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JUGLANS REGIA L. LEAVES AND GREEN HUSK EXTRACTS: QUALITATIVE AND QUANTITATIVE ANALYSIS OF FLAVONOID COMPOUNDS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract: In this study, the quantitative and qualitative analysis of flavonoid compounds in Juglans regia L. leaves and green husk extracts was performed using high-performance liquid chromatography (HPLC)**.** The results of the study provide a scientific basis for assessing the biological activity, pharmaceutical value, and quality of walnut raw materials.

Keywords: Juglans regia, flavonoids, HPLC, extraction, salidroside, rosavin.

Introduction. The significance of herbal remedies has considerably increased in recent years. Walnut (Juglans regia L.) leaves and green husk have been traditionally used in folk medicine, and their phenolic and flavonoid compounds have been shown to possess antioxidant, antibacterial, and hypoglycemic properties**.** This study analyzes the quantitative biological effects of walnut leaves and husk against diabetes mellitus and tonsillitis.

Type 2 diabetes mellitus and inflammatory diseases such as tonsillitis are widespread worldwide. Due to the side effects and limited efficacy of conventional treatments**,** phytotherapy approaches based on plant-derived materials are gaining increasing attention. Walnut (Juglans regia L.) leaves are rich in phenolic compounds, flavonoids, juglone, and other bioactive substances, and several studies have demonstrated their effects on glucose and lipid profiles**,** as well as their anti-inflammatory and antimicrobial properties**.**

Iranian physiologist and pathophysiologist researchers, Zahra Abbase et al.**,** investigated the antidiabetic pharmacological properties of ethanol extracts of walnut leaves by administering them to diabetic rats for 30 days [1].

Iranian researchers, Gholamali Jelodar, Maleki Mohsen, and Sirus Shahram**,** studied the hypoglycemic effects of three medicinal plants - walnut leaves (Juglans regia L.)**,** coriander leaves (Coriandrum sativum L.), and pomegranate seeds (Punica granatum L.) - in diabetic rats, as well as the morphological changes occurring in pancreatic tissues [2].

Masoud Mohammadi et al. investigated the effects of cyclohexane extracts of walnut leaves administered to animals for 28 days, observing positive impacts on body weight, blood glucose, lipid profile, antioxidant enzyme activity, and homocysteine levels, as well as confirming its therapeutic potential in diabetic conditions [3].

Researchers from the Nadali Esmaeili Research Institute (Iran)**,** including Khadijeh Rabiei et al.**,** demonstrated that walnut (Juglans regia L.) leaf extract is effective in reducing body weight and blood pressure**,** and that microcrystalline cellulose (used as placebo in clinical trials) also contributed to a reduction in blood glucose levels [4].

Researchers from the National Medical University of Ukraine, including Vasyl Popovych et al.**,** conducted a study involving 238 children in which Imupret® (walnut leaf extract) was administered as a supplement to standard symptomatic therapy. Analyses showed that within 5 days, pain and

discomfort during swallowing, throat redness, and overall condition were significantly improved, and the need for antipyretic medications decreased within 4 days. As a result, the incidence of tonsillitis was reduced by 66.56% [5].

Researchers from the Rzeszów University (Poland), including Natalia Zurek et al., demonstrated that walnut (Juglans regia L.) leaf extract at a concentration of 10 mg/mL exhibited bactericidal activity against Gram-positive Streptococcus pyogenes, while at 1 mg/mL, it retained inhibitory effects against certain bacteria [6].

Researchers from the Faculty of Pharmacy, Tripoli University, Libya, including Asma Eswayah et al., reported that the methanol extract of walnut (Juglans regia L.) leaves exhibits antibacterial and antifungal activity against Staphylococcus aureus and Candida albicans, with minimum inhibitory concentrations (MICs) of approximately 25 mg/mL for S. aureus and ~50 mg/mL for C. albicans [7].

Scientific objective: To determine the quantitative content of flavonoids in walnut (Juglans regia L.) leaves and green husk using high-performance liquid chromatography (HPLC) and to provide a scientific basis for their pharmaceutical and biological activity potential.

