The Influence of Bioproduct Zamin–M On Some Flavonoids of Artichoke Cynara Scolymus L.

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ABSTRACT

In this paper, the experimental data on the effect of the biological product Zamin-M, based on a group of phenolic compounds in artichoke leaves are provided. Experimental results showed that the amount of quercetin flavonoids in 70% alcoholic extract was 0.09 mg/g, which is 1.6 times higher in control variants than control plants treated with NH4NO3 + biological product.

The amount of rutin in the control variant was 0.0879 mg/kg, which is 2.2 times less than in plants treated with Zamin-M, 2.3 times more than in plants treated with NH4NO3 and NH4NO3 + Zamin -M ".

The amount of cynaroside in 70% of the alcoholic extracts of the leaves of the experimental variant was 8.25 mg/g, which is 1.62 times more than in control plants treated with NH4NO3, 2.3 times more than in plants treated with NH4NO3 + a bioproduct, 2.3 times more than untreated plants.

The obtained results indicate that the treatment of plants with the bioproduct Zamin-M with compared to mineral fertilizers NH4NO3 has an effective influence on the metabolic profile, which is associated with the role of secondary metabolites in the plant Cynara scolymus L.

Keywords: Cynara scolymus L. Pseudomones stubzeri SKB 308, Bacillus megeterium SKB 310, Bacillus subtilis SKB 30, bioproduct, flavonoid, cymaroside

Introduction

Based on the identification of internal biotic connections in the "plant-soil-microorganism" system of agricultural production in the world, the widespread involvement of microbial groups in practice is required in terms of increasing crop yields, accelerating plant growth and development, and improving plants' resistance to various stressors.

Mainly, in the recent practice of modern agricultural biotechnology, special attention has been paid to the bacteria around the roots, which accelerate the growth and development of plants in saline conditions. Stimulatory, fungicidal, bactericidal and phytostimulatory properties of salt-resistant rhizobacteria determine the creation of biopreparations based on local rhizobacterial strains that increase the resistance of agricultural crops to various adverse conditions and increase their efficiency.

PGPR is a special group of microorganisms that strongly colonizes the rhizosphere and rhizoplane and significantly improves plant growth and productivity using direct or indirect mechanisms. In addition, microbial contamination, as well as physiological, genetic, and environmental factors, are key factors influencing the accumulation and composition of secondary metabolites.

By means of minimal use of PGPRs in the cultivation of medicinal plants, it is possible to ensure stable high yields of important secondary metabolites in the industry [1]. Thus, the use of microorganisms in vivo not only increases the productivity and phytochemical performance of medicinal plants, but also reduces the use of chemical fertilizers [2].

Applying *Azotobacter chroococcum* and *Azospirillum lipoferum* microorganisms in *Coriandrum sativum L.* has been reported to increase the number of umbels and seeds with fungicidal, bactericidal and anthelmintic properties [3.4]. As a result of in vivo use of *Azospirillum spp* and *Azotobacter spp,* the highest level of anesol was recorded in the *Rauwolfia serpentine* plant [5]. An increase in the amount of alpha-pinene in the plant *Coriandrum sativum L.* has been reported [6]. The use of *Bacilus spp* in the cultivation of the plant *Matricaria chamomilla L.* has been shown to be important in strengthening the root system and in the production of essential oil [7]. Ratti et al. (2001) studied the effects of several types of phosphorus-absorbing solvent bacteria on *Lemon Grass* yields and noted that the height of these plants increased relative to the biomass control variant [8].

Another research has determined that seedlings of *Caharanthus roseus* growing under stress conditions with high humidity had increased plant biomass and alkaloids when treated with *Pseudomonas fluorescent* bacteria [9]. In addition, the use of biological fertilizers has led to significant growth of the *Timus vulgaris* plant [10]. Abo-Baker, A.A. and G.G. Mostafa, (2011) reported that treatment of *Gibiskus sabdariffa* with a mixture of biological fertilizers improved the growth characteristics of the plant [11]. In their research, Fatma A. et al. reported on the beneficial effects of *Azospirillum* and *Azotobacter*, as well as phosphorus-absorbing bacteria on the medicinal plant *Majorana Hortensis* [12]. Incubation of papaya (*Carica papaya Linn*) with bacteria has also resulted in improved germination rate, percentage rate, root and stem length, and an increase in essential oil (28%) and its components (*camusulin* and *bisabolene* compounds). [13].

