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DETERMINATION OF FLAVONOIDS IN THE FRUIT PERICARP OF HORSE CHESTNUT (*AESCULUS HIPPOCASTANUM L.*)

OT KASHTANI (*AESCULUS HIPPOCASTANUM L.*) MEVASI PERIKARPI TARKIBIDAGI FLAVONOIDLARNI ANIQLASH

ОПРЕДЕЛЕНИЕ ФЛАВОНОИДОВ В ОКОЛОПЛОДНИКЕ ПЛОДОВ КОНСКОГО КАШТАНА (*AESCULUS HIPPOCASTANUM L.*)

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Abstract

In recent decades, medicinal plants have garnered significant scientific attention as natural alternatives to synthetic pharmaceuticals due to their low toxicity, high efficacy, and minimal side effects. Among these, horse chestnut (Aesculus hippocastanum L.) is one of the most widely used medicinal plants in both traditional medicine and modern pharmaceutical formulations. Horse chestnut is well known for its content of biologically active compounds, including esculin, escin, aescin, and a diverse range of flavonoids. These compounds have demonstrated considerable potential in the treatment of vascular diseases. Flavonoids, in particular, are recognized for their potent venotonic, anti-inflammatory, and antioxidant activities. Their use is widespread in the treatment of chronic venous insufficiency, varicose veins, hemorrhoids, and thrombophlebitis. Numerous studies have confirmed that horse chestnut extracts can enhance blood circulation, strengthen capillary walls, and effectively reduce swelling and inflammation.

Annotatsiya

So'nggi o'n yilliklarda dorivor o'simliklar past zaharliligi, yuqori samaradorligi va minimal nojo'ya ta'sirlari tufayli sintetik dori vositalariga tabiiy alternativ sifatida jiddiy ilmiy e'tiborni qozondi. Shular orasida ot kashtani (Aesculus hippocastanum L.) an'anaviy tibbiyot va zamonaviy farmatsevtik preparatlarda eng keng qo'llaniladigan dorivor o'simliklardan biridir. Ot kashtani biologik faol birikmalar – eskuin, eskin, aeskin hamda turli xil flavonoidlar tarkibi bilan mashhur. Ushbu birikmalar qon tomir kasalliklarini davolashda sezilarli salohiyatga ega. Xususan, flavonoidlar kuchli venotonik, yallig'lanishga qarshi va antioksidant xususiyatlari bilan tanilgan. Ularning qo'llanilishi surunkali venoz etishmovchilik, varikoz kengayishi, gemorroy va tromboflebitni davolashda keng tarqalgan. Ko'plab tadqiqotlar shuni tasdiqlaydiki, ot kashtani ekstraktlari qon aylanishini yaxshilaydi, kapillyar devorlarini mustahkamlaydi va shish hamda yallig'lanishni samarali kamaytiradi.

Аннотация

В последние десятилетия лекарственные растения привлекли значительное внимание научного сообщества как природные альтернативы синтетическим фармацевтическим препаратам благодаря их низкой токсичности, высокой эффективности и минимальным побочным эффектам. Среди них конский каштан (Aesculus hippocastanum L.) является одним из наиболее широко используемых лекарственных растений как в традиционной медицине, так и в современных фармацевтических препаратах. Конский каштан известен содержанием биологически активных соединений, включая эскулин, эскнин, аэсцин, а также разнообразные флавоноиды. Эти соединения продемонстрировали значительный потенциал в лечении сосудистых заболеваний. Особенно флавоноиды отличаются выраженными венотоническими, противовоспалительными и антиоксидантными свойствами. Их применение широко распространено при хронической венозной недостаточности, варикозном расширении вен, геморрое и тромбофлебите. Многочисленные исследования подтвердили, что экстракты конского каштана улучшают кровообращение, укрепляют стенки капилляров и эффективно уменьшают отёчность и воспаление.

Key words: *Horse chestnut, varicose veins, flavonoids, gallic acid, quercetin, rutin.*

Kalit so'zlar: *ot kashtani, varikoz kengayishi, flavonoidlar, gallik kislota, kversetin, rutin.*

Ключевые слова: *конский каштан, варикозное расширение вен, флавоноиды, галловая кислота, кверцетин, рутин.*

INTRODUCTION

In recent ears, the growing interest in medicinal plants has stimulated extensive research into their chemical composition and pharmacological properties [1,2]. This is primarily driven by the increasing demand for natural therapeutic agents that offer minimal side effects and high biological activity compared to synthetic drugs. Among the most studied medicinal plants, horse chestnut (*Aesculus hippocastanum L.*) occupies a special place due to its long-standing use in traditional and modern medicine [3,4]. Various parts of the plant—including seeds, flowers, and pericarp—are rich in a wide spectrum of bioactive compounds such as triterpenoid saponins (escin), coumarins (esculin), tannins, and flavonoids [3,5,6].

