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Biologically Active Substances Contained in The Dry Extract of Woad

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Abstract

Biologically active substances of woad (Isatis tinctoria), such as flavonoids, vitamins, and polysaccharides, have been studied. It was found that the dry extract obtained from the leaves of woad contains vitamins C, B_2, and PP, as well as the flavonoids dihydroquercetin, quercetin, and rutin. The total polysaccharide content in the studied extract was also determined and amounted to 11.07%.

The obtained data allow us to conclude that the dry aqueous extract obtained from the leaves of woad contains vitamins C, B_2, and PP, as well as the flavonoids dihydroquercetin, quercetin, and rutin, which indicates that the dry extract possesses antioxidant, antiviral, anti-inflammatory, vasodilatory, and other properties. Furthermore, the polysaccharide content in the studied extract was identified. The total polysaccharide content amounted to 11.07%.

Keywords: Woad, biologically active substances, flavonoids, vitamins, polysaccharides.

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1. Introduction

Isatis tinctoria L. (woad) has been used in medicine for centuries and has demonstrated anti-inflammatory effects. However, to date, no well-defined extracts with precise analysis of active substances have been developed. The aim of this study was to develop extracts of Isatis tinctoria L. and characterize their active ingredients. The dry aqueous extract obtained from the leaves of Isatis tinctoria L. was analyzed for active ingredients. (1)

In recent years, a set of measures has been implemented to improve the system of providing the population of the Republic with medicines, medical devices, and medical equipment, and favorable conditions have been created for the development of the domestic pharmaceutical industry. At the same time, insufficient production capacity and the narrow range of domestic pharmaceutical products do not meet the needs of the domestic market and create a high dependence on imports. (2)

To date, data have been collected on the biological activity of about 12,000 chemical compounds isolated from

medicinal plants. As a result of chemical reactions occurring in the plant cell, products of primary and secondary metabolism accumulate in plants, which determines the multiplicity and harmony of their effects on the human body. The composition of such medicinal plants contains amino acids, macro- and microelements, flavonoids, and vitamins, which contribute to a positive effect on the human body, strengthen the cardiovascular system, and promote recovery after inflammatory processes (3). One such plant is woad, which grows widely throughout the territory of the Republic of Uzbekistan. Consequently, in this study, we aimed to investigate the active ingredients contained in the dry aqueous extract of woad leaves.

According to the World Health Organization, more than 60% of available medicines are preparations derived from medicinal plant raw materials, because they are high-quality, cost-effective, and have few side effects on the body. For this reason, our government pays great attention to the creation of industrial plantations of medicinal plants for the production of medicines and biologically active supplements. (4)

Woad (Latin Isatis tinctoria) (from the Greek word isadzo – to heal) is a biennial herbaceous plant of the Brassicaceae family, 40–120 cm tall, with a multi-headed root. The composition of woad is rich in organic acids, flavonoids, minerals, alkaloids, and phytosterols, which allow it to strengthen, nourish, and revitalize weakened hair follicles, as well as to heal the cornea of the eye, etc. (5) Woad has long been used in the treatment of various diseases in folk and traditional medicine of many countries. Currently, the study of its chemical composition and therapeutic properties has become relevant, as woad has a wide spectrum of action. An extract from the roots and leaves is used to treat various respiratory diseases, as well as viral diseases such as COVID-19. In addition, woad has antithrombotic, antitumor, and antidiabetic effects. (6)

Aim of the study. Determination of biologically active substances in the dry aqueous extract obtained from the medicinal plant raw material of woad grown in Uzbekistan.

2. Methods

A dry aqueous extract was obtained from the leaves of woad. An analysis of water-soluble vitamins, the total flavonoid content (by HPLC method), and the total sugar content was carried out.

3. Results and Discussion

HPLC analysis of water-soluble vitamins was performed on an Agilent Technologies 1200 chromatograph using an Eclipse XDB C18 (reversed-phase) column, 3.5 µm, 4.6×150 mm. A diode-array detector (DAD) was used at 254 and 290 nm. Mobile phase A: 0.5% acetic acid, pH 1.7; mobile phase B: CH_3CN (acetonitrile). Flow rate was 1 mL/min. Gradient %B/min: 0–5 min/96:4%, 6–8 min/90:30%, 9–15 min/80:20%, 15–17 min/96:4%. The column thermostat was set to 25°C.

