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DETERMINATION OF FLAVONOID AND PHENOLIC COMPOUNDS IN *CARTHAMUS TINCTORIUS L.* PLANT MATERIAL BY HPLC

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Annotation. The article presents the results of HPLC determination of flavonoid and phenolic compounds in the 70% ethanol extract of Carthamus tinctorius L. plant material cultivated in Uzbekistan. The analysis was performed on an Agilent 1200 system with an Eclipse XDB C18 column at 254 and 276 nm. Dihydroquercetin, rutin, rosavin, luteolin and salidroside were found at 5.03, 9.14, 5.18, 0.89 and 5.69 mg/g, respectively.

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Safflower (*Carthamus tinctorius L.*) is an annual oilseed and dye-producing plant belonging to the family Asteraceae (Compositae). Approximately 20–25 species of the genus *Carthamus* are known, most of which are distributed throughout the Mediterranean region, Western and Central Asia. Ethiopia and Afghanistan are considered among the centres of origin of safflower. The plant can reach a height of up to 120 cm and has an erect, highly branched stem. Its leaves are arranged alternately along the stem and are lanceolate in shape; in some varieties, their margins are spiny. The flowers, which are yellow, orange or reddish-orange, are arranged in large capitulum-type inflorescences. The flowering period usually occurs from June to August, while fruit formation and ripening take place from August to September. In agricultural practice, the cultivated species is primarily safflower (*Carthamus tinctorius L.*). This species is widely cultivated, with its main distribution area covering steppe and semi-desert regions [1].

According to published literature, various organs of safflower, including its flowers and seeds, are used in pharmaceutical practice worldwide. In different countries, safflower-based preparations include infusions, tinctures, decoctions prepared from the flowers, seed oil and dried plant materials. The presence of natural pigments and biologically active compounds in the flowers increases the importance of this plant in the pharmaceutical and food industries. In the pharmaceutical industry of the Russian Federation, safflower is mainly used as a component in the production of biologically active food supplements. Pharmacopoeial monographs relating to the fruits and flowers of safflower are available in several foreign pharmacopoeias. In particular, information on this plant material is included in the 6th edition of the European Pharmacopoeia, the Chinese Pharmacopoeia (2008), the British Herbal Pharmacopoeia (1991, 1996), the British Pharmacopoeia (2008), and the American Herbal Pharmacopoeia (2008) [2].

In Uzbekistan, safflower has also traditionally been used in folk medicine. The presence of various groups of biologically active substances, including flavonoids, phenolic compounds, pigments and other

natural metabolites, makes the pharmacognostic investigation of this plant relevant. Flavonoids are particularly important as a group of natural compounds known for their antioxidant properties and their contribution to the biological activity and quality characteristics of medicinal plant materials. Therefore, the qualitative and quantitative determination of flavonoids and phenolic compounds in *Carthamus tinctorius L.*, the investigation of their composition, and the development of methods for standardising the plant material are of considerable scientific and practical importance. Spectrophotometric methods and high-performance liquid chromatography (HPLC) are widely used to investigate the chemical composition of plant materials. Spectrophotometric methods make it possible to evaluate the total content of groups of biologically active substances, whereas HPLC provides high sensitivity and selectivity for the separation, identification and quantitative determination of individual components. The combined application of these methods enables a more comprehensive assessment of the chemical composition of plant materials.

Spectrophotometric and HPLC methods were used to investigate the biologically active compounds present in the aerial parts of *Carthamus tinctorius L.* In this study, extracts prepared from the aerial parts of the plant, including leaves, stems and flowers, were analysed. Ethanol solutions at concentrations of 50%, 60% and 70% were used for extraction, and the effect of solvent concentration on the extraction efficiency of biologically active compounds was compared. The obtained chromatographic results revealed peaks characteristic of flavonoids such as rutin, quercetin and luteolin. It was also observed that extraction efficiency depends not only on the solvent concentration but also on the chemical composition and physicochemical properties of the plant material.

Based on the above considerations, the comprehensive investigation of biologically active compounds in the aerial parts of safflower (*Carthamus tinctorius L.*), the identification of flavonoids and phenolic compounds, and the assessment of their quantitative characteristics represent important scientific objectives. Accordingly, the aim of this article is to determine flavonoids and phenolic compounds in the aerial parts of *Carthamus tinctorius L.* using HPLC, evaluate their qualitative and quantitative composition, and establish a scientific basis for the pharmacognostic standardisation of the plant material.

Aim of the Study. To identify flavonoids and phenolic compounds in *Carthamus tinctorius L.* plant material using HPLC, assess their quantitative content, and characterise the chromatographic profile of the raw material.

