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DETERMINATION OF WATER-SOLUBLE VITAMINS IN "AS-MIR" BY THE HPLC METHOD AND THEIR SIGNIFICANCE IN INFLAMMATION

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Abstract: This study investigates the content of water-soluble vitamins in the AS-MIR mixture prepared from red cabbage (Brassica oleracea var. capitata f. rubra) and horseradish (Armoracia rusticana) in a 3:1 ratio using HPLC (LC-40 Nexera Lite). The results demonstrated that the extract contains relatively high levels of vitamins B1, B2, B3, B6, B9 and vitamin C, while vitamin B12 was not detected. These vitamins play crucial roles in antioxidant defense, DNA repair, cellular metabolism, and the reduction of oxidative stress. Their combined biological activity suggests potential anti-inflammatory and anticancer benefits. Based on the obtained results, the AS-MIR mixture may be recommended as a supplementary natural product for supporting anti-inflammatory processes and reducing cancer risk.

Keywords: AS-MIR extract; red cabbage; horseradish; water-soluble vitamins; HPLC; inflammation; antioxidant activity; DNA repair; cancer prevention.

Introduction. Red cabbage (Brassica oleracea var. capitata f. rubra) and horseradish (Armoracia rusticana), which are widely consumed today, are included in the group of plants with many medicinal properties. They are very beneficial for human health because they contain vitamins, minerals, and antioxidants. Therefore, medicinal products made from these plants have antibacterial, antifungal, and expectorant effects, relieve bronchitis, colds, and coughs, increase immunity, and fight inflammation. The antioxidants they contain reduce the effects of free radicals [1-3].

Among the water-soluble vitamins, the B vitamins (B1, B2, B3, B6, B9, B12, etc.) function as cofactors or coenzymes in cellular energetics, DNA synthesis, and key processes such as DNA repair, methylation, and antioxidant defense. Therefore, their status may affect the level of inflammation and genome stability, and consequently the risk of cancer or the effectiveness of cancer treatments. For example, the main function of vitamin B1 (Thiamine) is essential for carbohydrate metabolism (as a cofactor for pyruvate dehydrogenase and thiamine-dependent enzymes), and supports nerve and heart cell function. Thiamine deficiency in inflammation may increase metabolic stress and oxidative damage; some studies suggest that thiamine and its derivatives may reduce inflammatory signaling (e.g., NF-κB), but these results are complex and context-dependent.B1 supports cellular energy in cancer — Since the energy metabolism of tumor cells is altered, thiamine deficiency or excess can have different effects. Some studies have shown that thiamine supplementation can reduce tumor cell growth or reduce oxidative stress, while others have reported a risk of tumor promotion in some cases[4,5,6]. Vitamin B2 (Riboflavin) is a coenzyme in flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) — coenzymes in many oxidative-reductive enzymes; it is important in cellular energy and antioxidant defense. In inflammation, riboflavin may support mitochondrial function and reduce mitochondrial ROS production; some studies have also shown that riboflavin reduces the activity

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of the inflammasome (NLRP3, etc.) — which may help reduce inflammation. Epidemiological and experimental studies in cancer suggest that adequate riboflavin intake may have a protective role against some types of cancer (e.g., lung, cervical); however, this relationship is highly dependent on the species and other nutritional factors. Riboflavin helps maintain genome stability by supporting cellular DNA repair and antioxidant systems[7,8]. Vitamin B3 (niacin) is the main precursor for the synthesis of NAD^+ and $NADP^+$ - cellular energetics, response to oxidative stress, and plays a central role in DNA repair and immune cell function. Clinical and experimental studies in inflammation have shown that niacin can reduce inflammatory markers (such as CRP, TNF-α) and have immunomodulatory effects; however, side effects (e.g., vasodilation - "flushing", effects on the liver) are also observed at high doses. In cancer, niacin and NAD metabolism directly affect DNA repair and cellular energetics; recent animal and some preclinical studies have shown that VB3 can stimulate the immune system and enhance T-cell-mediated surveillance against liver tumors[9,10]. Vitamin B9 (folic acid) is essential for DNA and RNA synthesis and methylation reactions in the body, as well as for homocysteine metabolism and cell division. Its deficiency can lead to increased homocysteine levels, leading to inflammation and endothelial dysfunction; some studies suggest that folate supplementation may help reduce inflammation. In cancer, folic acid plays an important role in cell proliferation, and it has been reported in the literature that deficiency may increase DNA damage and increase cancer risk[11-14].

