DOI: https://doi.org/10.36719/2663-4619/127/269-276

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Quantification and Validation of Flavonoids in *Astrantia Maxima* Pall. Using Spectrophotometry

Abstract

This study aimed to quantify the flavonoid content in *Astrantia maxima* Pall. raw material using a spectrophotometric approach and to assess the method's validation parameters. Flowering-stage raw material of *Astrantia maxima* Pall. was collected in June 2025 from the Dashkasan district, Republic of Azerbaijan. Spectrophotometric determination of flavonoid content was performed, and method validation including accuracy, precision, and linearity was conducted following international regulatory guidelines. The total flavonoid content in *Astrantia maxima* Pall. was found to be 1.01% per gram of raw material. Validation outcomes indicated high reliability: accuracy demonstrated a confidence interval of 0.51; linearity was established over a concentration range of 0.01–0.06 mg/mL ($R^2 = 0.9989$); precision exhibited coefficients of variation of 0.0005%, 0.0006%, and 0.0006% across three consecutive days. These results confirm that *Astrantia maxima* Pall. is a rich source of flavonoids, highlighting its potential for incorporation into local medicinal plant-based resources and further pharmacological studies.

Keywords: *Astrantia maxima Pall., flavonoids, spectrophotometric analysis, method validation, raw material*

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Astrantia maxima Pall. bitkisində flavonoidlərin spektrofotometriya üsulu ilə miqdarı təyini və metodun validasiyası

Xülasə

Tədqiqatın məqsədi *Astrantia maxima* Pall. xammalında flavonoidlərin miqdarını spektrofotometrik yanaşma ilə təyin etmək və metodun validasiya parametrlərini qiymətləndirmək olmuşdur. *Astrantia maxima* Pall. bitkisinin çiçəkləmə mərhələsində olan xammalı 2025-ci ilin iyun ayında Azərbaycan Respublikası Daşkəsən rayonu ərazisindən toplanmışdır.

Flavonoidlərin miqdarının spektrofotometrik təyini aparılmış, metodun validasiyası isə beynəlxalq tənzimləyici qaydalara uyğun olaraq düzgünlük (accuracy), təkrarlanma dəqiqliyi (precision) və xəttilik (linearity) parametrləri üzrə həyata keçirilmişdir.

Astrantia maxima Pall. xammalında ümumi flavonoidlərin miqdarı 1 qram xammala görə 1,01% təşkil etmişdir. Validasiya nəticələri metodun yüksək etibarlılığını göstərmişdir: düzgünlük üçün etibarlılıq intervalı 0,51 olmuşdur; xəttilik 0,01–0,06 mq/mL konsentrasiya intervalında müəyyən edilmişdir ($R^2 = 0,9989$); təkrarlanma dəqiqliyi isə üç ardıcıl gün üzrə variasiya əmsallarının müvafiq olaraq 0,0005%, 0,0006% və 0,0006% olması ilə xarakterizə olunmuşdur.

Əldə olunan nəticələr *Astrantia maxima* Pall. bitkisinin flavonoidlərlə zəngin mənbə olduğunu təsdiqləyir və onun yerli dərman bitkisi resurslarına daxil edilməsi, eləcə də gələcək farmakoloji tədqiqatlar üçün perspektivli olduğunu göstərir.

Açar sözlər: *Astrantia maxima Pall., flavonoidlər, spektrofotometrik analiz, validasiya metodu, bitki xammalı*

Introduction

Astrantia maxima Pall. is a perennial species belonging to the Apiaceae family, distinguished by its characteristic inflorescence morphology. As is typical for members of the Umbelliferae, individual flowers are borne on stalks that converge at a common point; however, the structure resembling a

single flower is in fact a dense inflorescence composed of numerous small florets (Asgarov, 2016; Red Book of The Rebuplic of Azerbaijan Flora, 2023).

The leaves of *Astrantia maxima* Pall. are palmately lobed with serrated margins, exhibiting a glossy dark-green adaxial surface with well-defined venation, contrasted by a paler and more textured abaxial surface (Ibadullaeva, Gasimov, et al., 2024).

