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ANALYSIS OF PHENOLIC COMPOUNDS IN THE FOOD ADDITIVE AS-RAZZOQ

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Annotation

This article examines the content of polyphenolic compounds in a mixture of rose hips (Rosa canina L.) and licorice (Glycyrrhiza glabra L.) roots in a ratio of 3:1, utilizing high-performance liquid chromatography for analysis. The findings are presented, alongside a discussion on the potential use of flavonoids from the sample for treating upper respiratory tract colds and the biological activity of polyphenols.

How to Cite

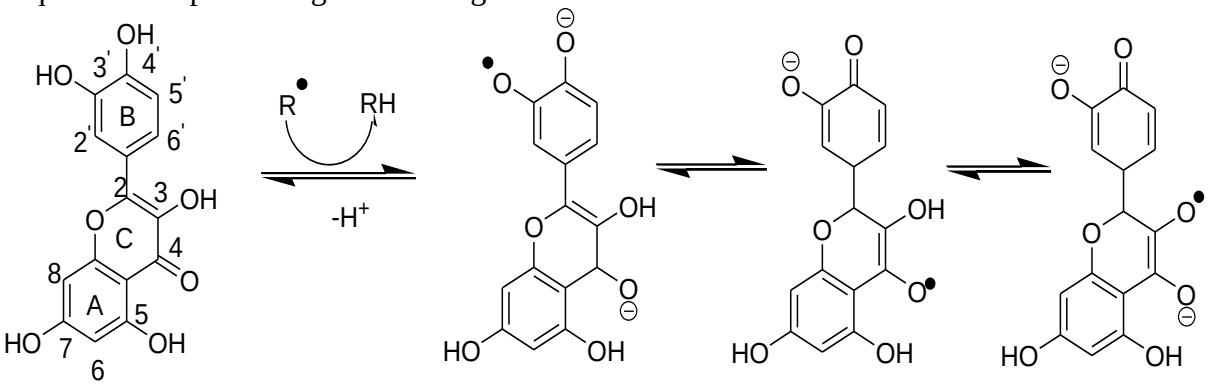
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Introduction: To prevent and treat colds and infectious diseases within the population, scientific research is being conducted to develop synthetic drugs and natural food additives that contain chemical compounds with immunostimulating properties. This includes identifying the chemical composition of these substances. In this context, it is essential to find medicinal plants that contain biologically active compounds capable of improving metabolism and stimulating the immune system. These plants can be supplements to synthetic drugs in traditional medicine to strengthen the body's defence mechanisms. Therefore, the search for natural products with biological activity is of great significance for advancing this field.

Polyphenolic compounds occupy a significant position among biologically active substances. For centuries, plants rich in these compounds have been used to treat bacterial diseases. Many of these compounds can reduce the virulence of pathogenic strains and bolster the body's defences.

In terms of flavonoid content, plant organs generally contain higher levels than animal organs. These substances maintain their protective functions and are consistently present in the blood, lymph, and intercellular fluids. They affect the receptors involved in cell signalling. Furthermore, flavonoids can penetrate the cytoplasm and directly influence the activity of specific enzymes. Rutin, a glycoside of quercetin, is the most well-known and extensively studied flavonoid, and it is also one of the most common in the plant kingdom.

Research involving quercetin conducted on several volunteers with inflammation and oxidative stress demonstrated a significant positive effect. However, it showed no notable effects in healthy individuals. The following presents the biochemical equation illustrating the anti-radical action of quercetin in preventing and treating inflammation:



Biochemical process diagram of the anti-inflammatory effect of quercetin. Figure 1.

The image above illustrates the quercetin molecule, which features three aromatic rings that interact with free radicals produced in the human body. This interaction occurs at the expense of its B ring (catechol group), specifically at the third carbon atom, which serves as the initial reaction point for the radicals. Numerous in vitro experiments conducted by scientists have demonstrated that quercetin does not exhibit any toxic effects on healthy cells. Additionally, kaempferol is a compound associated with the wild ornamental plant *Kaempferia galanga*, which belongs to the ginger family.