Vitamin C (Ascorbic Acid) is a potent antioxidant, supports collagen synthesis, immune function, iron absorption, and participates in many biosynthetic processes. Vitamin C can reduce inflammation, neutralize ROS, and support immune cell function. Clinical and experimental studies have demonstrated its clear anti-inflammatory effects. In cancer, vitamin C has a dual role—it protects cells as an antioxidant at low concentrations (which is beneficial prophylactically), but there are preclinical and some clinical studies that suggest that high (pharmacological) doses (e.g., high-dose infusions) can selectively kill some tumor cells by exerting a pro-oxidant effect [15-17]. Thus, the potential of vitamin C in the normal diet as a prophylactic agent, and the potential of high doses in therapy, require further testing.

Experimental part. Experimental part. Reagents and equipment used. The substances used in the experiment were obtained in the following order: vitamin B12 from “Rhydburg Pharmaceuticals” (Germany), vitamin C from “Carl Roth GmbH” (Germany), B9 from “DSM Nutritional Products GmbX” (Germany), vitamins B1, B2, B3, B6, PP from “BLDPharm” (China). Water, acetonitrile, acetic acid of chemically pure brand and sodium hydroxide were used as reagents of HPLC purity.

The content of water-soluble vitamins in the plant was determined using an LC-40 Nexera Lite high-performance liquid chromatograph manufactured by Shimadzu, Japan [18].

Procedure for preparing standard solutions. Solutions (100 mg/l) of vitamins C (CAS 50-81-7), B1 (CAS 59-43-8), B6 (CAS 58-56-0), B3 (CAS 59-67-6), B12 (CAS 68–19–9) and PP (CAS 98-92-0) were prepared by dissolving 5 mg of each vitamin in 50 ml of 0.1 N HCl solution. Standard solutions of vitamins B2 (CAS 83-88-5) and B9 (CAS 59-30-3) were prepared by dissolving 5 mg of these vitamins in 50 ml of 0.025% sodium hydroxide solution. Then, 200 µl of the initial B1, B6, B3, B12, PP vitamins were mixed and a solution with a concentration of 14.286 mg/l of each vitamin was prepared. In this way, standard solutions of 7.143, 3.571, 1.786 mg/l were prepared. Standard solutions of vitamin C with concentrations of 286, 143, 71.5, 57.2

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mg/l were also prepared. Pure water was used for the concentration of 0 mg/l to create a calibration graph.

Preparation of "AS-MIR" extract. To obtain the "AS-MIR" mixture, red cabbage leaves and horseradish roots obtained from the local market were dried and ground, mixed in a ratio of 3:1, and packaged. To extract water-soluble vitamins, 1 g of "AS-MIR" was measured, placed in a 50 ml conical flask, and 25 ml of 0.1 N HCl solution was added. The mixture was extracted in a GT SONIC-D3 (China) ultrasonic bath at 60 °C for 20 minutes. Then the mixture was cooled, filtered, and made up to 25 ml with water in a volumetric flask. 1.5 ml of the extract was filtered through a 0.22 µm syringe filter, placed in a vial, and used for analysis.

Chromatographic conditions. Determination of vitamins. Standard solutions and sample extracts were analyzed using an LC-40 Nexera Lite high-performance liquid chromatograph equipped with an LC-40D pump, a SIL-40 autosampler, an SPD-M40 photodiode array detector (PDA), and LabSolutions ver. 6.92 software.

Table 1. Mobile phase gradient program for the determination of vitamins.

Vaqt, daqiqa	Atsetonitril (A), %	0,5 % li sirka kislota (B), %
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Tugatish	

Shim pack GIST C18 (150 × 4,6 mm; 5 mkm, Shimadzu, Yaponiya) teskari fazali kolonkasi hamda asetonitril (A) va sirka kislotaning suvdagi 0,25 % li eritmasi (B) dan tashkil topgan gradientli harakatchan faza (1-jadval) qo'llanildi.

2-jadval. Vitamin C miqdorini aniqlashda harakatchan faza gradiyent dasturi.

Time, minutes	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
2	0	100
6	50	50
6,01	0	100
15	Finish	

A 15-minute gradient was used to determine vitamin C (Table 2) and the analytical signal was measured at a wavelength of 265 nm.