Flavonoids are of particular scientific and clinical interest due to their potent antioxidant, anti-inflammatory, and venotonic effects[7,8]. They are widely utilized in the treatment of chronic venous insufficiency, varicose veins, hemorrhoids, and vascular-related conditions. Recent pharmacological studies have highlighted that rutin and gallic acid—two key flavonoid compounds—play an important role in reducing capillary permeability, improving microcirculation, and providing antioxidant protection.

Despite the well-documented therapeutic properties of horse chestnut seeds and flowers, there is limited research on the flavonoid profile of the fruit pericarp. In this context, the present study focuses on the qualitative and quantitative determination of flavonoids, particularly rutin and gallic acid, in the fruit pericarp of *Aesculus hippocastanum L.* using high-performance liquid chromatography (HPLC). The results aim to contribute to a deeper understanding of the plant's phytochemical composition and its potential application in the development of functional food products and nutraceutical formulations.

MATERIALS AND METHODS

This study focused on determining flavonoids in the pericarp of horse chestnut fruits cultivated in the Andijan region of Uzbekistan. Ten mature horse chestnut fruits were collected, and the seeds were separated from the pericarp. The pericarp and seeds were crushed using a BOSCH BSG107 juice extractor to obtain a fine powder. Precisely 100 g of powder was prepared from both the pericarp and seeds. For extraction, 0.5 g of powdered sample was accurately weighed using an OHAUS NAVIGATOR™ NV222 balance (China) and placed in 25 ml of 96% ethanol. The extraction was performed using an ultrasonic bath at 50 °C for 15 minutes, followed by centrifugation at 3000 rpm. The supernatant was filtered through a 0.45 µm membrane filter before analysis [5,6,10].

Preparation of Reagents and Standards. A 0.5% acetic acid solution (pH = 1.05) was prepared by diluting 2.4 ml of glacial acetic acid with HPLC-grade water in a 500 ml volumetric flask. Quercetin standard solutions (70, 140, and 280 mg/l) were prepared in methanol for calibration.

Chromatographic Conditions. HPLC was performed using a C18 column (4.6×150 mm, 5 µm), a mobile phase of 0.5% acetic acid and acetonitrile (35:65), a flow rate of 1 ml/min, column temperature of 40 °C, and detection wavelength of 370 nm.

Preparation of rutin standard solutions and calibration curve construction. To prepare a standard solution of rutin at a concentration of 640 mg/l in ethanol, 16 mg of rutin was accurately weighed using an NV222 analytical balance (manufactured by OHAUS, USA) with a precision of 0.01 g. The rutin was dissolved in 25 ml of ethanol in an ultrasonic bath at 40 °C for 10 minutes. From this stock solution (640 mg/l), a series of standard dilutions were prepared to obtain concentrations of 96 mg/l, 64 mg/l, and 32 mg/l. These standard solutions were used to construct a calibration curve.

Chromatographic conditions for rutin analysis included the use of a chromatographic column (4.6×150 mm, 5 µm, LC-18). The mobile phase consisted of water: 0.5% acetic acid solution: acetonitrile in a ratio of 30:20:50. The column temperature was maintained at room temperature, with a flow rate of 1 ml/min. The detection wavelength was set at 357 nm.

Preparation of gallic acid standard solutions and calibration curve construction. To prepare a standard aqueous solution of gallic acid with a concentration of 510 mg/l, 25.5 mg of gallic acid

was weighed with 0.01 g accuracy using the NV222 analytical balance (OHAUS, USA). It was dissolved in 50 ml of HPLC-grade water in an ultrasonic bath at 60 °C for 17 minutes. From this stock solution (510 mg/l), dilutions were prepared to obtain standard solutions with concentrations of 340 mg/l and 170 mg/l, which were used to construct a calibration curve.

Chromatographic conditions for gallic acid analysis involved a chromatographic column (4.6×150 mm, 5 µm, LC-18) with a mobile phase composition of water: 0.5% acetic acid solution: acetonitrile in a ratio of 20:20:60. The column was operated at room temperature, with a flow rate of 1 ml/min, and detection was performed at 273 nm.