The development of the flower-head proceeds in a centripetal pattern, whereby the central florets reach maturity prior to those at the periphery (Bussman, Narel, et al., 2024; Lambion, Delvosalle, Duvigneaud, 2004).

The aerial parts of *Astrantia* contain a diverse range of phytochemicals, including flavonoids (quercetin, isoquercetin, astragalin, rutin, nicotiflorin, kaempferol, and kaempferutin), steroids, organic and phenolcarboxylic acids (malic, oxalic, malonic, citric, and angelic acids), as well as polyacetylene compounds. In contrast, the roots are characterized by a high content of sucrose, triterpene saponins, and triterpenoids (Kozhanova, Alimanova, et al., 2025; Nicolescu, Bunea, Mocan, 2025).

Compounds including flavonoids, triterpenes, and volatile oils have been reported in the genus *Astrantia*. The leaves of plants contain significant bioactive components that are believed to contribute to a variety of pharmacological activities (*Saini, et al.,* 2021; Kukhtenko, et al., 2024)

The chemical profile of *Astrantia* is notably rich in vitamins, minerals, and numerous biologically active constituents that collectively contribute to its therapeutic potential. Studies conducted by European researchers have demonstrated that flavonoids present in *Astrantia* exhibit detoxifying properties, enhance blood microcirculation, and exert anti-inflammatory effects. Sucrose has been shown to support physiological recovery during intoxication and to positively influence liver function, while saponins promote digestive activity by stimulating gastric juice secretion (Lyles, Sture, Walker, 2026).

Phytochemical investigations have revealed that *Astrantia* species possess a complex chemical profile, encompassing polyacetylene compounds, carbohydrates, and organic acids such as angelic, malic, and oxalic acids. The plant is also a source of triterpenoids, including gypsogenin, apigenin, oleanolic, and hypsogenic acids, together with triterpene saponins.

Astrantia maxima Pall. is a non-pharmacopoeial species and is not included in official medical practice. Nevertheless, due to its reported anti-inflammatory, antipyretic, blood-purifying, and related biological properties, the plant has long been used in traditional medicine.

Preparations derived from *Astrantia*, including decoctions and infusions, are generally regarded as well tolerated and are associated with a low incidence of adverse effects. However, despite its traditional use, comprehensive toxicological and clinical evaluations remain limited.

Both the aerial parts and roots are employed medicinally, typically in the form of decoctions or alcoholic tinctures.

The present study is aimed at the quantitative determination of flavonoid content in *Astrantia maxima* Pall. herb using a spectrophotometric method, alongside the validation of the proposed analytical procedure. Despite the growing interest in plant-derived bioactive compounds, *Astrantia maxima* remains a relatively underexplored species in terms of its phytochemical profile and standardization parameters. In particular, there is a lack of reliable and validated methods for the quantitative assessment of its flavonoid content, which represents a significant gap in the scientific literature. Flavonoids are known to contribute substantially to the pharmacological potential of medicinal plants, making their accurate determination essential for both quality control and further pharmacognostic investigations (de Lima, et al., 2024; Yuniarto, et al., 2025).

The scientific novelty of this research lies in the application and validation of a spectrophotometric method specifically for the quantitative analysis of flavonoids in *Astrantia maxima* Pall. herb. The proposed approach is designed to ensure accuracy, precision, and reproducibility, thereby providing a dependable analytical tool for routine use. By establishing validated parameters, this study contributes to the development of a standardized methodology for this plant species, which has not been previously reported (Shin, et al., 2025; Kushwah, et al., 2020; Khokrale, et al., 2024).

Furthermore, the results obtained are expected to expand the existing knowledge on the phytochemical composition of *Astrantia maxima*, supporting its potential use in pharmaceutical and herbal applications. The validated method may also serve as a reference for future studies focusing on related species or similar plant matrices.