Materials and methods: Each sample (1 g) was placed in a 300 mL flask with 50 mL of 70% ethanol. The flask was heated intensively at 70–80°C for 1 hour under a magnetic stirrer and a reflux condenser. Then, the mixture was stirred additionally at room temperature for 2 hours. After filtration, the residue was re-extracted twice with 25 mL of 70% ethanol. All filtrates were combined and transferred to a 100 mL volumetric flask. The solution was centrifuged at 6000–8000 rpm for 20–30 minutes. HPLC Analysis Conditions:

Analyses were performed using an Agilent 1200 system. Column: Exlips XDB C18, 5 µm, 4.6 × 250 mm, detector: Diode Array Detector (DAD), wavelengths: 254 nm and 276 nm, flow rate: 0.8 mL/min, column temperature: 30°C, injection volume: 10 µL. Eluents: phosphate buffer – acetonitrile, gradient program: 0–5 min: 95:5; 6–12 min: 70:30; 12–13 min: 50:50; 13–15 min: 95:5. Standard solutions were injected first, followed by the sample solutions.

Results: The obtained data demonstrated that the total flavonoid content in walnut leaves is higher compared to the green husk. In particular, the salidroside content in leaves was 11.6 mg/g, making it a valuable raw material with strong antioxidant and adaptogenic properties. Additionally, the presence of rutin, luteolin, and dihydroquercetin at high levels further enhances the pharmaceutical potential of the leaves.

The quantitative composition of flavonoids in walnut leaves and green husk is presented in Table 1.