When *Ocimum sanctum and Withania somniferum* plants were treated with with biological fertilizers based on *Azospirillum and Azotobacter*, phosphate soluble bacteria, nitrogen-fixing bacteria and a combination of these fertilizers, these microorganisms have a positive effect on the growth and productivity of medicinal plants. *Azospirillum* improves root growth by forming stimulant compounds in addition to nitrogen fixation, which increases water and nutrient absorption and overall plant productivity [14]. Co-application of arbuscular mycorrhizal fungi (VAM) with biological fertilizers has increased the growth and yield of some medicinal plants such as *Hemigraphis colorata, Tinospora cordifolia, Ocimmum basilicum, Gymnema sylvestre, Coleus amboinicus, Bacopa moninierii and Artemisia vulgaris* [15]. Studies conducted by Sajid Masood et al. have shown that tomato plant has an effective influence on plant growth by treatment with *B. pumilus* bacteria [16]. In their experiments, Ghorbanpour M. et al noted that treatment of *Hyoscyamus niger* plant with strains of *Pseudomonas putida (PP) and Pseudomonas fluorescens* (PF) resulted in a significant increase in alkaloids in the plant's roots, buds, and leaves [17]. Mahfouz S.A. et al. in field experiments conducted over two consecutive seasons (2003-2004 and 2004-2005), the effects of biological fertilizers on fennel plant growth, yield, and fat content were studied. The use of biological fertilizers (*Azotobacter chroococcum, Azospirillum liboferum and Bacillus megatherium*) in combination with chemical fertilizers has been reported to have a positive effect on vegetative organs (plant height, number of branches and wet and dry mass). Treatment of the plant with biological fertilizers resulted in an increase in the amount of carbohydrates in the dry mass of the plant as well as the amount of nitrogen, phosphorus and potassium in the plant tissue. As a result of the use of biological fertilizers, the amount of oxygen compounds, essential oil has increased. The highest levels of anethole (trans-1-methoxy-4- (prop-1-enyl) gasoline; $C_{10}H_{12}O$) in fennel essential oil were observed

at half the dose of N, P, and K, and in the variant treated with *Bacillus megatherium* [18]. The use of microorganisms serves to improve the physicochemical properties of the soil, increase the availability of nutrients for medicinal plant species, produce phytohormones, induce the root system, secrete exudates, increase the synthesis of primary and secondary metabolites [19]. Literally biological fertilizers (beneficial microbial populations) represent a unique alternative to sustainable agriculture and safe production. The use of microbial fertilizers not only increases the absorption of nutrients (nitrogen, phosphorus and potassium) in plants, but also improves the amount of total nitrogen, organic matter, soluble organic carbon, soluble organic nitrogen in the soil [20.21].

Thus, biological fertilization can reduce the cost of agricultural production and can be used to rehabilitate plants in areas of low commercial value, such as contaminated with heavy metal residues [22]. Yadegari et al. (2014) studied the influence of biological fertilizers on the growth properties, yield and quality of medicinal plants, Patora et al. (2003) studied these features in terms of lemon, mint, and Yadegari (2015) studied cucumber, mountain jam, and cloves [22.23.24].

The genus *Cynara* L is a perennial plant belonging to the family *Asteraceae*, of which 11 species are known. One of these species is *Cynara scolymus* L. (thorny artichoke). Artichoke is a vegetable crop and its thickened flowers are used as food. Its coin-wrapped leaves are fleshy, tasty, and rich in a variety of vitamins and can be eaten cooked or eaten raw. In England, France, Spain and other European countries, its freshly grown young leaves are used as a flavoring spice in food. Fluids from the young baskets, sap, flowers, leaves, roots and other parts of the plant have been used as a diuretic in chronic diseases of the kidneys, abdomen and intestines, in particular, constipation, liver pathologies (jaundice, cholecystitis, etc.). Its juice is mixed with honey and used in the treatment of stomatitis, paleness of the tongue in children, cracking [25.26].