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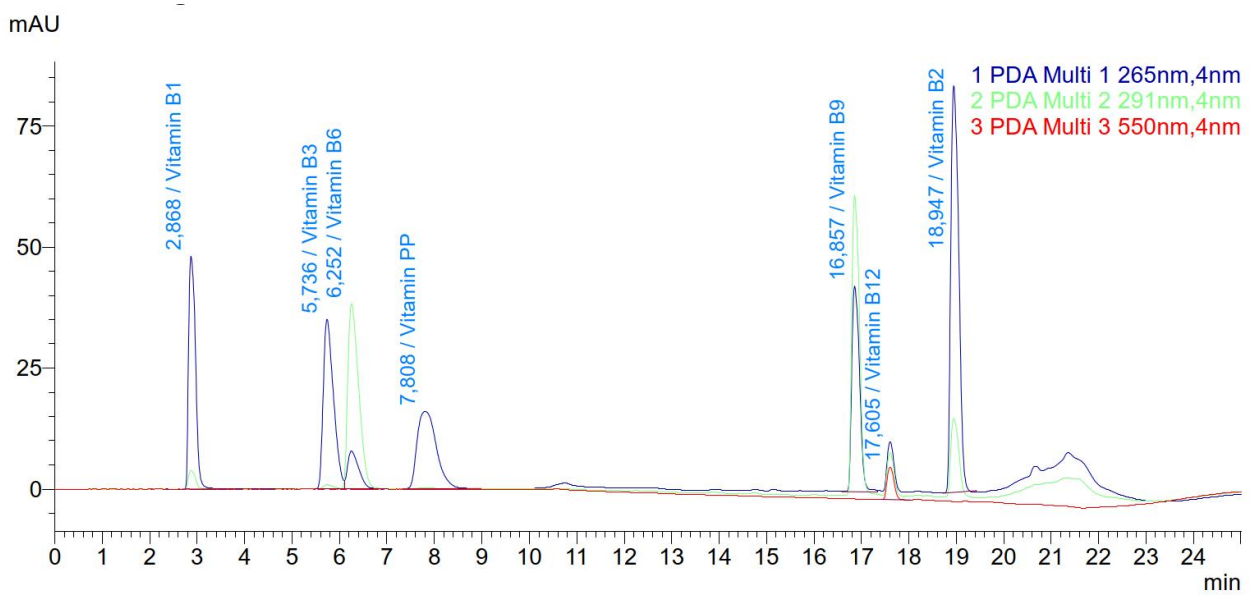

Figure 1. Chromatogram of a standard solution of vitamins.

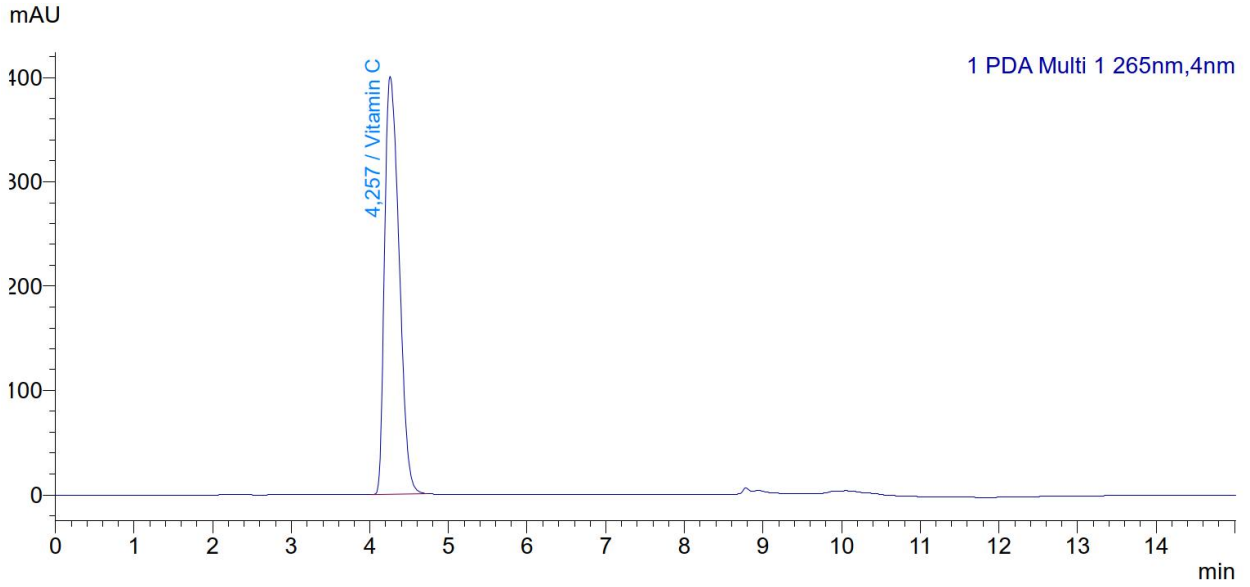

Figure 2. Chromatogram of a standard solution of vitamin C.

The injection volume was set to 10 µl, the flow rate to 0.6 ml/min, and the column thermostat temperature to 40 °C. The analytical signal (peak area) of each vitamin was recorded at three wavelengths: 265, 291, and 550 nm (Figures 1-3).

Results obtained. Determination of vitamins in the sample extract. A chromatogram of the sample extract (Figures 3-4) was obtained and based on the results, the amount of vitamins in 100 g of fruit was calculated using the following formula and presented in Table 3.

$$X = \frac{C_{vit} \cdot V_{ekstrakt}}{m_{namuna}} \cdot 100\ g$$

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Here, X – the amount of vitamins in 100 grams of fruit, mg;
Cvit – the concentration of vitamins in the extract determined by the YuSSX method, mg/l;
Vextract – the volume of the sample extract, l;
mnamun – the mass of the sample taken for extract reparation.

