded in root samples collected at the end of the ashwagandha vegetation period, i.e. at harvest date II. It was also observed that the level of antioxidant properties of the herb and the

roots of ashwagandha depended on the type of solvent used to prepare the extracts. However, further research in this direction is required for the results obtained to provide implementation instructions for the cultivation of this species.

Several years of research results will allow the development of a detailed cultivation technology under permaculture cultivation conditions, and will also indicate the optimum time for harvesting the herb and roots, in order to obtain a raw material with the highest antioxidant properties. Continued research on ashwagandha should in future also include traditional field cultivation and the size and quality of the raw material obtained. It also seems necessary to determine the withanolides in the herb and roots of ashwagandha depending on the cultivation technology used.

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References

[1] Holmgren D., Permaculture – Principles and Pathways beyond Sustainability, Holmgren Design Services, Victoria, Australia 2002.

[2] Alakendu P.R., Afiya S., Senthilkumar S., Manivannan S., Permaculture: A Sustainable Farming Approach for Modern Era, Haya: Saudi Journal of Life Science, 2024, 9(7), pp. 305–312.

[3] Bhandari D., Bisto B., Permaculture: A Key Driver for Sustainable Agriculture in Nepal, International Journal of Applied Sciences and Biotechnology, 2019, 7(2), pp. 167–173.

[4] Fadaee S., The permaculture movement in India: A social movement with Southern characteristics, Social Movement Studies, 2019, 18(6), pp. 720–734.

[5] Rhodes C.J., Permaculture: Regenerative – not merely sustainable, Science Progress, 2015, 98(4), pp. 403–412.

[6] Maye D., Examining innovation for sustainability from the bottom up: An analysis of the permaculture community in England, Sociologic Ruralise, 2018, 58(2), pp. 331–350.

[7] Krebs J., Bach S., Permaculture – Scientific evidence of principles for the agroecological design of farming systems, Sustainability, 2018, 10(9), p. 3218.

[8] PN-R-04031: 1997. Instrukcja pobierania próbek glebowych.

[9] Mocek A., Gleboznawstwo, Wydawnictwo Naukowe PWN, Warszawa 2015.

[10] Kostecka-Gugała A., Kruczek M., Ledwożyw-Smoleń I., Kaszycki P., Antioxidants and Health-Beneficial Nutrients in Fruits of Eighteen Cucurbita Cultivars: Analysis of Diversity and Dietary Implications, Molecules, 2020, 25, p. 1792.

[11] Cybul M., Nowak R., Przegląd metod stosowanych w analizie właściwości antyoksydacyjnych wyciągów roślinnych, Herba Polonica, 2008, 1, pp. 68–78.

[12] Obidoska G., Sadowska A., Próby uprawy polowej *Withania somnifera* (L.) Dun. oraz ocena plonu i wartości surowca krajowego, Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin, 2004, 233, pp. 173–180.

[13] Williamson R.F., Reay M., Sgouridis F., Permaculture Management of Arable Soil Increases Soil Microbial Abundance, Nutrients, and Carbon Stocks Compared to Conventional Agriculture, Agronomy, 2024, 14, p. 1446.

[14] Prajapati R.K., Sunil P., Nagauria M.N., Koulini S.R., Effect of sowing method on growth and yield of Ashwagandha in black soil under Gmelina arboria based agroforestry system, [in:] Multipurpose Trees in the tropics: Management and improvement strategies, (eds.) V.P. Tewari and R.L. Srivastava, Arid Forest Research Institute, 2005, pp. 343–346.

[15] Pandey P.K., Shukla P.K., Studies on propagation of Ashwagandha (*Withania somnifera* Dunal), Journal of Non-Timber Forest Products, 2004, 11, pp. 131–134.

[16] Anand R.K., Dwivedi S.V., Sagar V., Effect of sowing methods on growth, yield and economics of ashwagandha (*Withania somnifera* Dunal) under rainfed conditions, Progressive Horticulture, 2016, 48(2), pp. 136–140.

[17] Noworolnik K., Warunki glebowe a plonowanie zbóż i ich współdziałania z czynnikami agrotechnicznymi, Studia i Raporty IUNG-PIB, 2015, 44(18), pp. 119–133.

[18] Helfenstein J., Müller I., Grüter R., Bhullar G., Mandloi L., Papritz A., Siegrist M., Schulin R., Frossard E., Organic Wheat Farming Improves Grain Zinc Concentration, PLoS ONE, 2016, 11(8), pp. 1–20.

[19] Ukalska-Jaruga A., Smreczak B., Klimkowicz-Pawlas A., Maliszewska-Kordybach B., Rola materii organicznej w procesach akumulacji trwałych zanieczyszczeń organicznych (TZO) w glebach, Polish Journal of Agronomy, 2015, 20, pp. 15–23.

[20] Księżak J., Kęsik K., Wpływ nawożenia mineralnego i organicznego na plonowanie i jakość nasion bobiku, Polish Journal of Agronomy, 2017, 31, pp. 52–62.