Preparation of seed coat extracts from horse chestnut. From the seed coat of horse chestnut, 0.5 g of powdered sample was accurately weighed using the OHAUS NAVIGATOR™ NV222 balance (China) and extracted with 25 ml of 96% ethanol in an ultrasonic bath at 50 °C for 15 minutes. The extract was then centrifuged at 3000 rpm and filtered through a 0.45 µm membrane filter. The polyphenol content was determined according to the standard methods described above.

Results. The experimental results revealed that quercetin was not detected in the seed coat extract of horse chestnut. However, rutin was identified at a concentration of 62.06 mg/l, while gallic acid was detected at 340 mg/l (Table 1). Therefore, it was established that 0.5 g of horse chestnut seed coat contains 1.5515 mg of rutin (0.3109%) and 8.5 mg of gallic acid (1.7%).

Table 1. Results of polyphenol content determination in the seed coat extract of horse chestnut

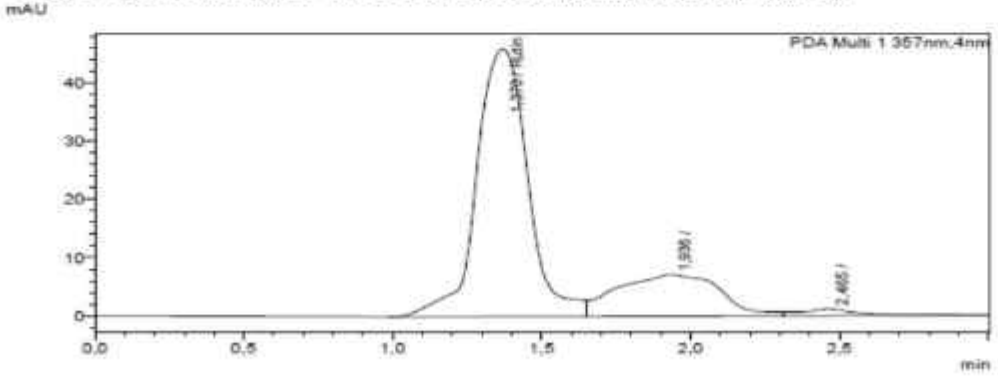
Extractant	Sample mass, g	Quercetin, mg/l	Rutin, mg/l	Gallic acid, mg/l
96% Ethanol	0,5	0	62,060	340

The chromatographic analysis of flavonoids in the ethanolic extract of horse chestnut (*Aesculus hippocastanum L.*) fruit pericarp revealed distinct and well-resolved peaks corresponding to the standard compounds. The identification of flavonoids was carried out by comparing the retention times and UV absorption maxima of the analyzed samples with those of standard solutions of quercetin, rutin, and gallic acid [7].

According to the obtained chromatograms, quercetin was not detected in the pericarp extract, as no characteristic peak was observed at the expected retention time of approximately 3.9 minutes at a detection wavelength of 370 nm. This indicates the absence or extremely low concentration of free quercetin in the analyzed sample.

In contrast, a prominent peak corresponding to rutin was observed at a retention time of 4.3 minutes, detected at a wavelength of 357 nm. The height and area of the rutin peak demonstrated a high signal-to-noise ratio, confirming its significant presence in the sample. Quantitative calculations based on the calibration curve revealed that the concentration of rutin in the extract was 62.06 mg/l, which corresponds to 1.5515 mg per 0.5 g of raw material, or 0.3109% of the pericarp dry weight (Fig.1 a).

Additionally, the chromatogram recorded at 273 nm showed a distinct and sharp peak at 5.7 minutes, corresponding to gallic acid. The peak was symmetrical and well-resolved from other minor components, indicating good chromatographic separation and purity of the analyte. The concentration of gallic acid in the ethanolic extract was determined to be 340 mg/l, equivalent to 8.5 mg per 0.5 g of raw material, or 1.7% of the pericarp dry weight (Fig.1 b).