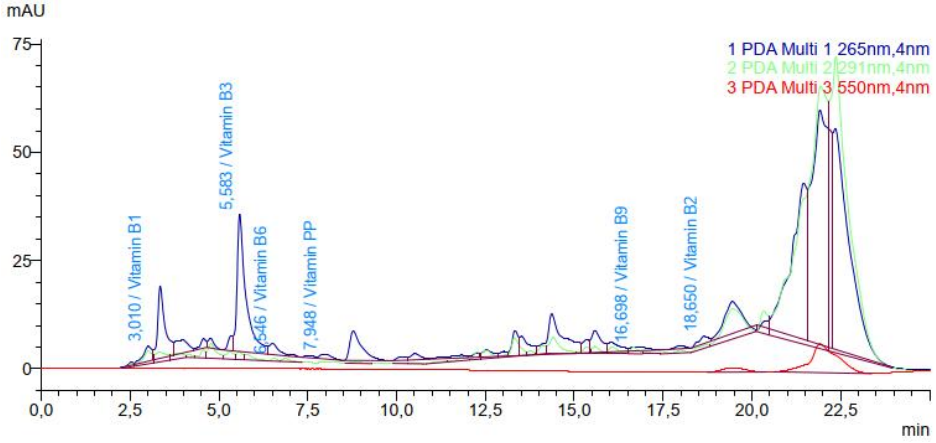

Figure 3. Chromatogram of the determination of vitamins in the sample extract.

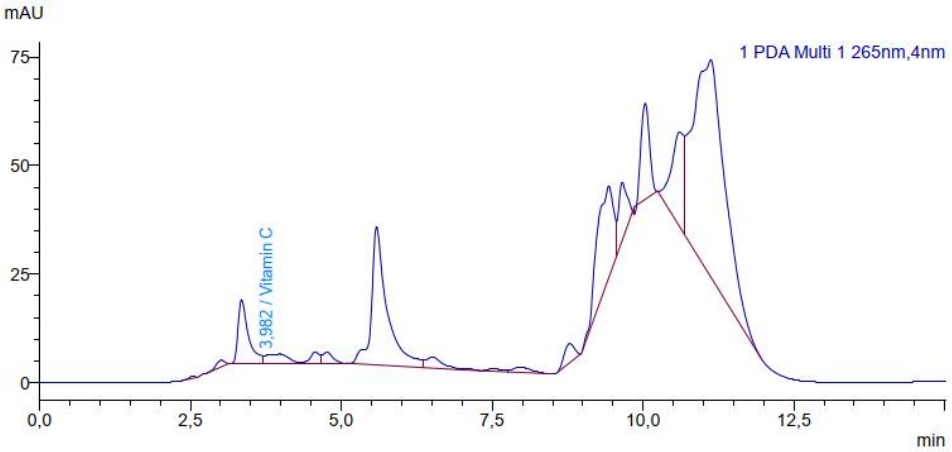

Figure 4. Chromatogram for determining the amount of vitamin C in the sample extract.

Table 3. Amount of vitamins in the extract and retention times.

Vitamin	Retention time, sec	Concentration, mg/l	Amount in 100 g sample, mg
Vitamin B_1	3,01	1,821	4,553
Vitamin B_3	5,583	14,876	37,190
Vitamin PP	7,948	0,823	2,058
Vitamin B_9	16,698	0,869	2,173
Vitamin B_2	18,65	0,74	1,850
Vitamin B_6	6,546	0,191	0,478

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Vitamin B_{12}	Not detected	0	0,000
Vitamin C	3,982	2,473	6,183

As can be seen from Table 3, the water-soluble vitamin B12 was not detected in the 3:1 mixture of the above-ground part of Brassica oleracea var. capitata f. rubra and the root of Armoracia rusticana (AS-MIR), but B1- 4.553 mg, B2-1.850 mg, B3-37.190 mg, B9-2.173 mg, B6-0.478 mg, C-6.183 mg per 100 g of the sample. Due to their antioxidant, DNA repair and metabolic activity supporting effects, these vitamins reduce cellular stress, which may contribute to a decrease in inflammation and a decrease in the risk of cancer. The combination of B2, B3, B6 and C activates the anti-inflammatory mechanisms of immunity. Folate (B9) reduces mutations caused by inflammation through DNA repair. The high content of B1, B2, B3, and B9 in the product may be beneficial in reducing oxidative stress in the body.

Conclusion. The results of the determination of water-soluble vitamins in 'AS-MIR', a mixture of the above-ground part of the red cabbage plant and horseradish root in a 3:1 ratio, showed that it contains high amounts of B1, B2, B3 and B9. On this basis, we recommend the use of "AS-MIR" in the treatment of inflammatory and cancer diseases.

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