The leaves of *Cynara scolymus L*. contain cynarin, cinaropicrin, one of the phytochemicals. The chemical composition of the plant is well studied and is of great importance in medicine. The leaves contain caffeolxin acid, cinnabar and leoteolin. An extract prepared from its leaves ensures the normal production of bile in the liver. It lowers the amount of cholesterol in the body.

In our previous research, an associative structure based on the strains of *Bacillus subtilis* SKB 309, *Bacillus megaterium* SKB 310 and *Pseudomonas stutzeri* SKB 308 rhizobacteria was developed and named Zamin-M. [31].

The patent for the invention of the Intellectual Property Agency of the Republic of Uzbekistan was obtained for the Association of bacteria that increase the fertility of saline soils (№IAP 05254. 16.01.2014).

The aim of our study was to study the effect of the bioproduct "Zamin-M" on the flavonoids of *Cynara scolymus L.*

Materials And Methods

Place of research. Our research was conducted on the experimental site of the Polytechnic Institute in Jizzakh, Jizzakh region.

The experimental area was initially cleared of weeds. The ground was well loosened and rows with a spacing of 70 cm were prepared. These experiments were conducted on the basis of 4 variants, and 4 rows were selected for each variant. The row length in each variant was 4 m, row spacing was 70 cm, and the total area was 32 m^2, width 8 m, and length 4 m. Seeds were sown at a depth of 5 cm at intervals of 70 cm. Planning and analysis of experiments were conducted based on the method by P. A. Dospekhov [32].

Bioproduct "Zamin-M" was used as a bacterial fertilizer, which has a complex effect, grown in saline soils of different levels, NH_4NO_3 was used as a mineral fertilizer. In field experiments, a working solution was prepared by mixing the composition of microorganisms with water in a ratio of 1: 100. Prickly artichoke seeds were treated with a suspension of the drug before sowing. During processing, the seeds were soaked for 1 hour. The seeds

were dried in a cool, sunny place and then planted. During the vegetation of artichokes, the soil was treated by spraying 300-350 l/ha of working solution.

Analysis of vegetative experiments was carried out during seed germination, leaf emergence, budding, flower formation and maturation. Biometric indicators were performed comparatively with respect to the control variant on the length of the main stem, sympodial branches, buds, number of flowers [27]. In the experiments conducted in the study, thorny artichoke agrotechnics was organized according to generally accepted methods [28].

Research materials. Extracts of dried extracts of leaves of *Cynara scolymus L.* in water, 40% and 70% ethanol.

Standards: cynnaroside, hyperoside (hyperin), luteolin, quercetin are dissolved in methanol, 30-50% water is added.

Preparation and analysis of standards: Aqueous liquid extract chromatography (ALEC) analysis was conducted with the usage of Agilent model 1100 (USA) in the revision (Agilent) of the program Chemstation (Agilent) program in A.10.02 (collection 1757), in 4-degree pump G1311A, G1322A degasser, in gradient mixer, in the variable wavelength detector (VWD) G1314A and Rheodyne 7725i injector (Rheodyne, SSHA) 100 µl. Analysis was conducted on Supelco Discovery HS C18 4.6x75 mm / 3 um (Supelco, Bellefonte, PA, USA) Discovery HS C18 4.0x20 mm / 3 um / 120A, equipped with a front column; mobile phase - acetonitrile gradient (R Chromasolv [Signa-Aldrich, Germany], channel B, in distilled water containing 0.05% orthophosphoric acid pH 2.5 (channel A), 15-45% B (0-15 min) flow rate 0.7 ml/min, pressure 92-102 bar, sample size 10 µl, detection was performed spectrophotometrically (UV) at 350 nm wavelength at room temperature (25 ° C). The following were used as standards (1- table).

Table 1. Standard flavonoids used to analyze the flavonoids' content of dried extracts of the leaves of *Synara scolymus L.*

Standard	UV λ_{max}
Cinnaroside (luteolin 7-O-glucoside)	**260, 348 nm 257, 268 pl, 351**
Hyperoside (hyperin)	**257, 358 nm (EtOH)**
Luteolin	**260, 356 nm**
Quercetin	**256, 370 nm**

Using analytical scales of the standards, the parts weighed to the nearest 20 mg to 0.1 mg were placed in a pre-prepared flask and then dissolved in methanol in a volume of solution with a concentration of 1 mg / ml. Primary calibration standards with a concentration of 250 µg / ml were obtained by taking equal volumes of the resulting solutions. Calibration solutions at partial concentrations (total 4 solutions: 31.25; 62.5; 125 and 250 mg / ml) were obtained by placing them sequentially in beakers with equal amounts of 50% aqueous methanol. Calibration data were obtained by sequential analysis of these solutions from low concentration to high concentration, which were then used to calculate the unknown concentration of flavonoids in artichoke leaf extracts.