Figure 1 shows the chromatogram of standard vitamin samples. Below, Figure 2 shows the chromatogram of vitamins contained in the woad extract.

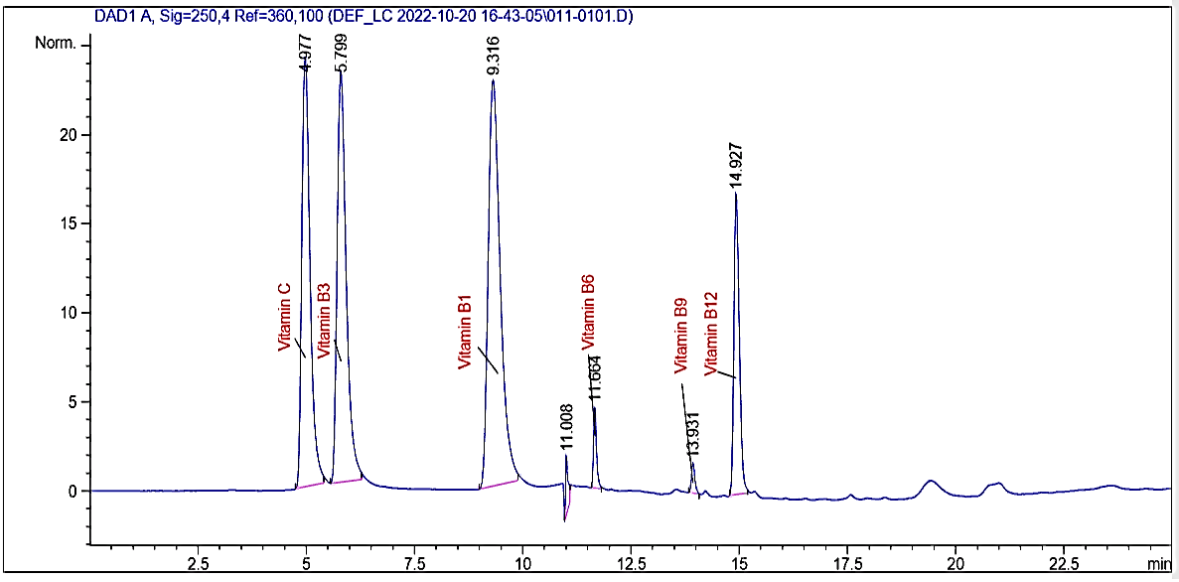

Fig. 1. Chromatogram of standard vitamin samples.