Research Materials. The object of the study was the aerial parts of safflower (*Carthamus tinctorius L.*). Plant samples were collected in June–July 2025 in the Tashkent region during the flowering stage. The plant material consisted of leaves, stems and flowers. The collected samples were cleaned of foreign impurities and dried at room temperature in a well-ventilated area protected from direct sunlight in order to minimise changes in the content of natural biologically active compounds. The dried plant material was ground and stored in tightly sealed, dry containers at room temperature, protected from moisture and sunlight. The same dried and powdered plant material was used in subsequent investigations.

To evaluate extraction efficiency, 50%, 60% and 70% ethanol solutions were used. Preliminary chromatographic analysis showed that the extract obtained using 60% ethanol exhibited more distinct separation of peaks characteristic of certain flavonoids. However, the selection of 70% ethanol for subsequent analysis was based on the possibility of simultaneously investigating flavonoids and other phenolic compounds in the extract. Therefore, the extract obtained using 70% ethanol was selected for subsequent quantitative analysis.

Research results. Numerous chromatographic peaks were recorded at wavelengths of 254 and 276 nm on the chromatogram of the *Carthamus tinctorius L.* sample obtained with a diode-array detector (DAD) (Fig. 1). The markers rutin, rosavin, luteolin, salidroside and dihydroquercetin are indicated on the sample chromatogram. According to the chromatographic analysis, the rutin content was the highest, 9.14 mg/g, and the luteolin content was 0.89 mg/g (Table 1).

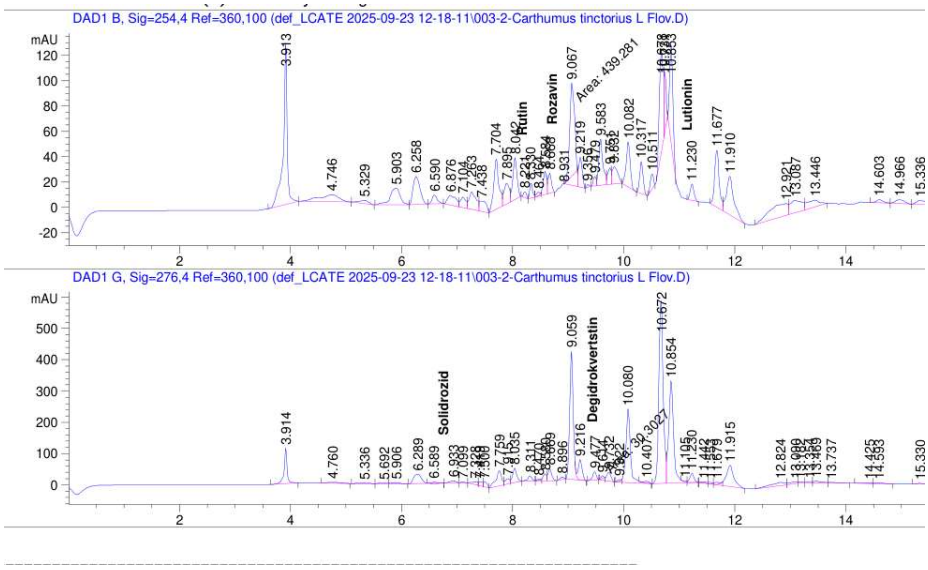


Figure 1. HPLC-DAD chromatogram of the *Carthamus tinctorius L.* sample (254 and 276 nm).

Table 1. Content of compounds determined in *Carthamus tinctorius L.* raw material

No.	Compound determined	Content, mg/g
1	Dihydroquercetin	5.03
2	Rutin	9.14
3	Rosavin	5.18
4	Luteolin	0.89
5	Salidroside	5.69

One important terminological point should be distinguished when interpreting the results: rutin, luteolin and dihydroquercetin are flavonoid compounds, whereas rosavin and salidroside, unlike flavonoids, are indicator compounds belonging to the group of phenolic glycosides. Therefore, in this article they are presented as additional phenolic compounds characterizing the HPLC profile. This approach retains all the markers identified in the analysis and describes the chemical classification more precisely.

The chromatograms of the reference standards (Figs. 2–6) demonstrated the identification capabilities of the HPLC method. According to the analysis, the main chromatographic peak of rosavin at 254 nm was recorded at about 9.156 min, that of salidroside at 6.793 min, and that of dihydroquercetin at about 8.595 min. For the quercetin standard, a dominant peak with a retention time of 10.781 min was observed in the 254 nm channel. Since the studied sample was analyzed in a different analytical series and under updated chromatographic separation conditions, a direct comparison of the absolute retention times of the standards between different analytical series is not appropriate. Therefore, the agreement between the standard and sample chromatograms obtained in the same analytical series was adopted as the main identification criterion.