Specimens

The dried samples were weighed on an analytical balance with an accuracy of approximately 20 mg to 0.1 mg. Aqueous and 40% alcohol extracts were dissolved in 2 ml of 40% aqueous ethanol, 70% alcohol extracts in 2 ml of 70% aqueous ethanol (ratio 1: 100 (m / v), slightly heated in a water bath, stirred in a vibrating stirrer. The samples were centrifuged at 3000 rpm for 5 min for clarification and analyzed by the reverse phase SSEX method

under the same conditions as the standard analysis.

Research Results And Their Discussion

It is known that some plants synthesize many flavonoid compounds. They are secondary metabolites that hold the most important phenolic compounds in plants.

Recently, interest in these compounds has been growing. Because these biologically active substances are widely used in medicine and in the food industry as agents that strengthen the vascular system, protect the liver and gastrointestinal system, stimulate the brain and heart, have antioxidant, anti-cancer and anti-inflammatory effects. The antimicrobial properties of flavonoids are also noteworthy. For example, the negative effects of quercetin on gram-positive bacteria, chalcone and flavonoids on staphylococci have been identified. From flavonoids quercetin, kempferol, isoramnetin affect protein metabolism (stimulates synthesis and stops breakdown). Quercetin, rutin and other flavonols restore hypodynamic heart strength and normalize pulse.

Prickly artichoke is an important source of bioactive phenolic compounds, including flavonoids. The antioxidant activity of plant polyphenols is related to the presence and location of hydroxyl and methoxyl groups [29]. The presence of flavonoids promotes chelation of metal ions by destroying free oxygen species [30]. However, the total amount of phenol does not precisely determine its ability to clear free radicals or its ability to reduce iron.

When the effect of biofertilizers on the group of phenolic compounds in artichoke leaves was determined, the results showed that the amount of quercetin from flavonoids in the plant was not detected in the aqueous and 40% alcohol extracts of the control variant. The content of quercetin in 70% alcohol extract was 0.09 mg/g, which was found to be 1.6 times higher in plants treated with mineral + biofertilizers than in controls, and 1.3 times higher in plants treated with Zamin-M bioproduct.

The amount of rutin in the control factor was 0.0879 mg/kg, which is 2.2 times higher than in the plants treated with "Zamin-M", 2.3 times in the plants treated with minerals, mineral+"Zamin-M" was found to have increased 2.5-fold in the given plants.

The amount of cinaroside in 70% alcohol extracts of the control variant was 8.25 mg/g, which is 1.62 times higher than the control in mineral-treated plants, 2.3 times higher than the control in plants treated with mineral+bioproduct, and 2.4 times higher than the control in plants treated with bioproduct. These results show that the treatment of plants with bioproducts and the use of bioproducts in combination with mineral fertilizers has a strong effect on the metabolite profile, which is a good factor in enhancing the biosynthesis of secondary metabolites in this plant.