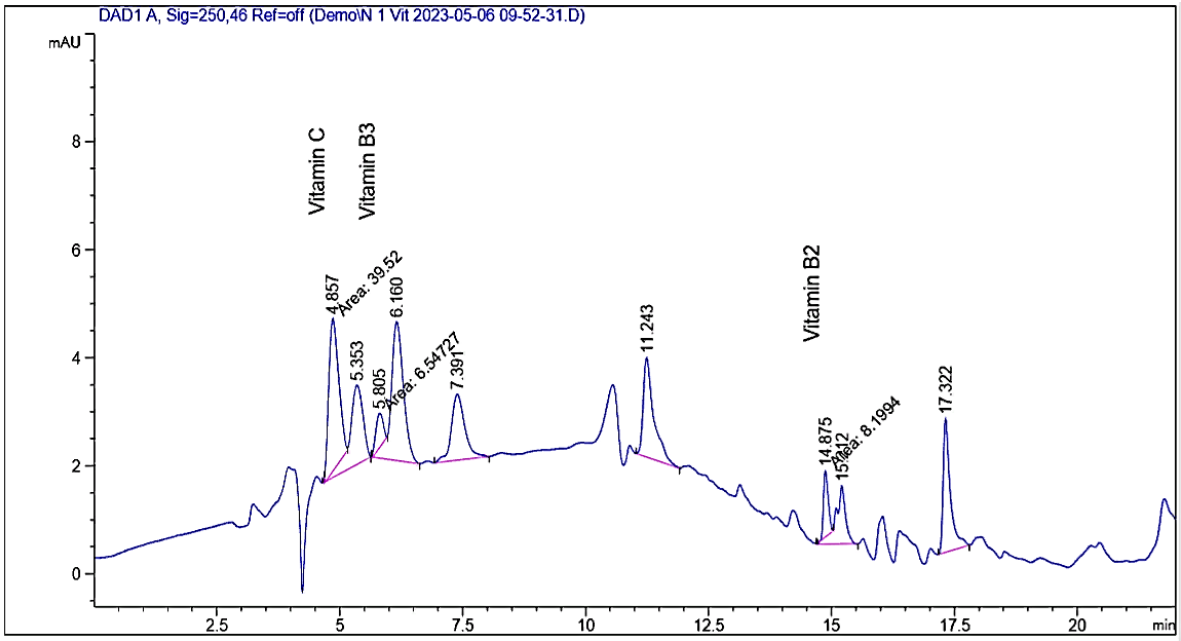

Fig. 2. Chromatogram of vitamins contained in the woad extract.

From this chromatogram, it follows that the dry extract (obtained on an aqueous basis) contains vitamins B_2, B_3 (PP), and C in the concentrations shown in Table 1.

Table 1.

Vitamin content in the dry extract of woad

Vitamins	**Woad**
	Concentration, mg/g
B-1	0
B-2	0,91
B-6	0
B-9	0
PP (B-3)	1,71
C	5,65

From these data, it can be concluded that the dry extract of woad possesses antioxidant, anti-inflammatory, and antiplatelet effects, and exerts a beneficial influence on the cardiovascular and nervous systems.

The determination of flavonoids was also carried out by HPLC using an Agilent Technologies 1200 instrument, with an Agilent C18 column (5 µm, 4.6 × 250 mm). Elution was performed in isocratic mode. The mobile phase consisted of a mixture of 0.1% orthophosphoric acid and acetonitrile in a ratio of 70:30. The flow rate was 1.0 mL/min, and the injection volume was 10 µL. The detection wavelengths were 254 and 276 nm.

Below, Figures 3–7 show the chromatograms of standard flavonoid samples.

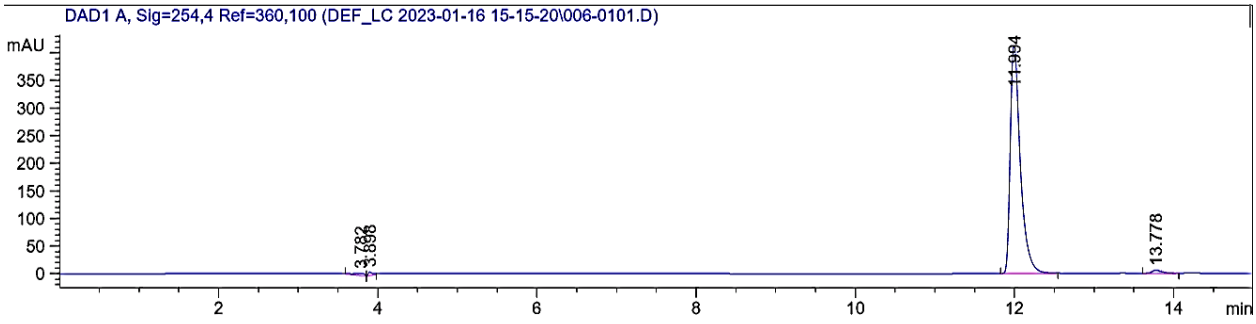

Fig. 3. Chromatogram of the Luteolin standard sample.