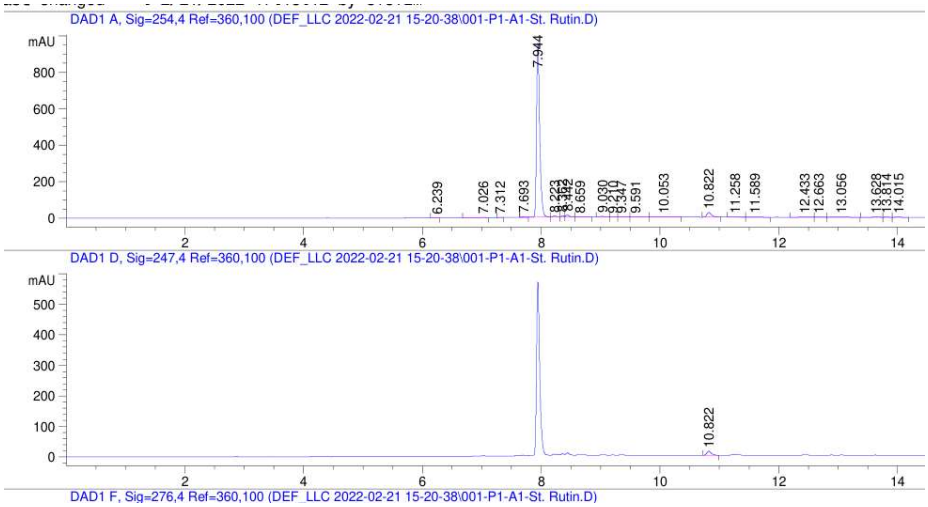


Figure 2. HPLC chromatogram of the rutin reference standard.

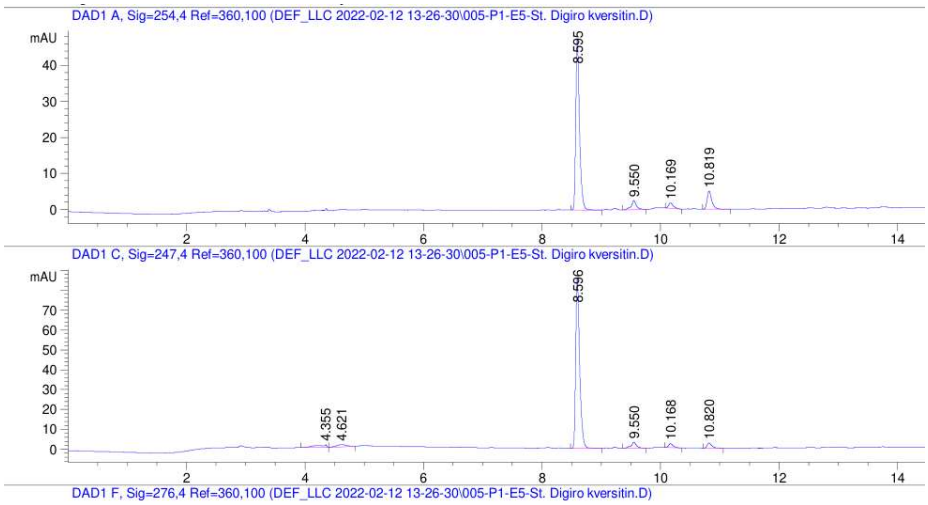


Figure 3. HPLC chromatogram of the dihydroquercetin reference standard.

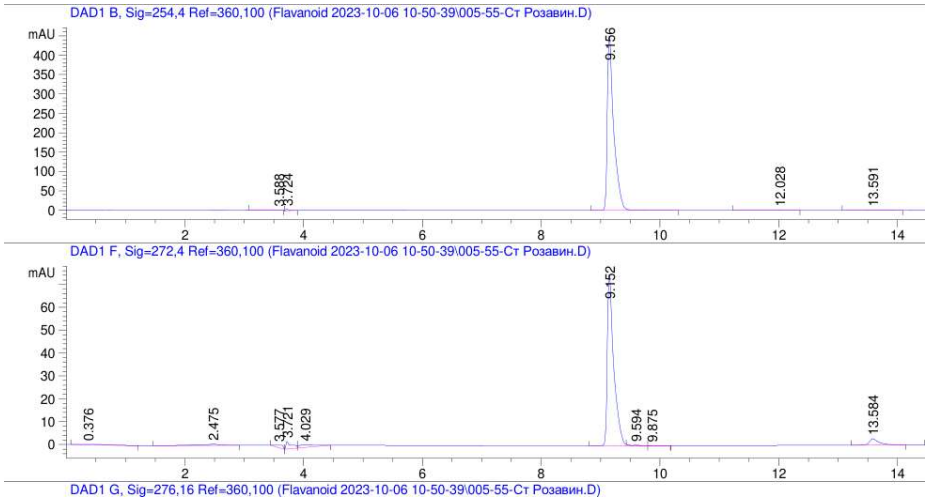


Figure 4. HPLC chromatogram of the rosavin reference standard.