[21] Trawczyński C., Bilans azotu, fosforu i potasu drugiej rotacji płodozmianu w systemie produkcji ekologicznej na glebie lekkiej, Fragmenta Agronomica, 2015, 32(2), pp. 87–96.

[22] Janowiak J., Spychaj-Fabisiak E., Próchnicotwórcza rola słomy przyorywanej bez obornika i razem z obornikiem na tle zróżnicowanego nawożenia azotem na glebie lekkiej, Zeszyty Problemowe Postępów Nauk Rolniczych, 2006, 512, pp. 201–207.

[23] Neina D., The role of soil pH in plant nutrition and soil remediation, Applied & Environmental Soil Science, 2019, pp. 1–9.

[24] Widłak M., Zmienność wybranych parametrów gleb ornych w okresie cyklu wegetacyjnego roślin, Annual Set The Environment Protection, 2016, 18, pp. 803–814.

[25] Kloc-Szatnik A., Wpływ pH na zawartość manganu w roślinach pochodzących z siedlisk naturalnych i uprawianych w kulturach wodnych, Acta Agrophysica, 2001, 57, pp. 139–146.

[26] Rajeswara B.R., Opportunities and challenges in the cultivation of Ashwagandha (*Withania somnifera* (L.) Dunal), Journal of Pharmacognosy, 2012, 3(2), pp. 88–91.

[27] Srivastava M.P., Gupta S., Dixit S., Yadav N., Yadav V., HinaS., Singh H., Kanaujia P., Sharma Y.K., *Withania somnifera* (Ashwagandha): A wonder herb with multiple medicinal properties, Asian Journal of Pharmacy and Pharmacology, 2018; 4(2), pp. 123–130.

[28] Nenadis N., Tsimidou M., Observations on the estimation of scavenging activity of phenolic compounds using rapid 1,1-diphenyl-2-picrylhydrazyl (DPPH) tests, Journal of the American Oil Chemists Society, 2002, 79, pp. 1191–1195.

29] Sanchez-Moreno C., Methods used to evaluate the free radical scavenging activity in foods and biological systems, Food Science and Technology International, 2002, 8(3), pp. 121–137.

[30] Tousif M.I., Nazir M., Saleem M., Tauseef S., Uddin R., Altaf M., Zengin G., Ak G., Ozturk R.B., Mahomoodally M.F., Exploring the industrial importance of a miracle herb *Withania somnifera* (L.) Dunal: Authentication through chemical profiling, in vitro studies and computational analyses, Process Biochemia, 2022, 121, pp. 514–528.

[31] Jezierska A., Sykuła A., Antioxidant properties of Moringa oleifera and *Withania somnifera* extracts and their use in cosmetics for men, Biotechnology Food Science, 2023, 85(1), pp. 34–42.

[32] Ghasemzadeh A., Omidvar V., Jaafar Z.H., Polyphenolic content and their antioxidant activity in leaf extract of sweet potato (*Ipomoea batatas*), Journal of Medicinal Plants Research, 2012, 6, pp. 2971–2976.

[33] Truong V.D., McFeeters R.F., Thompson R.T., Dean L.L., Shofran B., Phenolic acid content and composition in leaves and roots of common commercial sweet potato (*Ipomoea batatas* L.) cultivars in the United States, Journal Food Science**,** 2007, p. 72, pp. C343–C349.

[34] Gupta S., Pareek S., Ameta K.D., Sarolia D.K., Pilania S., Kaushik R.A., Shuklaand K.B., Kumari P., Analysis of nutritional composition of sweet potato vines, Journal of Pharmacognosy and Phytochemistry, 2018, 7(2), pp. 104–106.

[35] Fernando I.D.N.S., Abeysinghe D.C., Dharmadasa R.M., Determination of phenolic contents and antioxidant capacity of different parts of *Withania somnifera* (L.) Dunal. from three different growth stages, Industrial Crops and Products, 2013, 50, pp. 537–539.

[36] Sharma A.K., Basu I., Singh S., Efficacy and safety of ashwagandha root extract in subclinical hypothyroid patients: a double-blind, randomized placebo-controlled trial, Journal Altern Complement Medicine, 2018, 24(3), pp. 243–248.

[37] Sharma R., Samant S., Sharma P., Devi S., Evaluation of antioxidant activities of *Withania somnifera* leaves growing in natural habitats of North-west Himalaya, India, Journal of Medicinal Plants Research, 2012, 6(5), pp. 657–661.

[38] Dhanani T., Shah S., Gajbhiye N.A. Kumar G.S., Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*, Arabian Journal of Chemistry, 2017, 10(1), pp. 1193–1199.

[39] Ezez D., Mekonnen N., Tefera M., Phytochemical analysis of *Withania somnifera* leaf extracts by GC-MS and evaluating antioxidants and antibacterial activities, International Journal of Food Properties, 2023, 26(10), pp. 581–590.