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Quantitative Determination Of Berberine In The Aboveground Organs Of Mahonia Aquifolium (Pursh) Nutt.

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Abstract

Our study's objective was to use high-performance liquid chromatography (HPLC) to quantify the amount of berberine in the total amount of alkaloids extracted from *M. aquifolium.* Eclipse plus C-18 was used to separate the crude extract (250 x 4.6 x 5 µm). Methanol (0.1% formic acid): water (0.1 formic acid) 35:65 v v^{-1} made up the mobile phase. At 346 nm, ultraviolet detection was carried out. With a $r^2 > 0.9998$, a solid linear response was seen in the concentration range under study. The alkaloid extract from the aerial parts of *M. aquifolium* should contain at least 2% berberine, according to the results. With intra- and inter-day variances of less than 1.03%, the approach is accurate. Within the range under study, linearity, sensitivity, and precision are all adequate.

Keywords: alkaloids, berberine, bioactive compounds, quantitative analysis, HPLC.

1. Introduction

About two centuries ago, the discovery of alkaloids marked a new era in medicine and chemistry. In modern phytochemistry, the study of biologically active secondary metabolites encompasses several key areas: exploration of flora for alkaloid-containing plants, selection of optimal methods for extracting the total alkaloid content from plant materials, and isolation of individual compounds. Additionally, there is an emphasis on identifying the structure of previously unknown alkaloids and studying their pharmacological activity to develop effective medicinal drugs. This research also relies on the latest technological advancements and includes resource studies, as well as addressing any challenges related to the cultivation of medicinal plants [1-4].

Alkaloids are abundant in the kingdom of plants and have a high level of biological activity. It was previously believed that the alkaloid bases found in a plant's chemical composition were characteristic and specific to a particular family. However, studies on their distribution in plants have shown that the same base can be present in plants from entirely different families. For instance, berberine, one of the first alkaloids used in medicine, has attracted significant interest due to its low toxicity and broad biological activity. Berberine demonstrates antioxidant, anti-inflammatory, neuroprotective, antiviral, antibacterial, hypoglycemic, hypolipidemic, antidepressant, antidiarrheal, and anticancer properties, showing efficacy against liver tumors, stomach cancer, throat cancer, and more. It is also used to treat chronic hepatitis, hepatobiliary disease, cholecystitis, gallstone disease, and other conditions [5-26].

One of the rich sources of the biologically active alkaloid berberine is the genus *Mahonia* Nutt. (family *Berberidaceae*), which includes around 60 plant species native to Asia, North America, and Europe. In Georgia, *Mahonia* is represented at the Batumi Botanical Garden on the shore of the Black Sea (Adjara) by a collection of naturalized plants comprising six species, including *M. aquifolium* (Pursh) Nutt., which is well-known for its medicinal properties. This species is widely used in traditional and folk medicine (in Chinese and North American practices) as well as in modern practical medicine, especially for the treatment of skin diseases. *Mahonia aquifolium* (Pursh) Nutt also known as *Berberis aquifolium* Pursh [27-30].

However, there is a lack of scientific research data on the chemical, and particularly the alkaloid, composition of this species introduced in Georgia, as it has primarily been used for ornamental purposes in gardens and parks. Based on the above, preliminary qualitative analysis confirmed the presence of alkaloids in the introduced *M. aquifolium*. Phytochemical analysis further indicated that all vegetative organs of the plant contain isoquinoline alkaloids to varying degrees, including the biologically active berberine base. The total alkaloid extract from the aerial parts of the plants, which contains biologically active compounds, including berberine, exhibited below mentioned activities: cytotoxicity was evaluated in vitro using the Hoechst (DNA quantification) and Resazurine spectrophotometric assays which demosntrated high activity against lung cancer carcinoma A-549, colon cancer DLD-1, and normal fibroblast WS-1 cell lines. Antibacterial activity was evaluated using the microdilution method exhibited moderate activity. Inflamatory asay showed us is low toxicict to RAW 264.7 cells. The antiproliferative and antipsoriatic activity was evaluated quantitatively against human keratinocytes of the HaCaT line, which showed that it exhibits a pronounced cytotoxic effect on cultured human HaCaT cells and a significant antiproliferative effect on cultured human HaCaT cells at concentrations lower than the cytotoxic ones. For determination of antioxidant activity was used the DCFH-DA, compared to quercetin both in the ORAC test and in the in vitro test IC_{50} using a cellular model.

The main constituent in the extracted sum of *M. aquifolium*, are quinolone alkaloids. The pharmacologically and biologically active berberine is dominant compound within sum of alkaloids. The berberine was selected as a marker (chemical and biological) of sum. Therefore, our study's objective was to use high-performance liquid chromatography (HPLC) to quantify the amount of berberine in the total amount of alkaloids extracted from *M. aquifolium*.

Figure 1. *Mahonia aquifolium* (Pursh) Nutt.