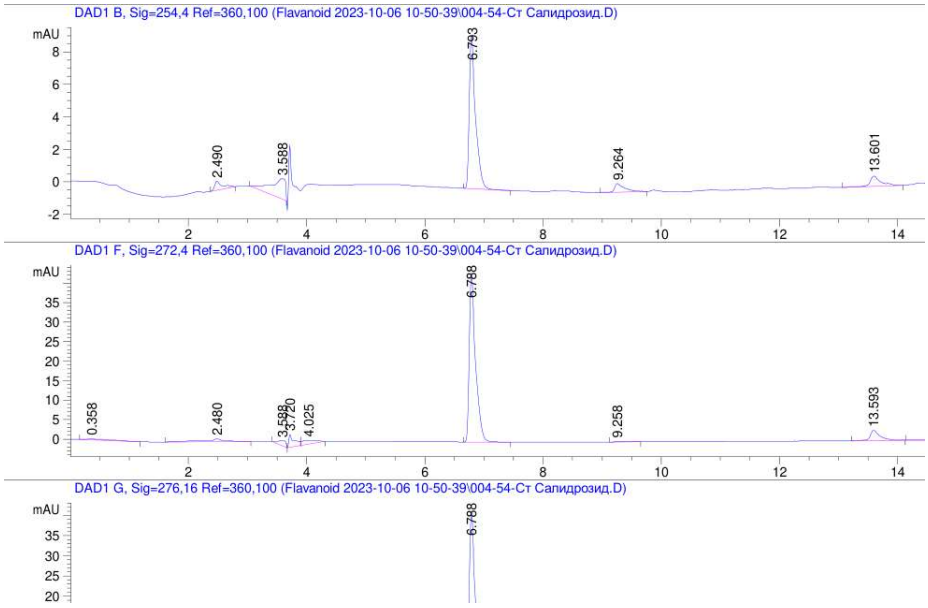


Figure 5. HPLC chromatogram of the salidroside reference standard.

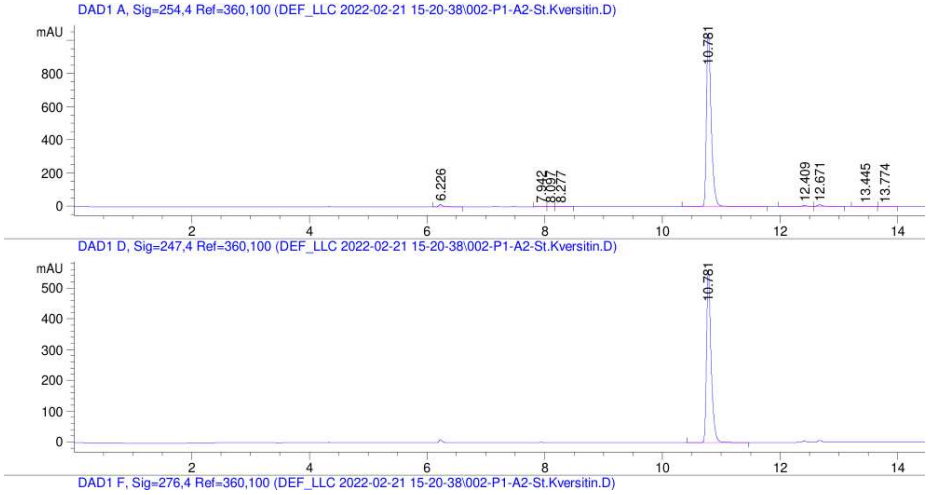


Figure 6. HPLC chromatogram of the quercetin reference standard (additional identification standard).

In the dissertation materials, a comparative study of 50%, 60% and 70% ethanol extracts showed that flavonoid peaks were separated more clearly and intensely in the 60% ethanol extracts, and that the rutin, quercetin and luteolin peaks in flower extracts were higher than in the aerial part. These data show that extraction conditions and the choice of plant organ significantly affect the flavonoid profile.