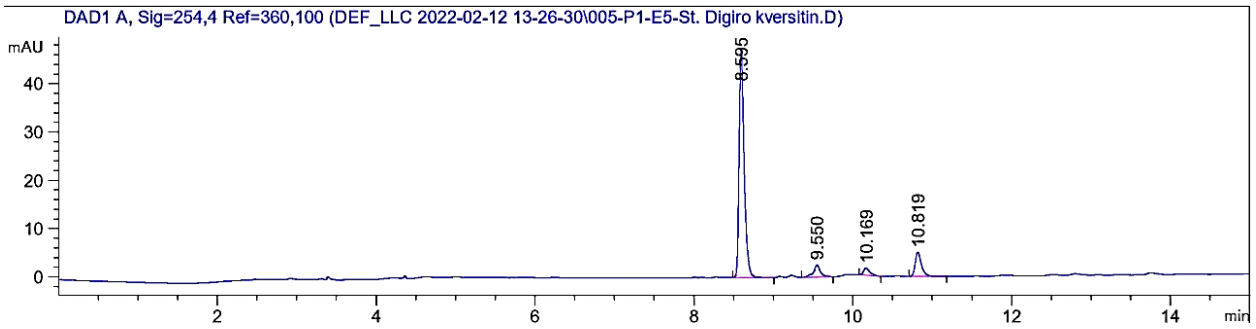

Fig. 4. Chromatogram of the Rutin standard sample.

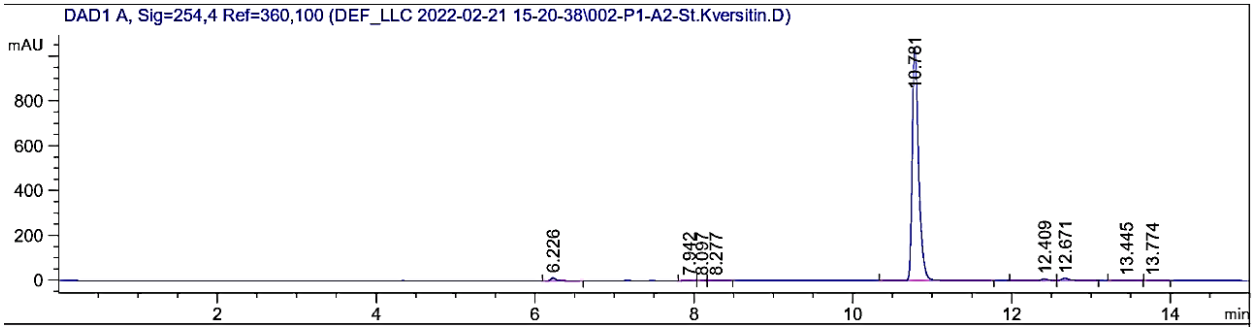

Fig. 5. Chromatogram of the Dihydroquercetin standard sample.

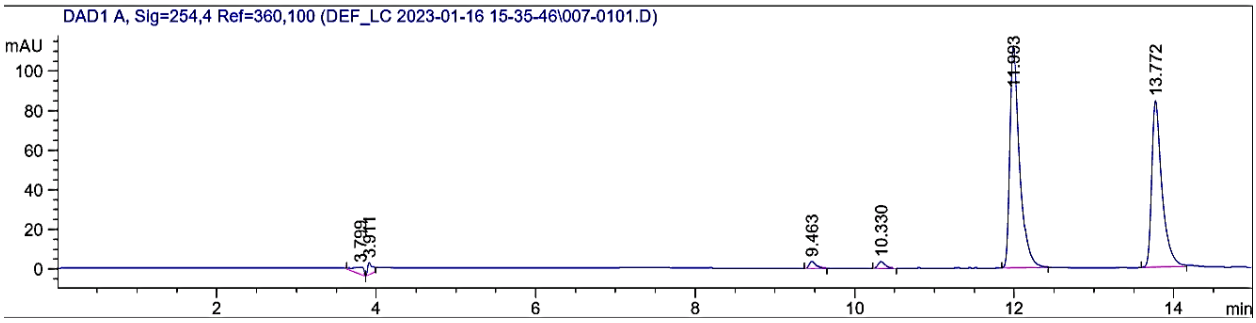

Fig. 6. Chromatogram of the Quercetin standard sample.