2. Materials and methods

2.1. Plant Material: The study object was *M. aquifolium* Nutt., collected in the Kartli floristic region: N41.757750 E044.762710, H-485m, in 2021. The experimental specimen is stored in the herbarium of TSMU's I. Kutateladze Institute of Pharmacochemistry (TBPH - No. 21343**)** Identification of study object was performed with comparison to mentioned sample of herbarium, which was performed by Direction of Pharmacobotany of Tbilisi state Medical Universitiy Iovel Kutateladze Institue of Pharmacochemistry. The newly collected partsof the plant were held up with air-dried at room temperature and protected from light and humidity until chemical analysis. Before analysis, the row materials were powdered.

***2.2. Sample Preparation*:** The extraction of total alkaloids from the aerial part of *M. aquifolium* followed a previously established classical method. Finely ground, air-dried plant material (50 g, above mentioned vegetative parts were selected 10 g each) was pre-alkalized with 12% NH_4OH, then extracted with $CHCl_3$ by maceration at room temperature, treated with 10% H_2SO_4, and re-alkalized with 25% NH_4OH (while cooling) to a pH of 9–10. The alkaloids were then transferred to $CHCl_3$ in their base form. The yield of total alkaloids from the aerial parts of *M. aquifolium*, obtained as an amorphous brown powder, was 0.51 g.

***2.4. Chemicals and reagents*:** HPLC grade methanol of analytical grade was purchased from Merck & Co. Ultrapure water for HPLC analysis was obtained from a Millipore Classic purification system. Berberine (HPLC 99.0%.) has been purchased from Sigma LTD.

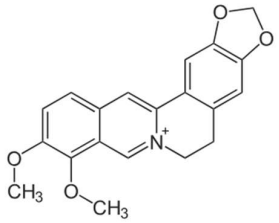

Figure 2. Structure of Berberine

Identification of Berberine was realized using the LC-MS spectra with comparison the reference data[31].

***2.5. Chromatographic instrument and conditions*:** The Agilent Technologies Model 1260 liquid chromatography, which was part of the HPLC system, has a photodiode array detector (DAD), an auto-sampler, a quaternary pump, a vacuum degasser, and an MS. Computer software: Chemstation. An Eclipse plus C-18 (250 x 4.6 x 5 µm) was used to accomplish the chromatographic separation. All separations were carried out at 20°C. The mobile phase was made up of methanol (0.1% formic acid): water (0.1 formic acid) 35:65 v/v; the flow rate was 1 ml/min, and the injection volume was 10 µl. All peaks' UV spectra were captured within the detection range of 200–400 nm. The sole wavelength used for quantification was 346 nm.

***2.6. Preparation of standard solution*:** To reach a final concentration of 1.0 mg/mL, a standard solution of berberine was made in methanol. To achieve different concentration levels (0.002-1.0 mg/mL), several working solutions of berberine (n=5) were made. A 10.0 mL volumetric flask was filled with the proper volume of Berberine solution, and the volume was adjusted to 10.0 mL using mobile phase. Prior to HPLC analysis, all prepared standard solutions were passed through a 0.45 µm membrane filter (Millipore).

***2.7. Preparation of sample solution*:** involved adding 20.0 mg of *M. aquifolium* crude extract (bought from the Institute of Pharmacochemistry in Tbilisi, Georgia) to a 100 mL volumetric flask, dissolving it in methanol, and then adjusting the volume to 100 mL using methanol. A syringe filter (0.45 mm Millipore) was then used to filter 2 ml of each solution into an HPLC vial.

3. Results and discussion

***3.1 Assay and Validation*:** The procedure was tested on *M. aquifolium* crude extract in accordance with ICH recommendations (ICH-Q2 (R1) (2005) Geneva. For Berberine, the HPLC method's linearity was tested. For every concentration, five distinct concentrations were made and examined in triplicate. Berberine has a concentration range of 0.002 to 1.0 mg mL^{-1}. Peak regions were plotted against concentrations to create calibration curves. Using least squares regression, the slope, y-intercept, and coefficient of correlation (r^2) were computed in order to evaluate the linearity. The concentrations that resulted in signal-to-noise ratios of 3:1 and 10:1, respectively, were the limits of detection and quantification.