Material and Methods

In this study, dried and powdered plant raw material of *Astrantia maxima* Pall., collected during the flowering period (July) from various regions of Azerbaijan, was used. Quantitative determination of flavonoids was carried out using a spectrophotometric method based on the formation of a flavonoid aluminum chloride complex, with measurement of absorbance at a wavelength of 410 nm. The absorbance measurements were performed using a Cary UV–Vis 60 spectrophotometer (Agilent Technologies). Rutoside was used as the reference (standard) compound. All analyses were performed in six replicates to ensure the reliability and reproducibility of the results.

Preparation of the sample solution

10 g of the powdered raw material were extracted with 70% ethanol at room temperature under constant stirring for 24 h. The extract was then filtered, and the volume was adjusted to 100 mL.

Preparation of the standard rutoside solution

10 mg of rutoside were dissolved in 100 mL of 70% ethanol until complete dissolution.

To 1 mL of the extract, 1 mL of a 2% aluminum chloride solution was added, mixed thoroughly, and allowed to stand at room temperature for 30 min. The absorbance of the resulting solution was measured using a spectrophotometer at a wavelength of 410 nm. The flavonoid content was calculated as rutoside equivalents using a calibration curve obtained with the standard rutin solution.

Method Validation

The method was validated by evaluating parameters such as linearity, precision, and accuracy. The results of the analysis fully complied with regulatory requirements, confirming the effectiveness of the method for the quantitative determination of flavonoids in the studied plant raw material.

Statistical Analysis

Standard methods of variance statistics were used for the statistical processing of experimental data. The mean, standard deviation (SD), standard error of the mean (S.E.), coefficient of variation (CV, %), and confidence interval at a significance level of $p = 0.95$ were calculated. All calculations were performed using Microsoft Excel.

Results

The quantitative determination of flavonoids in the plant raw material of *A. maxima* Pall. was performed using the spectrophotometric method. The validation parameters for the method of assessing flavonoid content in the herbaceous part of the plant were determined in strict accordance with the requirements of international regulatory documents, ensuring the accuracy and reproducibility of the obtained data (Matić, Sabljić, Jakobek, 2017; Smyslova, et al., 2019; Aishwarya, et al., 2024; Adamtsevich, et al., 2023; 15). Validation parameters such as accuracy, precision, and linearity were examined (Shin, 2025; Jain, 2011; Vyas, 2011).

Accuracy Assessment

To evaluate accuracy, a model mixture was prepared in a 25 mL volumetric flask. Sequentially, 1 mL of the extract, 1 mL of aluminum chloride solution, and one drop of acetic acid were added to the flask. After adding the reagents, the volume was adjusted to the mark with 95% ethanol, and the mixture was thoroughly mixed to obtain a homogeneous solution. The solution was allowed to stand at room temperature for at least 30 minutes to ensure complete reaction and complex formation.

For spectrophotometric measurements, 1.5 mL of the prepared solution was taken and mixed with 1.5 mL of extract. Spectrophotometric measurements of each model mixture were performed in triplicate. Based on the obtained results, the corresponding calculations were carried out to assess the accuracy of the quantitative determination method. The detailed data are presented in Table 1.

Thus, based on the conducted study, it can be concluded that the spectrophotometric method for the quantitative determination of flavonoids is reliable, and the associated statistical calculations comply with established requirements. The results are presented in Table 1.

Table 1.

Accuracy parameters of the quantitative determination of flavonoids in *Astrantia maxima Pall.* raw material by the spectrophotometric method.

Target volume, mL	Absorbance	Determined amount, mg	Interval, %
0.5	0.205	0.503	100.6
0.5	0.215	0.509	101.8
0.5	0.209	0.507	101.4
1	0.416	1.005	100.5
1	0.413	1.002	100.2
1	0.419	1.008	100.8
1.5	0.623	1.507	100.5
1.5	0.617	1.502	100.1
1.5	0.639	1.509	100.6
Standard deviation (%)			0.52
Coefficient of variation (%)			0.51
Mean value (%)			100.7
Lower limit of the confidence interval (%, p = 95%)			100.3
Upper limit of the confidence interval (%, p = 95%)			101.1
Minimum value (%)			100.1
Maximum value (%)			101.8

Linearity Assessment

The linearity of the quantitative determination method was evaluated using linear regression analysis, establishing the linear relationship between the concentration of the standard compound (x) and the absorbance (y).

