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PHENOLIC COMPOUNDS OF THE "AS-MIR" FOOD SUPPLEMENT

Mirzokhidbek M. Bokiyev
Andijan State Medical Institute, Assistant Professor of the Department of Biological Chemistry
https://orcid.org/0000-0002-4221-474X

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Abstract

An extract was obtained from the dried and powdered aerial part of red cabbage (Brassica oleracea var. capitata f. rubra) and the root of horseradish (Armoracia rusticana) mixed in a 3:1 ratio (AS-MIR) using ultrasonic extraction in 96% ethanol for 20 minutes. The content of phenolic compounds (flavonoids) in the extract was determined by the High-Performance Liquid Chromatography (HPLC) method. Chromatographic analysis showed that in 100 g of the dried mixture, there were 8.928 mg of quercetin, 2.973 mg of apigenin, 1.373 mg of rutin, 1.148 mg of salicylic acid, and 1.313 mg of gallic acid.

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Introduction. In recent years, the development of medicinal products based on bioactive substances of natural origin has become one of the most relevant directions in the fields of pharmaceuticals, food and biotechnology. Inflammatory processes play an important role in the pathogenesis of many chronic diseases, including cardiovascular, metabolic and even oncological diseases. Despite the effectiveness of modern synthetic anti-inflammatory drugs, their long-term use has adverse effects on the gastrointestinal, hepatic and renal systems. Therefore, the need for natural, plant-based complex preparations and biologically active supplements (BPS) is significantly increasing.

In folk medicine, red cabbage (Latin *Brassica oleracea var. capitata f. rubra*) is not only a tasty vegetable, but also a plant with many medicinal properties. Cabbage is very beneficial for human health because it is rich in vitamins (C, K, B6), minerals (potassium, magnesium), antioxidants, and especially sulforaphane.

The L-glutamine and sulforaphane in red cabbage juice help repair the stomach lining and fight bacterial infections (such as Helicobacter pylori), and it is rich in potassium and antioxidants, which help maintain normal blood pressure. Its flavonoids and polyphenols (gallic acid, quercetin, rutin) are effective in preventing cardiovascular diseases, while antioxidants and vitamin C help strengthen immunity and fight infections, rejuvenate the skin, reduce inflammation, and heal wounds faster. [1, 2].

Red cabbage is rich in anthocyanins, which have powerful antioxidant effects, sulforaphane and indole-3-carbinol, which support detoxification processes in the body and slow the development of cancer cells. It has a particularly protective effect against breast, lung and colon cancer. Applying cabbage leaves externally as a compress to painful areas reduces muscle and joint pain, and it has a low glycemic index, helping to control blood sugar levels. [3,4].

Sweet pepper (Latin *Armoracia rusticana*) is a plant with health benefits, the roots and leaves of which are used in folk medicine to treat many diseases. Sweet pepper is rich in biologically active substances, including vitamins (C, B6), minerals, antioxidants and essential oils. Its root part has a pungent taste and is enriched with strong-smelling essential oils, glucosinolates, flavonoids and ascorbic acid. Due to its sharp bitterness, horseradish is widely used not only as a spice in food, but also in folk medicine.

This article presents the results of the determination of flavonoids in ethanolic extracts of a 3:1 mixture (AS-MIR) of dried red cabbage (*Brassica oleracea var. capitata f. rubra*) aerial parts and bell pepper (*Armoracia rusticana*) roots using the HPLC method.

Reagents and equipment used. The main standards and reagents used in the research process were: gallic acid from Macklin (China), salicylic acid from Rhydburg Pharmaceuticals (Germany), as well as quercetin, apigenin and kaempferol from Regal (China). Rutin was isolated from natural raw materials by extraction and column chromatography. HPLC-purified water, acetonitrile, acetic acid and sodium hydroxide reagents were used for analysis. The amount of polyphenols was determined using a Shimadzu LC-40 Nexera Lite high-performance liquid chromatography from Japan.

The procedure for preparing standard solutions and plant extracts was as follows. Working solutions of standard substances were prepared in the following sequence: gallic acid (5.2 mg), salicylic acid (5.2 mg), rutin (5 mg), quercetin (5 mg), apigenin (5 mg) and kaempferol (5 mg) were measured in separate containers and dissolved in 96% ethanol. The solutions were allowed to dissolve completely in an ultrasonic bath for 20 minutes, then transferred to 50 ml flasks and made up to the specified volume with ethanol. The mixture, obtained from 200 µl of each standard solution, was diluted into four solutions of different concentrations. The prepared solutions were placed in vials and prepared for chromatographic analysis.