Research method. During our research, we began to study the flavonoid composition of the plants *Rosa canina* L and *Glycyrrhiza glabra* L, which are natural sources rich in flavonoids. The polyphenol content of the plant composition prepared from *Rosa canina* fruits and licorice roots (in a ratio of 3:1) was determined using the USSX.

Initially, the most important flavonoids were taken as standards for chromatographic analysis. Among them, gallic acid was obtained from "Macklin" (China), Salicylic acid from "Rhydburg Pharmaceuticals" (Germany), quercetin, apigenin, and kaempferols from "Regal" (China), rutin was isolated from natural sources by extraction and column chromatography. Water, acetonitrile, acetic acid of chemically pure brand and sodium hydroxide were used as reagents with HPLC purity. Gallic acid (5.2 mg), salicylic acid (5.2 mg), rutin (5 mg), quercetin (5 mg), apigenin (5 mg), kaempferol (5 mg) were dissolved in 96% ethanol for 20 minutes in an ultrasonic bath and transferred to a 50 ml flask and made up to the mark with ethanol. 200 µl of each solution was taken and mixed, and a total of 4 different solutions were prepared by diluting them. Each solution was poured into a vial and used for analysis. The amount of polyphenols in the plant was determined using an LC-40 Nexera Lite high-performance liquid chromatograph manufactured by Shimadzu, Japan.

In our previous research, we aimed to determine the optimal ratio of a mixture of rose hips and licorice plants that exhibits the highest biological activity. We assessed the antioxidant activity of various compositions of these samples using spectrophotometric analysis. The study focused on the antioxidant activity of extracts obtained with two different solvents—water and ethanol—as well as their ability to inhibit the autooxidation reaction of adrenaline.

The results indicated that the ethanol extract made from a 75:25 ratio of rose hips (*Rosa canina* L.) and licorice (*Glycyrrhiza glabra* L.) roots demonstrated superior antioxidant activity compared to

the other samples. Given its high antioxidant properties and the rich composition of biologically active substances in rose hips, which stimulate the immune system, along with the presence of glycyrrhizic acid in licorice root that offers antioxidant, antimicrobial, and anti-inflammatory benefits, we aimed to develop a new food supplement called “AS-RAZZOQ.” This supplement is designed to treat and prevent upper respiratory tract diseases while being rich in biologically active substances.

Furthermore, we analyzed the content of phenolic compounds—key biologically active substances in the formulation of this food supplement using a physicochemical method.

Experimental part. For the extraction of phenolic compounds, 1 g of the test sample was weighed with an accuracy of 0.01 g on an NV222 balance manufactured by OHAUS (USA), placed in a 50 ml conical flask and 25 ml of 96% ethanol was added. The mixture was extracted in an ultrasonic bath of the GT SONIC-D3 brand (China) at a temperature of 60 ^{o}C for 20 minutes. Then the mixture was cooled, filtered and made up to 25 ml with ethanol in a volumetric flask. 1.5 ml of the extract was centrifuged at 7000 rpm in a Mini-7 brand (BIOBASE, China) centrifuge filtered through a 0.45 µm syringe filter and used for analysis.

Standard solution, sample extract Shim pack GIST C18 reversed-phase column (150 × 4.6 mm; 5 µm, Shimadzu, Japan) and a gradient mobile phase consisting of acetonitrile (A) and 0.5% acetic acid in water (B) (Table 1) were used. The injection volume was 10 µl, the flow rate was 0.5 ml/min, and the column thermostat was set to 40 °C. The analytical signal (peak area) of phenolic compounds was recorded at 300 nm. A chromatogram of a sample extract weighing 1 g was obtained, and based on the results, the amounts of phenolic compounds in 100 g of sample were calculated using the following formula and are presented in Table 3.