To prepare the solutions required for linearity assessment, 0.05 g of the standard rutin was placed in a 100 mL volumetric flask, approximately 30 mL of 95% ethanol was added, and the mixture was shaken. The volume was then adjusted to the mark with 95% ethanol to obtain the stock solution.

From the stock solution, 2.0 mL was transferred to a 50 mL volumetric flask, approximately 20 mL of solvent was added, mixed, and the volume was adjusted to the mark with 95% ethanol to obtain a solution with a concentration of 0.02 mg/mL. Similarly, solutions with concentrations of 0.01, 0.03, 0.04, 0.05, and 0.06 mg/mL were prepared (Zebbiche, et al., 2023; Gvozdeva, 2026; Mullins, et al., 2014).

Spectrophotometric measurements of all prepared solutions were performed in triplicate. Based on the study, calculations corresponding to linearity parameters were carried out. The detailed results are presented in Figure 1 and Table 2.

Table 2.

Linearity parameters of the quantitative determination of flavonoids in *Astrantia maxima Pall.* raw material by the spectrophotometric method.

N	Concentration of the rutin standard (mg/mL)	Absorbance
1	0,01	0.4784
2	0,02	0.9568
3	0,03	1.4352
4	0,04	1.9145
5	0,05	2.3156
6	0,06	2.8721
Correlation coefficient (r)		0.9995
Slope		47.21

Intercept (y-intercept)	0.00968
Coefficient of determination (R^2)	0.9989
Standard error (S.E.)	0.033
Linear function	y = 47.212x + 0.0097

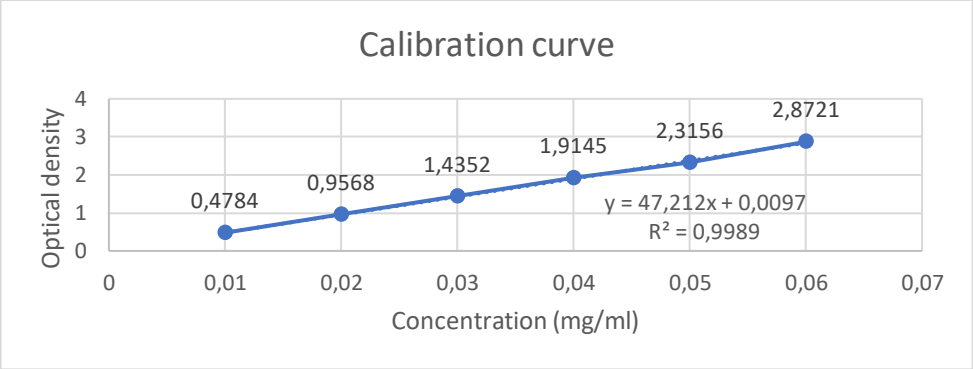

Fig. 1. *Linearity indicators for the quantitative determination of flavonoids in Astrantia maxima Pall. raw materials using spectrophotometry*

The results obtained indicate a linear relationship between the absorbance of the standard rutin solution and its concentration.

Precision Determination

To assess precision, a model mixture was prepared in a 25 mL volumetric flask. Sequentially, 1 mL of the extract, 1 mL of aluminum chloride solution, and one drop of acetic acid were added to the flask. After adding the reagents, the volume was adjusted to the mark with 95% ethanol, and the mixture was thoroughly mixed to obtain a homogeneous solution. To allow complex formation, the prepared solution was left at room temperature for 40 minutes. After this period, the spectral characteristics of the solution were measured five times, and corresponding calculations were performed based on the mean values. Analyses were carried out over a period of three days. The final results of the inter-assay precision of the method for determining flavonoid content in *A. maxima* Pall. raw material using spectrophotometry are presented in Table 3.

Table 3.