Method for extraction of phenolic compounds from the “AS-MIR” mixture. To isolate phenolic compounds, a sample of the mixture prepared in a 3:1 ratio was weighed on an OHAUS NV222 (USA) analytical balance to the nearest 0.01 g. 1 g of the mass was placed in a 50 ml conical flask, and 25 ml of 96% ethanol was added to it. The mixture was extracted in a GT SONIC-D3 (China) ultrasonic bath at 60 °C for 20 minutes. After cooling, the extract was filtered and made up to 25 ml in a volumetric flask with ethanol.

1.5 ml of the resulting extract was separated using a Mini-7 (BIOBASE, China) centrifuge at 7000 rpm. The solution after centrifugation was filtered through a 0.45 µm syringe filter and sent for HPLC analysis [5].

Chromatographic analysis procedure. The amount of phenolic compounds in the alcoholic extract of the “AS-MIR” mixture was determined on a Shimadzu LC-40 Nexera Lite high-performance liquid chromatograph. Standard solutions prepared for the analysis of phenolic components, extracts obtained on the basis of a 3:1 mixture of the above-ground part of the red cabbage (*Brassica oleracea var. capitata f. rubra*) plant and the root of horseradish (*Armoracia rusticana*) were separated on a reversed-phase Shim-pack GIST C18 (150 × 4.6 mm; 5 µm) column.

A gradient system consisting of two components was used as the mobile phase in the analysis: A–acetonitrile, B – 0.5% acetic acid solution in water.

The gradient program was carried out in the following sequence: 0–5 minutes: A – 5%, B – 95%. 5–17 minutes: isocratic mode with unchanged percentages. 17–22 minutes: A fraction A was increased to 40%, and B was reduced to 60%. 22–22.1 minutes: the system was returned to the initial conditions (A – 5%, B – 95%). 22.1–40 minutes: the analysis was completed while maintaining the initial ratio.

Chromatographic separation was performed at an injection volume of 10 µl, a flow rate of 0.5 ml/min, and a column temperature of 40 °C. Chromatograms of the standard mixture and plant extracts were recorded with an ultraviolet detector at a wavelength of 300 nm[6.]

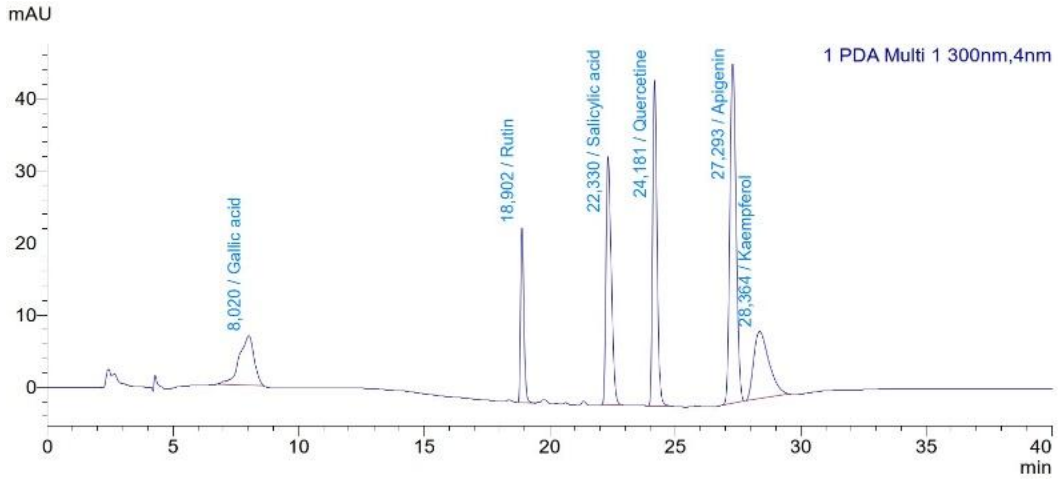


Figure 1. Chromatogram of the standard solution (at 300 nm)