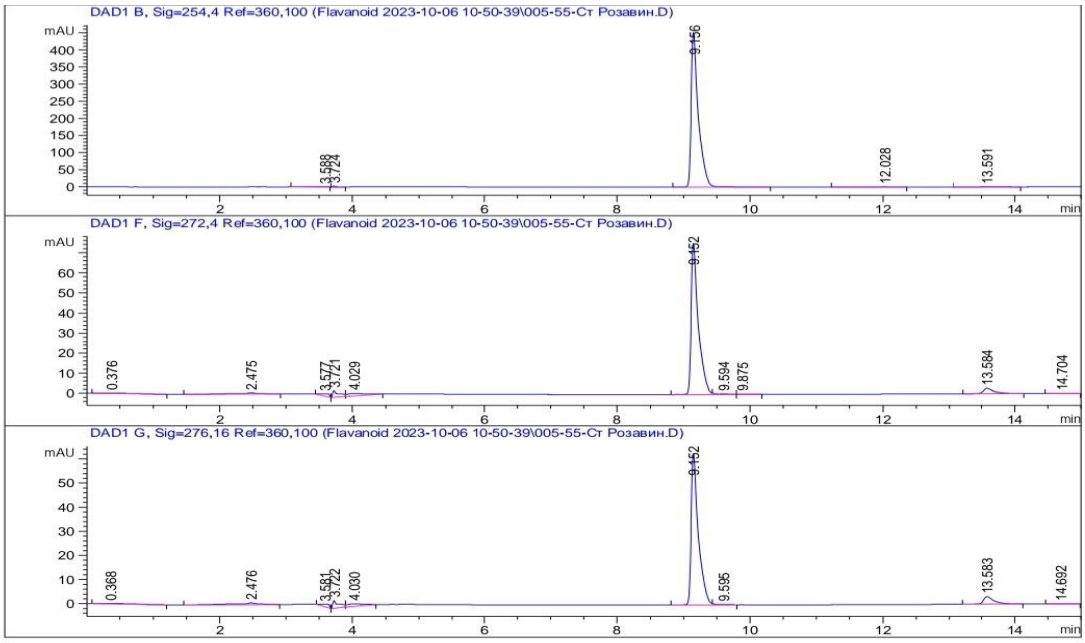


Figure 1. Peaks of the standard solution of Rosavin

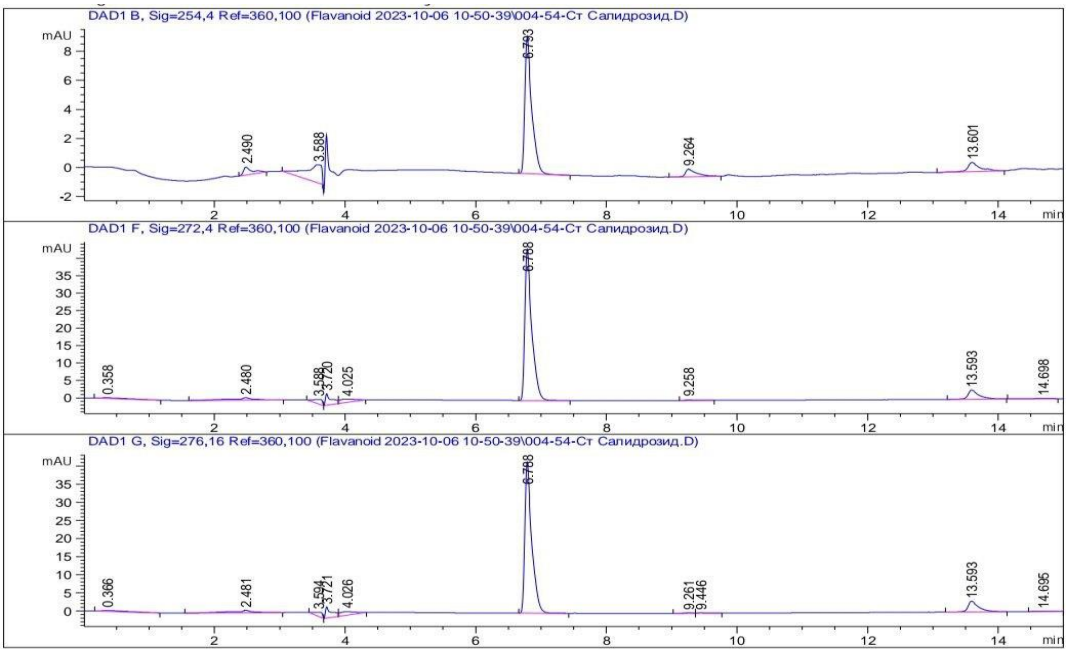


Figure 2. Peaks of the standard solution of Salidroside

The higher content of Rosavin in the husk indicates its significant role in the plant's stress-response mechanisms. Moreover, the elevated levels of Seneroside in the husk make it an important bioactive component in certain physiological processes**.**