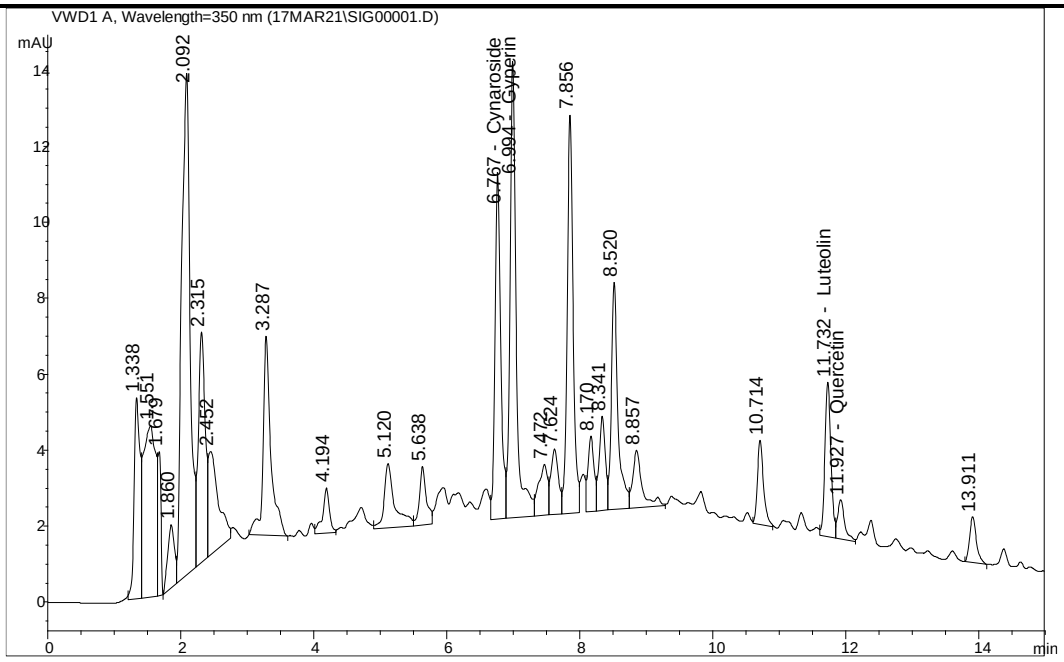

Figure 1. Chromatogram of the effect of treatment with Zamin-M bioproduct on the amount of flavonoids Cynara scolymus L. (prickly artichoke) (aqueous extracts)

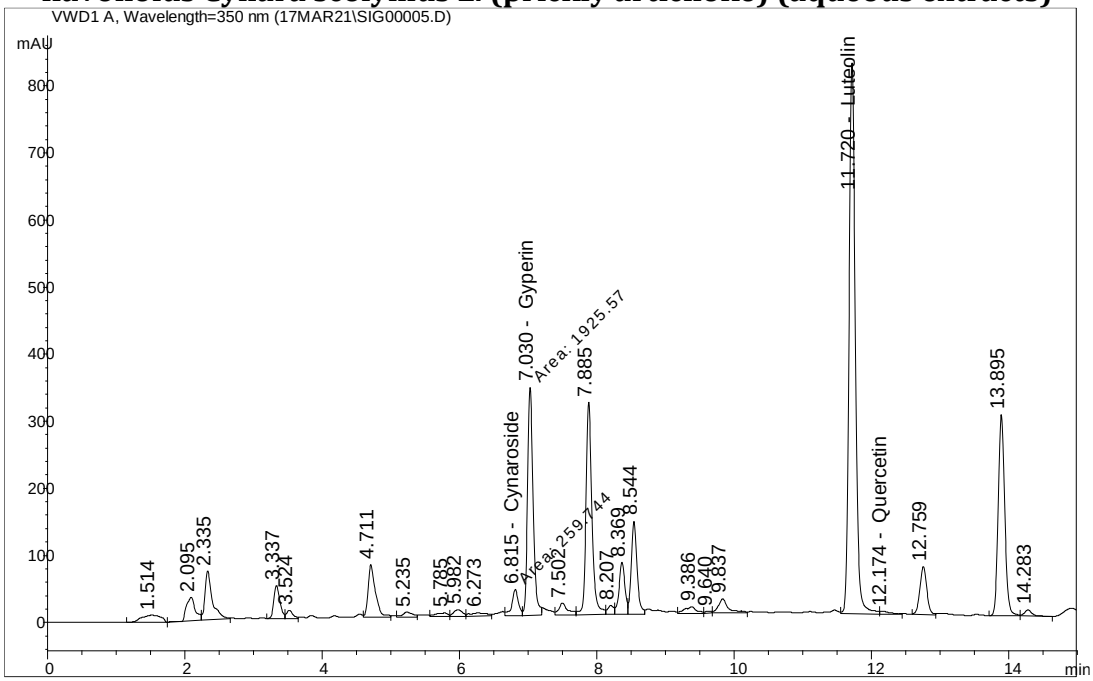

Figure 2. Chromatogram of the effect of treatment with Zamin-M bioproduct on the amount of flavonoids Cynara scolymus L. (prickly artichoke) (extracts in 40% alcohol)