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

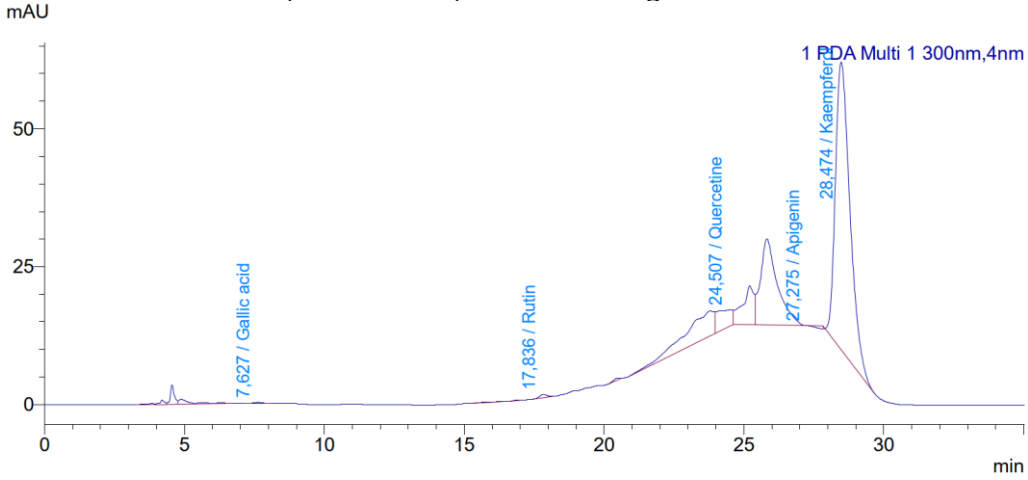
Here, X – the amount of phenolic compounds in 100 grams of sample, mg;

Cphen – the concentration of phenolic compounds in the extract determined by the HPLC method, mg/l;

$V_{extract}$ – the volume of the sample extract (l);

m_{sample} – the mass of the sample taken for the preparation of the extract.

Below is a chromatogram of the polyphenol content of a plant composition prepared from rosehip fruits and licorice roots (in a 3:1 ratio) obtained using HPLC:



Chromatogram for the determination of polyphenols in a sample extract. Figure 1.

The chromatogram indicates the following retention times for various compounds: gallic acid at 7.627 seconds, rutin at 17.36 seconds, quercetin at 24.507 seconds, apigenin at 27.275 seconds,

and kaempferol at 28.474 seconds. The extract is rich in flavonoids and contains gallic acid, rutin, quercetin, and kaempferol. However, apigenin and salicylic acid were not detected in the sample. The results of the study are presented in the table below:

Amount of polyphenols in the extract and retention times. Table 1.

Phenol compound name	Retention time, sec	Concentration, mg/l	Amount in 100 g of sample, mg
Gallic Acid	7,627	0,186	0,465
Rutin	17,836	0,777	1,943
Salicylic Acid	No peak detected	0	0,000
Quercetin	24,507	3,909	9,773
Apigenin	27,275	0	0,000
Kaempferol	28,474	80,084	200,210

The table indicates that 100 grams of the extract made from the fruits and roots of the narcissus plant, in a ratio of 3:1, contains the following compounds: gallic acid (0.465 mg), rutin (1.946 mg), quercetin (9.773 mg), and kaempferol (200.210 mg). Additionally, analysis using HPLC revealed that salicylic acid and apigenin were not present in the extract. Results from high-performance liquid chromatography indicate that the sample is particularly rich in quercetin, rutin, and especially kaempferol.

Conclusion. Chromatographic research has proven that the composition of the fruit of the narcissus and the roots of the narcissus (3:1) is rich in flavonoids. Since inflammation is the background of the disease in diseases of the upper respiratory tract, including colds, the importance of polyphenol compounds in the prevention and treatment of this pathological process in the patient's body, as recognized by scientists around the world, is very great. Therefore, we can conclude that we have scientifically substantiated the high effectiveness of using these local medicinal plants in strengthening the immune system and preventing and treating diseases of the upper respiratory tract. In addition, it can be recommended that they be used to remove phlegm accumulated in the lungs due to severe colds.

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