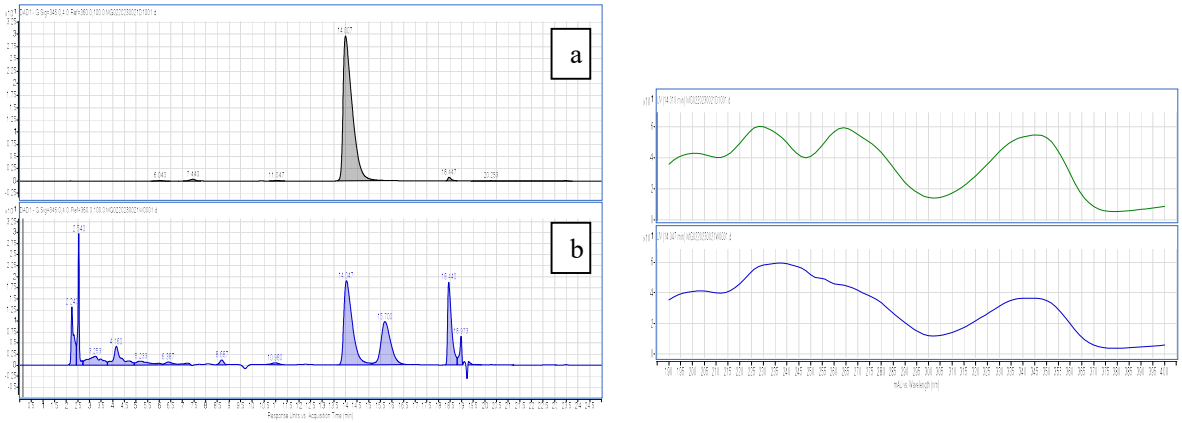

Figure 3. HPLC profile of a) Berberine and total alkaloids from the aerial parts b) *M. aquifolium*.

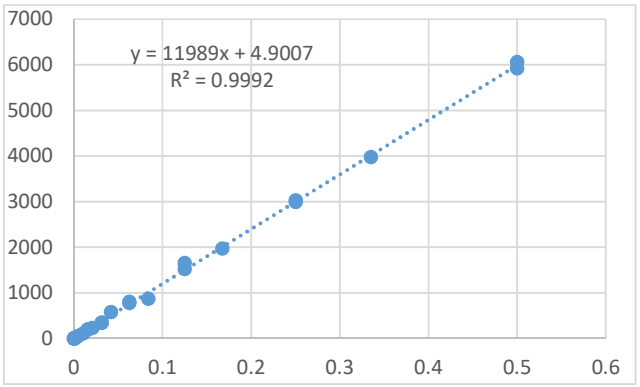

Figure 4. Calibration curve and linearity of Berberine.

Both intra- and inter-day precision were used to assess the method's accuracy. Six sample solutions were made independently on a single day, and their analyses were used to calculate intraday precision. The same process was repeated on two additional days to assess inter-day precision. For every day, the RSD (relative standard deviation) and standard deviation values were computed. The recovery test was used to assess the method's accuracy. To do this, known amounts of standard solutions containing berberine were spiked into the actual samples. Three concentration levels (50, 100, and 150%) were used to prepare the standard solutions. Samples were analyzed in triplicate at each level using the chromatographic conditions previously mentioned.

To obtain a good resolution, the HPLC separation conditions were improved. The reverse phase column Eclipse plus C-18 (250 x 4.6 x 5 µm) produced the best separation of the *M. aquifolium* crude extract. The

effect of the mobile phase was investigated in order to produce an ideal resolution. A mobile phase consisting of methanol (0.1% formic acid): water (0.1 formic acid) 35:65 v/v was used to detect a sufficient separation; a berberine solution was made in methanol. 10.3 minutes was the recorded retention time for berberine. A suitable chromatographic baseline and adequate sensitivity were demonstrated by the measurement at 346 nm. Consequently, under ideal circumstances, a baseline separation with symmetrical, crisp, and well-resolved peaks for Berberine was accomplished in 30 minutes.

The chromatogram reveals the full baseline separation of berberine in the *M. aquifolium* crude extract. The ICH recommendations for the validation of analytical methods were followed in the validation of this procedure. By comparing the retention times of Berberine and the crude extract of *M. aquifolium*, excellent resolution was seen with no interference from other substances. HPLC was used to confirm the peak purity (Agilent Chemstation software).

Table 1 shows the mahonia extract's intra- and inter-day precision results. The method's precision was demonstrated by the intra-day % RSD being less than 2.4% and the inter-day % RSD being less than 3.3%. The calculated recoveries varied between 99.68 and 100.28%. The detection limit was 0.5 μg/mL. 3.8 μg mL^{-1} was the quantitative limit. Berberine is successfully quantified using the established HPLC method. Berberine should make up at least 2% of the overall alkaloid concentration.

Table I. The results of precision measurements for Berberine within and between days

Linearity	**Range**	**0,005-1,0 mg/ml**	
	Calibration curve	**R^2 = 0.9992** **y=11989x + 4.9007**	
Accuracy	**99,8±0,3**		
Precision	**Intermediate precision %RSD**	**I day**	**2.1**
		II day	**2.3**
		III day	**1.9**
	Intermediate precision (Intra-day) %RSD (n=18)		**3.3**

4. CONCLUSIONS

A novel technique for measuring the pharmacologically active alkaloid berberine in the biologically active extract from the areal part of *M. aquifolium* was developed. In conclusion, the established method for the quantitation of berberine demonstrates significant novelty and innovation for the quantification of the biologically active extract from the areal part of *M. aquifolium*. The methodological advances in HPLC analysis enhance accuracy, sensitivity, and reproducibility. The innovation in analytical parameters reduces both the detection limit and the quantitative determination limit of berberine. Validation confirms the method's reliability and reproducibility, underscoring its practical value.

Disclosure statement

The authors report there are no competing interests to declare.

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