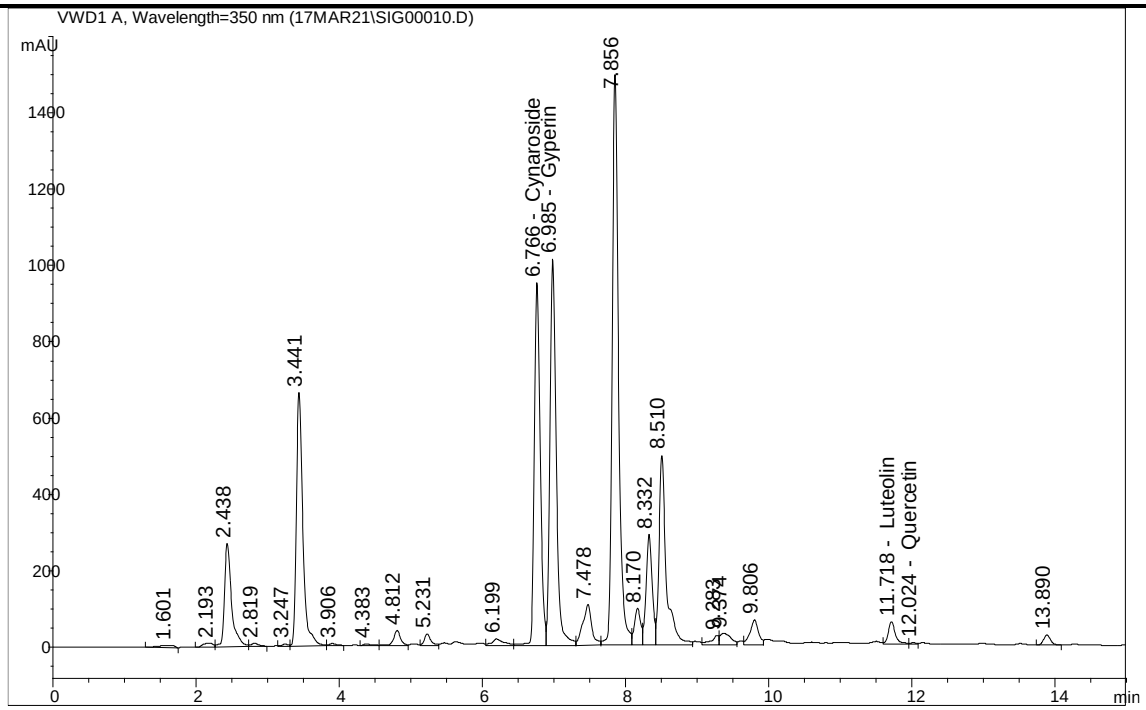

Figure 3. Chromatogram of the effect of treatment with Zamin-M bioproduct on the amount of flavonoids Cynara scolymus L. (prickly artichoke) (extracts in 70% alcohol)