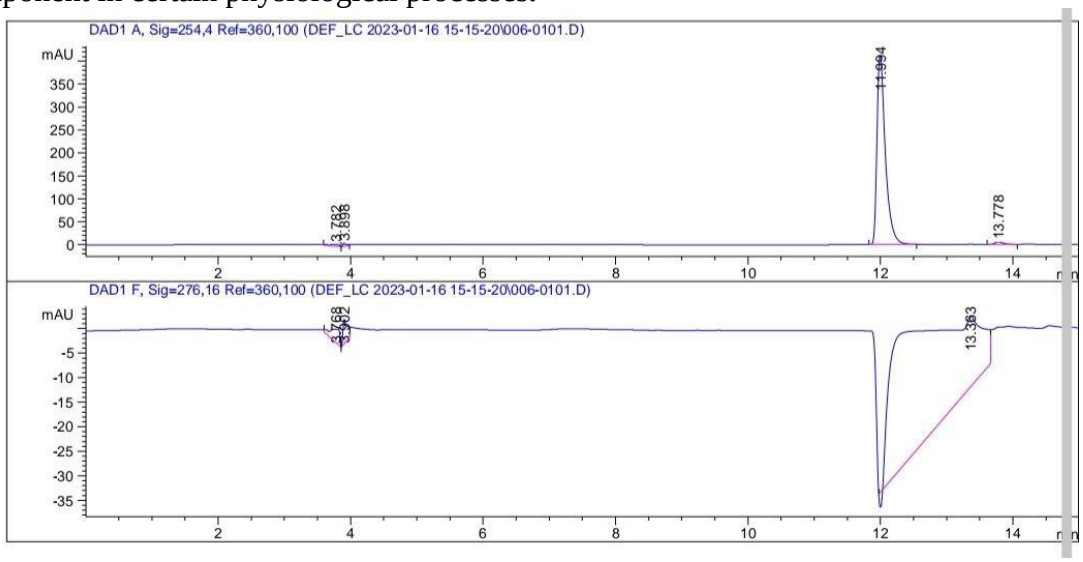


Figure 3. Peaks of the standard solution of Luteolin

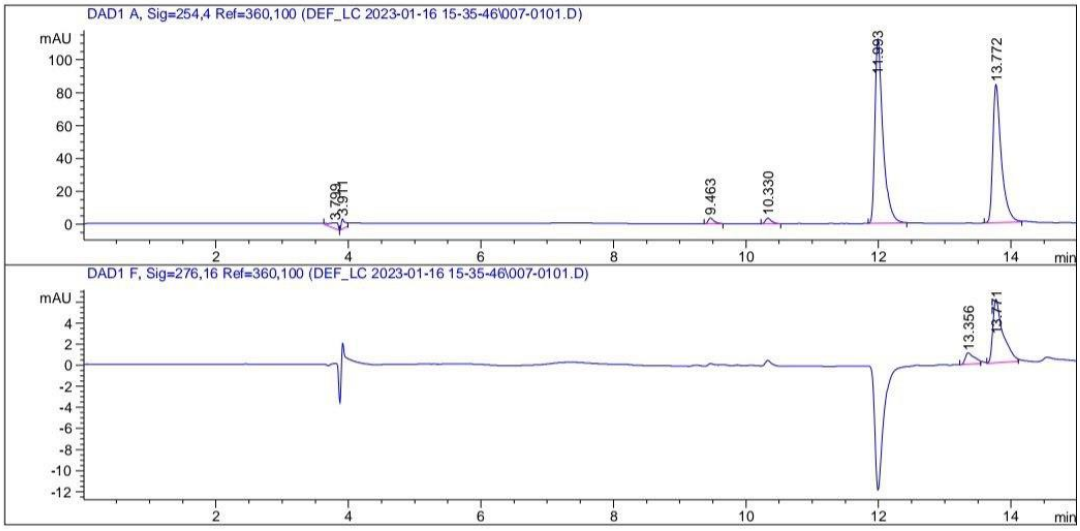


Figure 4. Peaks of the standard solution of Seneroside

Overall, walnut leaves are a rich source of flavonoids and can be recommended as a primary raw material for the production of phytopharmaceuticals **(**Table 1**).**

Table 1. Quantitative content of flavonoids in walnut leaves and husk

Flavonoids	**Leaves (mg/g)**	**Husk (mg/g)**
	Concentration (mg/g)	
Dihydroquercetin	2.21	1.36
Luteolin	0.99	0.37
Rutin	2.9	0.74
Seneroside	0.12	0.33
Quercetin	0.32	0.31
Salidroside	11.6	2.87
Rosavin	2.52	3.16