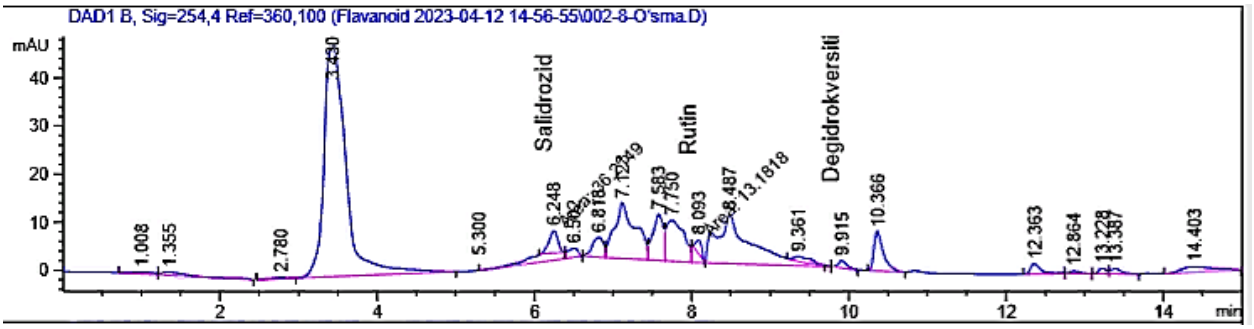

Fig. 7. Chromatogram of the Seneroside standard sample.

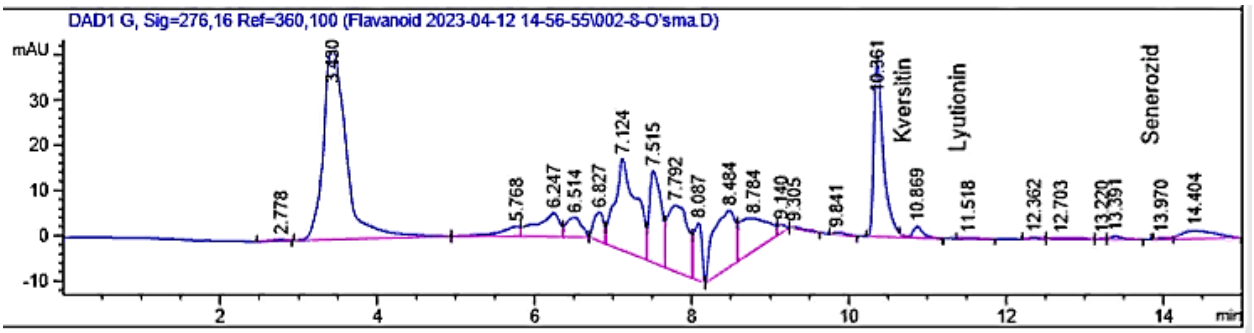

Fig. 8. Chromatogram of the total flavonoids contained in the dry extract of woad.

Figure 8 shows the chromatogram of the total flavonoids contained in the dry extract of woad, at a wavelength of 254.4 nm.

From these data, it can be concluded that the dry extract of the studied object — woad — contains the flavonoids in the concentrations shown in Table 2.

Table 2

Flavonoid content in the dry extract of woad.

Flavonoids	Woad
	Concentration, mg/g
Dihydroquercetin	*0,056*
Luteolin	*0*
Quercetin	*0,016*
Rutin	*0,015*

Determination of total sugar content was carried out using the phenol–sulfuric acid method. 0.5 mL of polysaccharide solution was added to 0.5 mL of a 5% phenol solution. Then, 2.5 mL of concentrated sulfuric acid was added, and the mixture was placed in a water bath for 10 minutes. A calibration curve was constructed for glucose solutions at concentrations of 10–120 μg/mL. The measurements were performed on a Shimadzu UV–VIS 1280 spectrophotometer (Shimadzu Europa GmbH, Germany) at a wavelength of 490 nm. The total polysaccharide content was found to be 11.07%.

4. Conclusion

The obtained data allow us to conclude that the dry aqueous

extract obtained from the leaves of woad contains vitamins C, B_2, and PP, as well as the flavonoids dihydroquercetin, quercetin, and rutin, which indicates that the dry extract possesses antioxidant, antiviral, anti-inflammatory, vasodilatory, and other properties. Furthermore, the polysaccharide content in the studied extract was identified. The total polysaccharide content amounted to 11.07%.

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