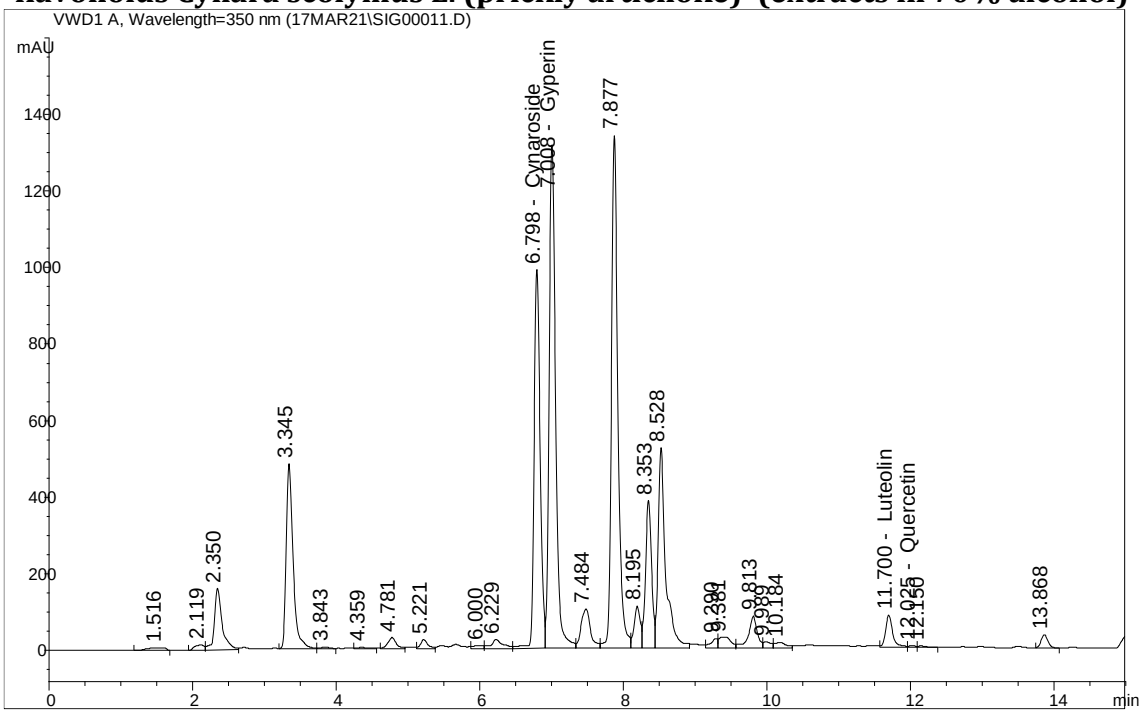

Figure 6. Chromatogram of the effect of treatment with Zamin-M bioproduct on the amount of flavonoids Cynara scolymus L. (prickly artichoke) (total extracts)

Table 2. Quantitative analysis of the influence of treatment with Zamin-M bioproduct on the amount of flavonoids *Cynara scolymus L.* (prickly artichoke).

№	Variants of the experiment	The amount of sample weighed,	Tsinnarozid, mkg/ml	Hyperoside Mkg/ ml	Luteolin mkg/ml	Quercetin mkg/ml	ω Ts mg/g	ω G mg/g	ω L mg/g	ω K mg/g
1	Mineral fertilizer (Aqueous extract)	21,5	1,79	1,55	0,51	0,17	0,17	1,73	0,66	0,67
2	Mineral+Zamin-M (Aqueous extract)	20,1	0,92	10,67	7,84	0,29	0,09	23,20	1,47	0,07
3	Zamin (Aqueous extract)	21,9	8,23	7,18	4,17	н/о*	0,75	1,74	1,16	0,00
4	Not processed extract (40% alcohol)	21,1	4,36	22,65	64,0	н/о	0,41	10,39	5,65	0,00
5	Extract treated with mineral fertilizers (40% alcohol)	21,0	8,40	43,01	109, 62	1,03	0,80	10,24	5,10	0,02
6	Extract treated with mineral fertilizer+Zamin (40% alcohol)	19,8	110, 26	126,7 7	30,6 3	0,37	11,1 4	2,30	0,48	0,02
7	Extract treated with Zamin+M (40% alcohol)	20,0	114, 47	128,8 5	40,5 1	н/о	11,4 5	2,25	0,63	0,00
8	Not processed extract (70% alcohol)	20,8	85,8 0	89,26	10,2 5	0,48	8,25	2,08	0,23	0,09
9	Extract treated with mineral fertilizers (70% alcohol)	20,1	135, 00	121,1 6	20,6 7	0,74	13,4 3	1,79	0,34	0,07
10	Extract treated with mineral fertilizer and Zamin+M (70% alcohol)	19,9	194, 68	137,4 9	8,39	0,64	19,5 7	1,41	0,12	0,15
11	Extract treated with 70%-Zamin+M (70% alcohol)	20,4	204, 55	182,3 7	11,7 3	0,68	20,0 5	1,78	0,13	0,12

Conclusion

Given the obtained experimental data and analysis of the literature, it can be concluded that sufficient in-depth studies have not been carried out on the use of complex biological products based on rhizobacteria, contributing to an increase in the amount of flavonoids. Taking into account a number of factors, starting with the choice of suitable microorganisms, promoting plant growth based on plant-host-target interactions, mechanisms of metabolic rate is important.

Based on this, research on the development of scientifically grounded technologies for the production of microbial preparations based on various rhizobacteria with useful properties and their introduction into the cultivation of medicinal plants are relevant and have great scientific and practical importance.

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