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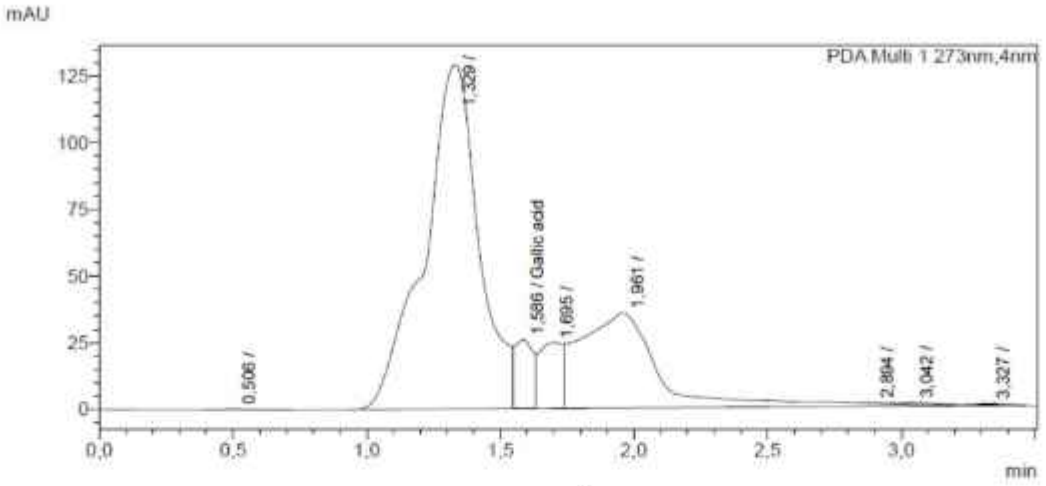

b

Fig.1. Chromatogram for the determination of rutin (a) and gallic acid (b) content in alcoholic extract of chestnut seed peel.

Overall, the chromatographic results demonstrate that the pericarp of horse chestnut fruit contains considerable amounts of rutin and gallic acid, both of which are known for their biological activity, especially antioxidant and vascular-protective effects. The absence of quercetin and the dominance of rutin and gallic acid suggest a specific flavonoid composition characteristic of the pericarp, which may differ from other parts of the plant, such as seeds and flowers.

DISCUSSION

The results of the current study demonstrated a significant presence of flavonoid compounds, particularly rutin and gallic acid, in the fruit pericarp of horse chestnut (*Aesculus hippocastanum L.*). The concentration of rutin was determined to be 62.06 mg/l, while gallic acid was quantified at 340 mg/l in the ethanolic extract. Notably, quercetin was not detected in the pericarp extract under the applied chromatographic conditions.

Our findings are consistent with previous studies indicating that rutin is one of the predominant flavonoids in horse chestnut. For instance, Bakhiyarova and Tursunova [3] reported high levels of rutin in the seeds and flowers of Aesculus hippocastanum, highlighting its key role in the plant's biological activity. However, their study primarily focused on seeds, while our data provides new insights into the flavonoid composition of the fruit pericarp, which has been less explored in scientific literature.

Studies by Kokareva et al. [5] have also confirmed the presence of flavonoids in horse chestnut seed extracts, with rutin and quercetin being the main components. Interestingly, in our analysis of the pericarp, quercetin was absent, suggesting that the distribution of flavonoids within the plant may vary significantly between different anatomical parts. This observation is supported by the work of Li et al. [6], who noted variability in flavonoid profiles depending on plant part, maturity stage, and extraction method.

Gallic acid was identified at a concentration of 1.7% relative to dry weight, which aligns with the findings of Dragović-Uzelac et al. [7], who described high gallic acid content in various fruit peels with potent antioxidant properties. The significant presence of gallic acid in the pericarp suggests its contribution to the antioxidant potential of horse chestnut, which is in agreement with earlier studies on phenolic compounds in medicinal plants [8].

Furthermore, the absence of quercetin in the pericarp extract contrasts with the results reported by Gupta et al. [8], who observed moderate quercetin content in seed extracts. This discrepancy may be attributed to differences in tissue localization of flavonoids, as well as the solvent polarity used during extraction. Our use of 96% ethanol likely favored the extraction of more polar flavonoids such as rutin and gallic acid, while quercetin, which is less soluble under these conditions, remained undetected.

Overall, these results expand the phytochemical knowledge of *Aesculus hippocastanum L.*, particularly regarding the pericarp, which can be considered a valuable source of biologically active

flavonoids. The high levels of rutin and gallic acid support the potential use of horse chestnut pericarp in the development of functional food ingredients and nutraceuticals aimed at improving vascular health and combating oxidative stress.

Conclusion. In this study, the presence and quantitative content of flavonoid compounds in the fruit pericarp of horse chestnut (*Aesculus hippocastanum L.*) were determined. The conducted analyses confirmed the presence of biologically active substances, particularly flavonoids such as rutin and gallic acid, in the seed coat extract of horse chestnut. These compounds are well-known for their antioxidant, anti-inflammatory, and capillary-strengthening properties. Their presence indicates the significant therapeutic potential of horse chestnut, especially in the treatment of venous insufficiency and varicose vein diseases.

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