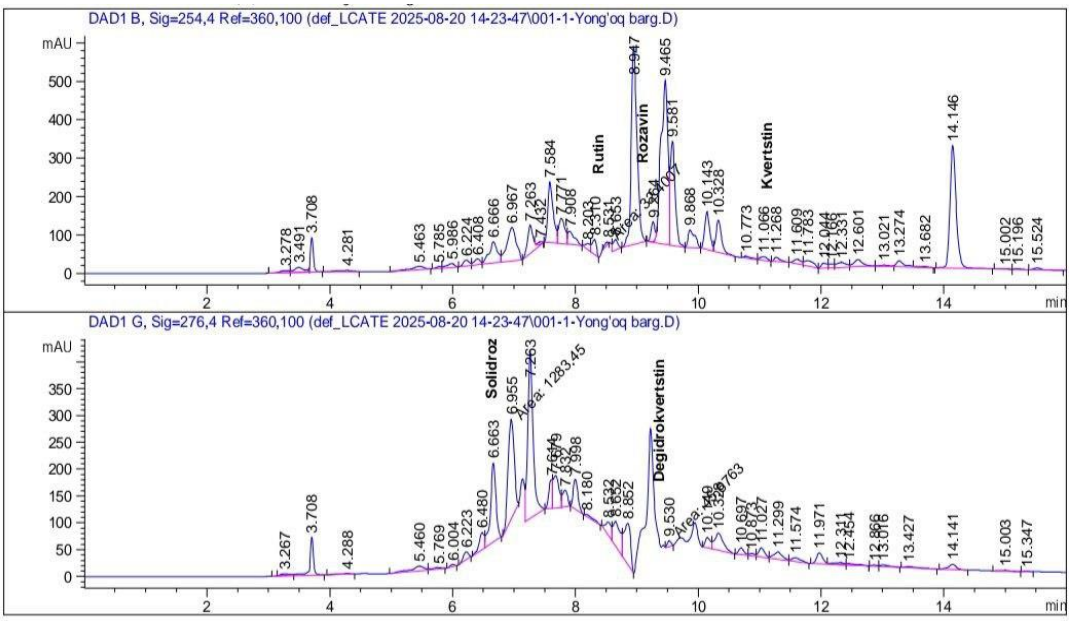


Figure 5. Quantitative content of flavonoids in walnut leaves

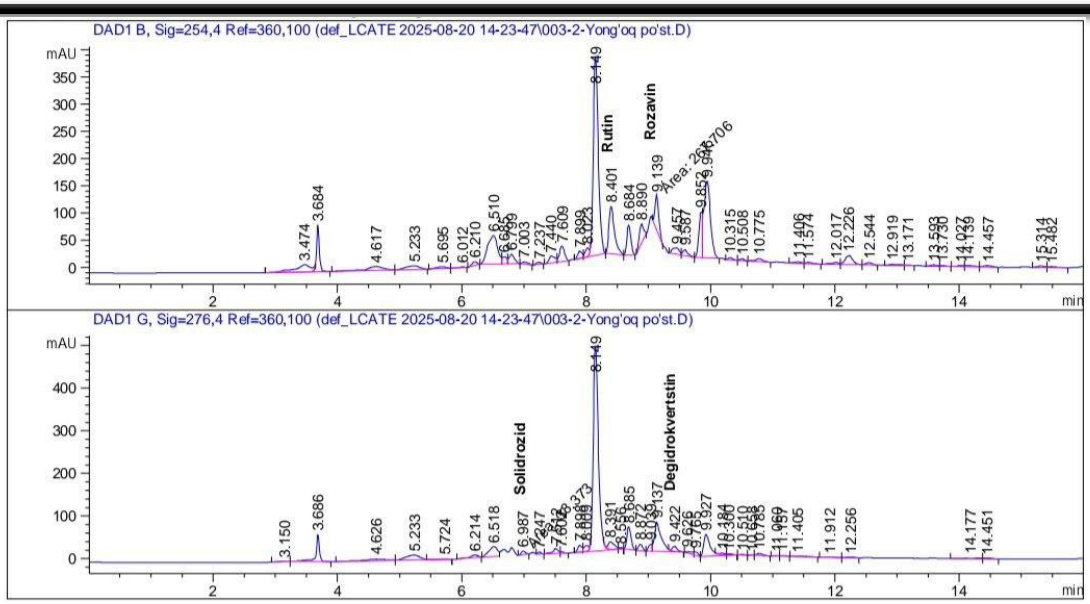


Figure 6. Quantitative content of flavonoids in walnut husk

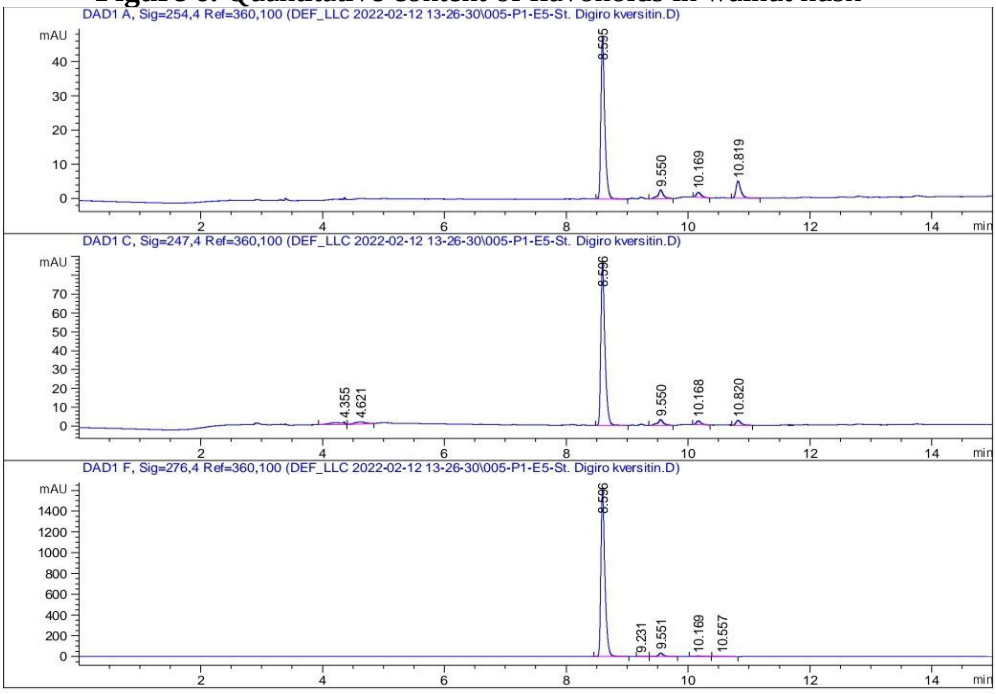


Figure 7. Chromatographic analysis of Dihydroquercetin

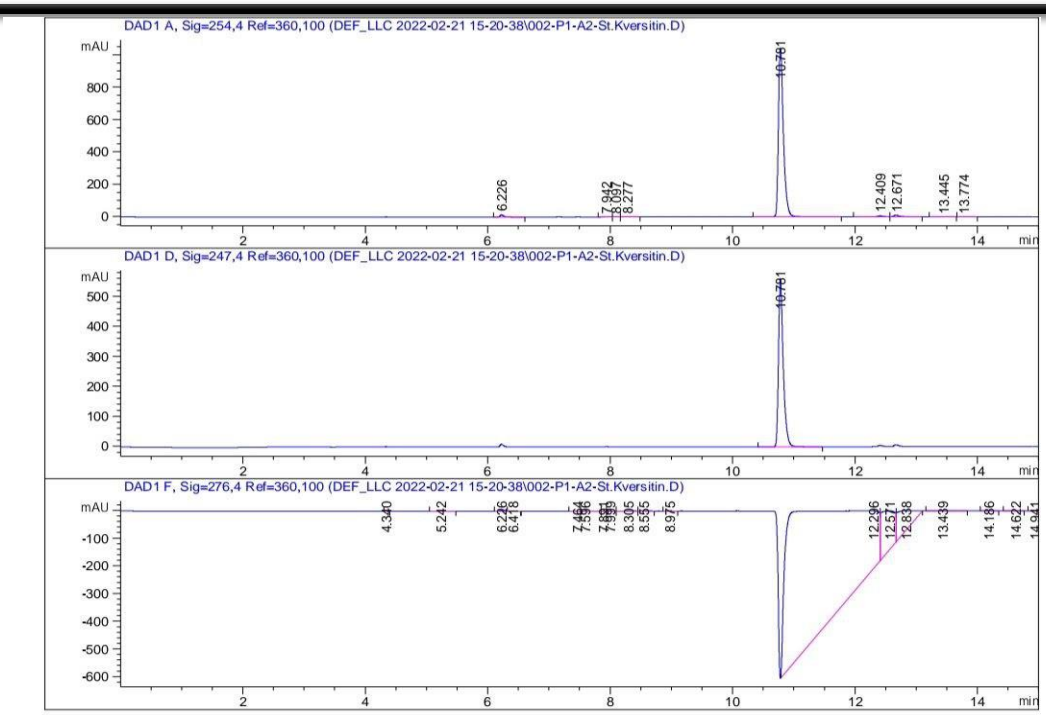


Figure 8. Chromatographic analysis of Quercetin

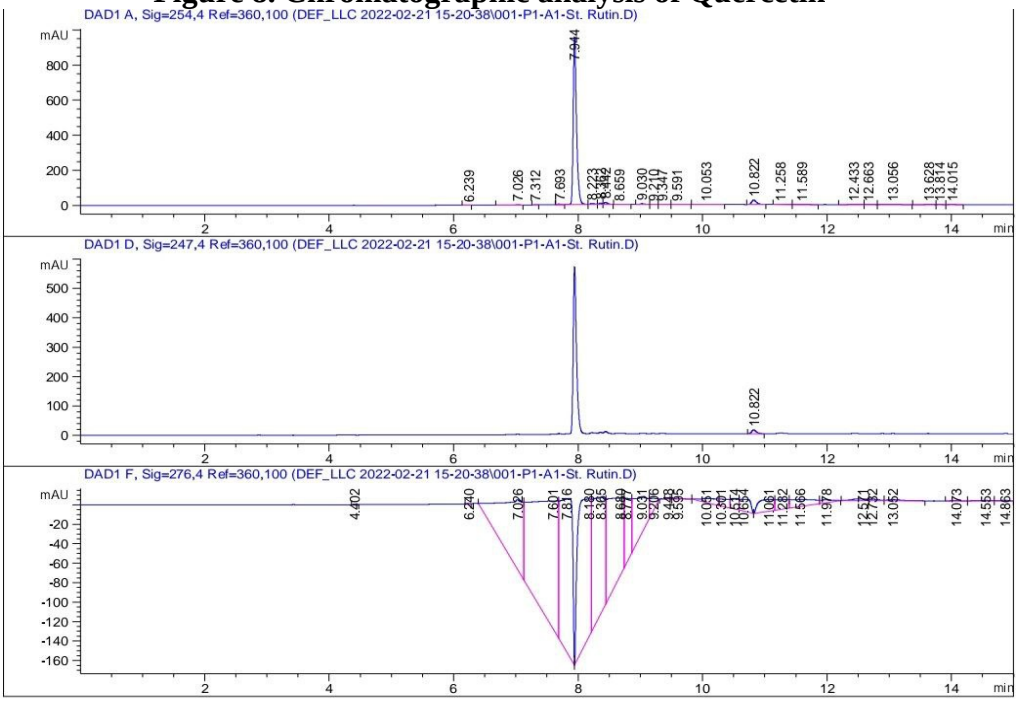


Figure 9. Chromatographic analysis of Rutin

Conclusion: The results of the study demonstrated that **the** flavonoid content in walnut leaves and husk differs significantly**.** The HPLC method allowed for the rapid, accurate, and reproducible quantitative assessment of flavonoids. Since the leaves are particularly rich in flavonoids, especially salidroside**,** they can be effectively used for the production of pharmaceutical, nutraceutical, and antioxidant preparations**.**

References:

1. Abbasi Z, Jelodar G, Geramizadeh B. Prevention of Diabetic Complications by Walnut Leaf Extract via Changing Aldose Reductase Activity: An Experiment in Diabetic Rat Tissue. J Diabetes Res. 2020 Aug 15;2020:8982676. doi: 10.1155/2020/8982676. PMID: 32879893; PMCID: PMC7448230.

2. Jelodar G, Mohsen M, Shahram S. Effect of walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreas of alloxan induced diabetic rats. Afr J Tradit Complement Altern Med. 2007 Feb 16;4(3):299-305. doi: 10.4314/ajtcam.v4i3.31223. PMID: 20161893; PMCID: PMC2816491.
3. Jelodar G, Mohammadi M, Akbari A, Nazifi S. Cyclohexane extract of walnut leaves improves indices of oxidative stress, total homocysteine and lipids profiles in streptozotocin-induced diabetic rats. Physiol Rep. 2020 Jan;8(1):e14348. doi: 10.14814/phy2.14348. PMID: 31960621; PMCID: PMC6971412.
4. Rabiei K, Ebrahimzadeh MA, Saeedi M, Bahar A, Akha O, Kashi Z. Effects of a hydroalcoholic extract of Juglans regia (walnut) leaves on blood glucose and major cardiovascular risk factors in type 2 diabetic patients: a double-blind, placebo-controlled clinical trial. BMC Complement Altern Med. 2018 Jul 4;18(1):206. doi: 10.1186/s12906-018-2268-8. PMID: 29973195; PMCID: PMC6031195.
5. Popovych V, Koshel I, Malofiichuk A, Pyletska L, Semeniuk A, Filippova O, Orlovska R. A randomized, open-label, multicenter, comparative study of therapeutic efficacy, safety and tolerability of BNO 1030 extract, containing marshmallow root, chamomile flowers, horsetail herb, walnut leaves, yarrow herb, oak bark, dandelion herb in the treatment of acute non-bacterial tonsillitis in children aged 6 to 18 years. Am J Otolaryngol. 2019 Mar-Apr;40(2):265-273. doi: 10.1016/j.amjoto.2018.10.012. Epub 2018 Oct 24. PMID: 30554882.
6. Żurek N, Pycia K, Pawłowska A, Potocki L, Kapusta IT. Chemical Profiling, Bioactive Properties, and Anticancer and Antimicrobial Potential of *Juglans regia* L. Leaves. Molecules. 2023 Feb 20;28(4):1989. doi: 10.3390/molecules28041989. PMID: 36838977; PMCID: PMC9966981.
7. Asma E, Nahla L, Fadilah A, Amal B, Fulwah A, Haya A. Chemical Profiling, Bioactive Properties, and Anticancer and Antimicrobial Potential of Juglans regia L. Leaves. MDPI Molecules.