Results of inter-assay precision for the determination of flavonoid content in *A. maxima Pall.* raw material using the spectrophotometric method.

Target volume (mL)	**Optical density**			**Detected volume (mL)**			**Detected concentration (%)**		
	Day I	**Day II**	**Day III**	**Day I**	**Day II**	**Day III**	**Day I**	**Day II**	**Day III**
1.0	0.413	0.415	0.412	1.0003	1.0001	1.0007	100.03	100.11	100.07
	0.416	0.416	0.416	0.9994	1.0007	1.0001	99.94	100.07	100.01
	0.419	0.415	0.415	1.0006	1.0009	0.9998	100.06	100.09	99.98
	0.416	0.418	0.411	1.0001	0.9997	1.0006	100.01	99.97	100.06
	0.418	0.417	0.418	1.0005	1.0005	1.0012	100.05	100.05	100.12
	0.413	0.419	0.417	1.0008	1.0016	0.9997	100.08	100.16	99.97
Minimum value (%)							99.94	99.97	99.97
Maximum value (%)							100.08	100.16	100.12
Mean value (%)							100.028	100.075	100.035
Standard deviation (%)							0.0496	0.0638	0.0582
Coefficient of variation (%)							0.0005	0.0006	0.0006
Confidence interval (p = 95%)							99.98 - 100.08	100.01- 100.14	99.97- 100.10
Standard error (S.E.)							0.0202	0.0260	0.0238

Discussion

Based on the obtained results, it can be concluded that the inter-assay precision parameters of the spectrophotometric method for determining flavonoid content in *Astrantia maxima* Pall. raw material fully comply with established analytical requirements. The very low coefficients of variation obtained over three consecutive days (0.0005%, 0.0006%, and 0.0006%, respectively) indicate a high level of method precision and reproducibility, confirming the stability of the analytical procedure under the tested conditions.

The quantitative determination of flavonoids in *Astrantia maxima* Pall. using the proposed spectrophotometric method revealed that 1 g of the plant raw material contains 1.01% total flavonoids expressed as rutoside equivalents. This result provides important insight into the phytochemical composition of this relatively underexplored species and suggests that it may represent a promising source of biologically active compounds. Given the known pharmacological relevance of flavonoids, their relatively high content further supports the potential applicability of this plant in pharmaceutical and herbal preparations.

Simultaneously, the applied spectrophotometric method was successfully validated according to key analytical parameters, including linearity, accuracy, and precision. The method demonstrated excellent linearity within the concentration range of 0.01–0.06 mg/mL, with a correlation coefficient (R^2) of 0.9989, indicating a strong relationship between absorbance and concentration. The accuracy of the method, characterized by a confidence interval of 0.51, falls within acceptable limits, confirming the reliability of the obtained results.

Overall, the validation data confirm that the proposed method is suitable for the quantitative determination of flavonoids in *Astrantia maxima* Pall. raw material. The combination of high precision, satisfactory accuracy, and strong linearity demonstrates that this method can be reliably applied in routine analysis. Furthermore, considering that this plant species has been insufficiently studied and that no previously validated spectrophotometric methods have been reported for it, the present findings contribute to filling an important gap in the scientific literature and provide a foundation for future phytochemical and pharmacognostic investigations.

Conclusion

The results of the present study demonstrate that Astrantia maxima Pall. herb contains a considerable amount of flavonoids (1.01% calculated as rutoside equivalents), confirming its richness in biologically active compounds. The successful application and validation of the spectrophotometric method, characterized by high precision, acceptable accuracy, and excellent linearity, indicate that the proposed analytical approach is reliable and suitable for the quantitative determination of flavonoids in this plant raw material.

Overall, the obtained results highlight the significant potential of Astrantia maxima Pall. herb for the future expansion of the local medicinal plant raw material base and support its перспектив istifadəsi (potential use) in pharmaceutical and herbal applications. Furthermore, this study creates a foundation for subsequent phytochemical, pharmacological, and standardization research on this species.

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Received: 02.11.2025
Accepted: 20.02.2026