Experimental part. *Sample preparation. Carthamus tinctorius L.* plant material was used as the object of study. For analysis, a 1.0 g portion of the sample was weighed on an analytical balance, placed in a 300 mL flat-bottom flask, and 50 mL of 70% ethanol was added. The mixture was intensively stirred at 70 °C for 1 h using a magnetic stirrer with a reflux condenser, and then extraction was continued for 2 h at room temperature. The mixture was allowed to settle and was filtered. The residue was re-extracted twice with 25 mL of 70% ethanol. The filtrates were combined and brought to the mark with 70% ethanol in a 100 mL volumetric flask. The solution was centrifuged at 6000 rpm for 20 min, and the supernatant was taken for chromatographic analysis.

Chromatographic conditions.

Chromatograph – Agilent-1200 with autosampler;

Column – Eclipse XDB C18, 5 µm, 4.6 × 250 mm, reversed-phase;

Detector – diode-array detector (DAD);

Detection wavelengths – 254 and 276 nm;
Flow rate – 0.8 mL/min;
Temperature – 30 °C;
Injection volume – 10 µL;
Mobile phase – phosphate buffer : acetonitrile;
Gradient – 0–5 min – 95:5; 6–12 min – 70:30; 12–13 min – 50:50; 13–15 min – 95:5.

The working standard solutions and then the prepared sample solution were injected into the chromatograph. Identification was based on comparing the retention times and detector responses of the peaks in the standard and sample chromatograms. The following expression was used to determine the amount of substance:

$X = S_{sam} \times 2 \times 200 \times 1000 / (S_{st} \times 10 \times 1000)$

Here:

S_{sam} – peak area of the sample,
S_{st} – peak area of the standard,
2 – concentration of the standard sample,
10 – amount injected into the chromatograph.

Conclusion. The 70% ethanol extract of *Carthamus tinctorius L.* plant material was analyzed by HPLC-DAD, and a multicomponent chromatographic profile was obtained. Dihydroquercetin (5.03 mg/g), rutin (9.14 mg/g), rosavin (5.18 mg/g), luteolin (0.89 mg/g) and salidroside (5.69 mg/g) were detected in the sample. Among the flavonoid markers, the rutin content was the highest, so it may be considered a promising analytical marker for the standardization of *Carthamus tinctorius L.* raw material. The HPLC conditions based on the Agilent-1200 system, the Eclipse XDB C18 column and the DAD detector proved to be a suitable analytical approach for assessing the phenolic profile of the raw material. In further studies, it is advisable to evaluate separately the validation parameters of the method, such as selectivity, linearity, accuracy, repeatability, LOD and LOQ.

References

1. Харисова А.В. Фармакогностическое исследование сафлора красильного (Carthamus tinctorius L.) : дис. ... канд. фарм. наук. – Самара, 2014. – 160 с.
2. Флора Узбекистана. – Ташкент : Изд-во АН УзССР. – Т. 5. – С. 340, 348.
3. Zhou Y.X., Chen Y., Ye X. et al. Carthamus tinctorius L.: A review of its botany, traditional uses, phytochemistry, pharmacology, and toxicology // Frontiers in Pharmacology. – 2016. – Vol. 7. – P. 370. – DOI: 10.3389/fphar.2016.00370.
4. Nawaz H., Shad M.A., Rehman N. et al. Therapeutic potential of safflower (Carthamus tinctorius L.): A review of traditional uses, phytochemistry and pharmacology // Journal of Ethnopharmacology. – 2017. – Vol. 209. – P. 50–60. – DOI: 10.1016/j.jep.2017.07.024.
5. Lin L.C., Pai Y.F., Tsai T.H. Isolation of luteolin 7-O-glucoside and pharmacokinetics // Journal of Agricultural and Food Chemistry. – 2015. – Vol. 63. – P. 7700. – DOI: 10.1021/jf505848z.
6. Carthamus tinctorius L. namuna xromatogrammasi va rutin, kversetin, digidrokversetin, rozavin hamda salidrozid standartlarining HPLC hisobotlari : taqdim etilgan laboratoriya materiallari.
7.O‘zbekiston Respublikasi Davlat farmakopeyasi, 2-nashr (2025).
8.Xian B. va hammualliflar (2022). Comprehensive review of two groups of flavonoids in Carthamus tinctorius L.
9.Wang S. va hammualliflar (2021). Chemical characterization of flavonoids and alkaloids in safflower (Carthamus tinctorius L.) by comprehensive two-dimensional hydrophilic interaction chromatography coupled with hybrid linear ion trap Orbitrap mass spectrometry