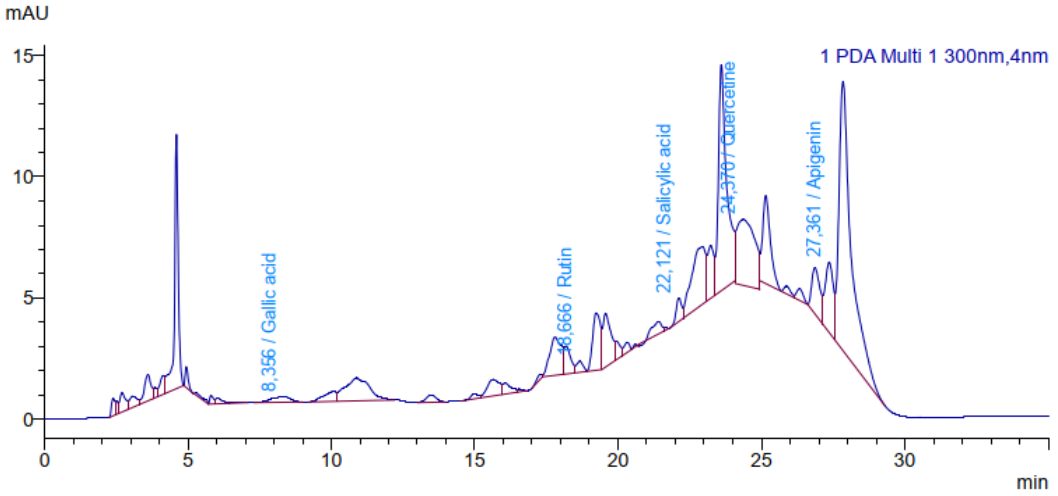


Figure 2. Chromatogram of the "As-MIR" extract

Discussion of the obtained results. Results of chromatographic determination of the amount of phenolic compounds. Based on the analysis of chromatograms 1 and 2, the retention times, measured concentrations and amounts of the main phenolic components present in the extract were determined in a 3:1 mixture of 100 g of dried *Brassica oleracea var. capitata f. rubra* aerial parts and *Armoracia rusticana* roots. Calculations were performed based on the following formula:

$$X = \frac{C_{phen} \cdot V_{extract}}{m_{samle}} \cdot 100\ g$$

Where: X – amount of phenolic compound in 100 g of dried AS-MIR mixture (mg); C_{phen} – concentration of phenolic substance in the extract (mg/l) determined by HPLC; $V_{extract}$ – final extract volume (l); m_{sample} – mass of the sample taken for extraction (g).

Phenolic compounds in the extract, their retention times and amounts.

Table 1. The amount of phenolic substances in 100 g of dried "ASMIR"

Phenolic Substance	**Amount (mg/100 g)**
Gallic Acid	1,313
Rutin	1,373
Salicylic Acid	1,148
Quercetin	8,928
Apigenin	2,973

Phenolic Substance	Amount (mg/100 g)
Kaempferol	0

According to the results of chromatographic analysis, gallic acid was retained for 8.356 seconds, rutin for 18.666 seconds, salicylic acid for 22.121 seconds, quercetin for 24.370 seconds, and apigenin for 27.361 seconds. Kaempferol was not detected in the AS-MIR mixture. The concentrations of these compounds in the analyzed extract were 0.525 mg/l, 0.549 mg/l, 0.459 mg/l, 3.571 mg/l and 1.189 mg/l, respectively.

The obtained data show that the composition of phenolic compounds in the AS-MIR mixture consists mainly of flavonoids, among which quercetin and apigenin are concentrated in the highest amounts. The absence of kaempferol indicates a specific feature of the biological composition of the mixture.

Quercetin and apigenin are natural bioflavonoids, belonging to the group of polyphenols that are commonly found in the daily diet. Their antioxidant, anti-inflammatory, immunomodulatory and anti-tumor properties have been widely described in the scientific literature. The significant accumulation of these substances in the AS-MIR mixture increases its potential nutraceutical value and indicates the possibility of therapeutic[7] use of the mixture.

In conclusion, the results of the chromatographic determination of phenolic compounds in a 3:1 mixture of the above-ground part of the red cabbage (*Brassica oleracea var. capitata f. rubra*) plant and the root of the bell pepper (*Armoracia rusticana*) (AS-MIR) showed that this mixture is rich in biologically active flavonoids such as quercetin and apigenin. High levels of these substances may enhance the anti-inflammatory, antioxidant and effects of the mixture. On this basis, the AS-MIR mixture is recommended as a promising herbal raw material for use in folk medicine and nutraceuticals, particularly in inflammatory diseases.

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